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Original Research

Dietary Habits of Adults Hypertensive Patients Admitted in Cardiology of Deido District Hospital, Cameroon

Nyangono Biyegue Christine Fernande, PhD[1,3*]; Mintom Medjo Pierre, PhD[2]; Nnang Ebanda Veronique, PhD[1]; Ngondi Judith Laure, PhD[3]

[1]*University of Douala, Advanced Teacher Training College for Technical Education, PO Box 1872 Douala, Cameroon*
[2]*Deido District Hospital, PO Box 8037 Douala, Cameroon*
[3]*University of Yaounde I, Laboratory of Nutrition and Nutritional Biochemistry, PO Box 812 Yaounde, Cameroon*

[*]***Corresponding author***
Nyangono Biyegue Christine Fernande, PhD
University of Douala, Advanced Teacher Training College for Technical Education, PO Box 1872 Douala, Cameroon; Tel. (+237) 675223409/690884697;
E-mail: spfernande@yahoo.fr

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ABSTRACT

Objective
To assess the dietary habits of patients suffering from hypertension in Cameroon in the context of nutrition transition (Nutrition transition is the shift in dietary consumption and energy expenditure that coincides with economic, demographic, and epidemiological changes. Specifically, the term is used for the transition of developing countries from traditional diets high in cereal and fiber to more Western pattern diets high in sugars, fat, and animal-source food).

Methodology
A cross-sectional descriptive study was carried out during six months on 206 hypertensive patients attending the Deido district hospital. BMI was calculated using body weight and height. Hypertension was defined as the 2017 ACC/AHA guidelines (SBP ≥ 130 mmHg, or DBP ≥ 80 mmHg). Eating habits and food consumption score were assessed by using Food Questionnaire validated by the Laboratory of Nutrition and Nutritional Biochemistry (University of Yaounde 1, Cameroon). Food mineral contents were also evaluated.

Results
Patients suffering from hypertension consumed all groups of food as fruits (pawpaw, avocado, pineapple, water melon and orange), vegetables (folong, okok and carrots), tubers (plantains, cassava, unripe banana and yam), cereals (rice and white bread), legumes (egusi, beans and groundnuts), fats (margarine and refined palm oil), spices (onion and garlic) and animal proteins (fish, red meat, milk, eggs and dairy products). Results of consuming foods rich in micronutrients revealed that hypertensive patients mostly eat foods rich in sodium. The food consumption score is limit means that the global quality diet is inadequate quality and adequate quantity.

Conclusion
Patients suffering from hypertension have inadequate quality of diet and most consume foods rich in sodium.

Keywords
Food habits; Hypertension; Hospital

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INTRODUCTION

Overweight and obesity because of their strong prevalence and their implications as major risk factors of chronic diseases related to nutrition, type 2 diabetes, cardiovascular diseases, and hypertension are today the most significant threats of public health on a worldwide scale.[1] Indeed, many developing countries have experienced a very significant increase in the prevalence of obesity, type 2 diabetes, cardiovascular diseases, and hypertension.[1] In 2005, 58 million deaths occurred worldwide; over 35 million deaths were attributed to the chronic diseases.[2] According to world health organization (WHO),[3] hypertension comes in second position, after smoking but before alcoholism, on the list of the factors decreasing the number of years of life in good health. Moreover, high blood pressure increases morbidity and cardiovascular mortality.[1] The recent data of the WHO indicate that nearly a billion people in the world suffer from hypertension. Because of the aging of the population, the forecasts suggest that this number could increase to 1.5 billion in 2025. According to the Cameroon Heart Foundation, the statistics relating to arterial hypertension are worrisome. This arterial hypertension causes 17.3 million deaths in the world. Among those, 80% occur in the countries with average or weak incomes. Africa in general and Cameroon in particular are not saved by high prevalence of hypertension because epidemiologic studies reveal the strong prevalence of obesity, diabetes and high blood pressure.[4] In Cameroon, 35% of the adult population suffers from arterial hypertension; 17,000 people there die each year.[5] In Cameroon, the situation of people suffering high blood pressure is alarming; besides, it worth noting that the nutritional management is neglected even non-existent in hospitals. Nowadays, in spite of the free screening and public awareness campaigns, more and more of people die of this pathology either by negligence or by ignorance, or even for lack of nutritional follow-up.

The aim of this study is to assess the dietary habits of patients suffering from hypertension in Cameroon.

METHODOLOGY

This cross-sectional and descriptive survey was conducted in Douala (economic town of Cameroon) particularly in Deido District Hospital.

The survey covered a sample of 206 Cameroonian hypertensive patients. The study proceeded between August 2015 and February 2016.

Hypertension was defined based on the 2017 American Heart Association/American College of Cardiology (AHA/ACC) guidelines (observed systolic BP (SBP) ≥ 130 mmHg, or diastolic BP (DBP) ≥ 80 mmHg).[6] Pregnant women, breastfeeding women and normotensive patients (SBP<130 mmHg and DBP<80 mmHg) were excluded. Blood pressure and anthropometric measurement were taken before the administration of the Food Frequency Questionnaire.

Anthropometric Measurements

Body weight was measured to the nearest 100 g, with participants in light clothing and without shoes, using an electronic scale of 150 kg capacity (TANITA BC 543). Height was measured to the nearest 0.5 cm using a portable locally built stadiometer, with the participants standing upright on a flat surface without shoes, with the back of their heels and the occiput against the stadiometer. Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters). Nutritional status was defined as follows[7]: underweight: BMI equal to 18.5 kg/m^2; normal weight: BMI ranged to 18.5 and 24.9 kg/m^2; overweight: BMI range to 25.0 and 29.9 kg/m^2; obese: BMI≥30.0 kg/m^2.

Blood Pressure Measurement

Blood pressure was measured using electronic radial tensiometer (OMRON M3 Comfort). Blood pressure was measured by the first author with acalibrated tensiometer on the right arm of seated participants after a minimum of 10 min rest. Systolic and diastolic pressures were measured twice with an interval of 10 min between the first and the second measurement. The mean of the two readings was used in the analyses. Hypertension for individuals without a prior diagnosis was defined as systolic blood pressure SBP≥ 140 mmHg and/or diastolic blood pressure DBP≥90 mmHg.[8]

Age

Age is a cardiovascular risk factor independently of other factors.[9] In this study, age was represented in sections of 24-30-years, 31-40-years, 41-50-years, 61-70-years, 61-70-years and 71-80-years, 81-90-years.

Measurement of Eating Habits and Food Consumption Score

Measurement of eating habits: The eating habits of the participants were explored using a pretested food frequency questionnaire validated by the laboratory of nutrition and nutritional biochemistry (University of Yaounde 1, Cameroon). This questionnaire included 09 groups of foods in which frequency was characterized as: at least once a week, 1-3 times a week, more than 4 times a week. For each participant, we documented during seven days period the frequency of "*Western*" food, "*traditional*" food consumption; consumption of fat and sugar.

Food consumption score (FCS): Food consumption score represents the quality of diet. It is used as an indicator of access to the feeding.[10] It is based on a 7-days recall on the food types/groups (diversity) and the food frequency of consumption. To measure this score, 09 groups of food were aggregated into 08 groups of food necessary to build a food consumption score. Each group of food was affected by a weighting, thus the score obtained was the product of the weighting and the number of days of consumption during the last 7-days. The resulting food consumption score was calculated as the sum of the scores representing the number of groups of consumed food. The higher the score, the more the feeding was regarded as adequate.

Thus, FCS<21: Poor (inadequate quantity and quality of diet)

21.5<FCS<35: Limit (inadequate quality and adequate quantity of diet)

FCS>35: Acceptable (adequate diet)

Measurement of Food Mineral Content

Four minerals implied in hypertension were chosen: potassium, sodium, calcium, and magnesium. It was evaluated using a food frequency questionnaire instrument, and the most food consumed by hypertensive patients was selected. The mineral composition of these foods was evaluated using the dietary composition tables. The presence of one group of mineral was noted « 1 » and his lack noted « 0 ». We considered that consumption of food rich in one of the selected micronutrients is regular when the patients consumed higher than 4 foods rich in one of selected mineral.

Statistical Analysis

Software SPSS (Statistical Social Package for Sciences) version 11.5 for Windows was used to carry out the statistical analyses. The descriptive statistics made it possible to calculate the frequencies. The tables and the histograms were obtained using Excel.

Ethical Approval

One month preceding the survey, the communities and their leaders were informed by the study investigators about the goals, the importance and the benefits of the study. Participation in the study was voluntary. All the participants gave their free and informed consent to participate in the study. This study was approved by the administration and ethical committee of this hospital (Registry number N°960/AV/MINSANTE/DRSPL/SSDD/HDD).

RESULTS

In this study, 30.1% of patients are in the age between 41-50 years, 62.6% were women. Based on ACC/AHA guidelines, the prevalence of hypertension (130/80 mmHg) was 77.09%. The hypertensive population included 67 males and 107 females. It is constituted by 38.3% of obese and 30.3% of overweight persons. The prevalence of systolic hypertension was 97.4% and diastolic hypertension was 78%.

The results of *t*-test of Student showed a significant difference in height and BMI between female and male of hypertensive population (Table 1).

Table 1. *Anthropometric and Hemodynamic Characteristics of Hypertensive Population*

Parameters	Gender	Means	*p* value
Age (years)	Male	50.33±1.70	*p*>0.05
	Female	50.01±1.15	
Weight (Kg)	Male	78.01±1.82	*p*>0.05
	Female	79.92±1.73	
Height (m)	Male	1.68±0.009	(*p*<0.01)
	Female	1.63±0.006*	
BMI (Kg/m2)	Male	27.63±0.70	(*p*<0.05)
	Female	29.97±0.66*	
SBP (mmHg)	Male	158.84±2.17	*p*>0.05
	Female	157.06±1.83	
DBP (mmHg)	Male	96.36±1.36	*p*>0.05
	Female	95.29±1.23	
Pool (bat/min)	Male	81.51±1.46	*p*>0.05
	Female	83.94±1.60	

Table 2. *Distribution of Hypertensive Patients Based on Consumption of Fruits*

Fruits	Never n (frequency)	Rarely (1 - 2 times/ week) n (frequency)	>3times/ week n (frequency)	Regular consumption (%)	Irregular consumption (%)
Pawpaw	34 (19.4)	50 (28.6)	91 (52.0)	70.3%	29.7%
Soursop	114 (65.1)	33 (18.9)	28 (16.0)		
Pineapple	38 (21.7)	39 (22.3)	98 (56.0)		
Water-melon	41 (23.4)	47 (26.9)	87 (49.7)		
Orange	34(19.4)	43 (24.6)	98 (56.0)		
Grapefruit	100 (57.1)	48 (27.4)	27 (15.4)		
Sweet banana	57 (32.6)	55 (31.4)	63 (36.0)		
Mangoe	93 (53.1)	37 (21.1)	45 (25.7)		
Avocado	47 (26.9)	41 (23.4)	87 (49.7)		
Lemon	76 (43.4)	48 (27.4)	51 (29.1)		
Apple	73 (41.7)	39 (22.3)	63 (36.0)		
mandarine	74 (42.3)	50 (28.6)	51 (29.1)		
guava	80 (45.7)	43 (24.6)	52 (29.7)		
kassemango	85 (48.6)	46(26.3)	44 (25.1)		

Food Habits of Hypertensive Patients

Hypertensive patients are divided according to the consumption of the groups of food, individual food by groups of food and consumption of foods rich in minerals.

Food Consumption

The population of study is divided according to the consumption of fruits, vegetables, tubers, cereals, animal proteins, legumes, fats, drinks, spices and sweet foods.

We observed that 70.3% of patients regularly consumed fruits. The more consumed Fruits (>3times/week) in the hypertensive patients population are: pawpaw (*Carica papaya*) (52.0%) avocado (*Persea Americana*) (49.7%), pineapple (*Ananas comosus*) (56.0%), watermelon (*Citrus lanatus*) (49.7%) and orange (*Citrus X sinensis*) (56.0%) (Table 2).

On the other hand, 66.3% of patients regularly consume vegetables like: zom (*Solanum nigrum*) (46.8%), ndole (*Vernonia amygdalina*) (49.1%) and carrots (*Daucus carota* sub sp sativus) (58.8%), cabbages (47.8%) and tomatoes (58.3%) (Table 3).

Concerning cereals, 86.3% of patients consumed cereals like rice (*Oryza sativa*) and white bread (Table 4).

Concerning starchy foods, the results showed a weak consumption (35.4%). The most consumed foods are Irish po-

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Table 3. *Distribution of Hypertensive Patients Based on Consumption of Vegetables*

Vegetables	Never n (frequency)	Rarely (1 - 2 times/ week) n (frequency)	>3times/ week n (frequency)	Regular consumption (%)	Irregular consumption (%)
Folong	66 (37.7)	71 (40.6)	38 (21.7)	66.3%	33.7%
Okok	68 (38.9)	51 (29.1)	56 (32.0)		
Sanga	78 (44.6)	52 (29.7)	45 (25.7)		
Zom	43 (24.6)	50 (28.6)	82 (46.8)		
Kpem	78(44.6)	62 (35.4)	35 (20.0)		
Ndole	34 (19.4)	55 (31.4)	86 (49.1)		
Eru	91 (52.0)	42 (24.0)	42 (24.0)		
Djamadjama	97 (55.4)	34 (19.4)	44 (25.1)		
Cocoyam leaves	104 (59.4)	36 (20.6)	35 (20.0)		
Melon leaves	96 (54.9)	37 (21.1)	42 (24.0)		
Tomatoes	38 (21.7)	35 (20.0)	102 (58.3)		
Cabbages	51 (29.1)	41 (23.4)	83(47.4)		
Carrots	32 (18.3)	40 (22.9)	103 (58.8)		
Green beans	51 (29.1)	54 (30.9)	70 (40.0)		

Table 4. *Distribution of Hypertensive Patients Based on Consumption of Cereals*

Cereals	Never n (frequency)	Rarely (1 - 2 times/ week) n (frequency)	>3times/ week n (frequency)	Regular consumption (%)	Irregular consumption (%)
Maize and products	26 (14.9)	59 (33.7)	90 (51.4)	86.3%	13.7%
Rice	20 (11.4)	38 (21.7)	117 (66.9)		
Pasta	25 (14.3)	57 (32.6)	93 (53.1)		
White Bread	19 (10.9)	31 (17.7)	125 (71.4)		

Table 5. *Distribution of Hypertensive Patients Based on Consumption of Starchy Foods*

Starchy foods	Never n (frequency)	Rarely (1 - 2 times/ week) n (frequency)	>3times/ week n (frequency)	Regular consumption (%)	Irregular consumption (%)
Plantain	98 (56.0)	13 (7.4)	64 (36.6)	66.3%	33.7%
Cassava	104 (59.4)	25 (14.3)	46 (26.3)		
Unripe bananas	106 (60.6)	26 (14.9)	43 (24.5)		
yams	114 (65.1)	23 (13.1)	38 (21.8)		
Yams	114 (65.1)	33 (18.9)	28 (16.0)		
Irish potatoes	101 (57.7)	24 (13.7)	50 (28.6)		
Sweet potatoes	121 (69.1)	25 (14.3)	29 (16.6)		
Ponded yams	126 (72.0)	31 (17.7)	18 (10.3)		

tato (*Solanum tuberosum*) (28.6%), plantain (*Musa paradisiaca*) (36.6%), cassava (*Manihot esculenta*) (26.3%), unripe banana (*Musa acuminata*) (24.5%), and cocoyam (*Dioscorea sp*) (21.8%). (Table 5).

Patients (61.7%) regularly consumed legumes like Egusi (*Cucumis sp*) (58.3%), beans (*Phaseolus vulgaris*) (52.0%) and peanuts (*Arachis hypogeae*) (61.1%). All of this is present in their diet three times per week. (Table 6).

Table 6. *Distribution of Hypertensive Patients Based on Consumption of Legumes*

Legumes	Never n (frequency)	Rarely (1 - 2 times/ week) n (frequency)	>3times/ week n (frequency)	Regular consumption (%)	Irregular consumption (%)
Peanuts	35 (20.0)	33 (18.9)	107 (61.1)	61.7%	38.3%
Soya	117 (66.9)	33 (18.9)	25 (14.2)		
Egusi	20 (11.4)	53 (30.3)	102 (58.3)		
Beans	34 (19.4)	50 (28.6)	91 (52.0)		

Table 7. *Distribution of Hypertensive Patients Based on Consumption of Animal Proteins*

Animal proteins	Never n (frequency)	Rarely (1 - 2 times/ week) n (frequency)	>3times/ week n (frequency)	Regular consumption (%)	Irregular consumption (%)
Eggs	42 (24.0)	43 (24.6)	90 (51.4)	77.1%	22.9%
Red meat	27 (15.4)	43 (24.6)	105 (60.0)		
White meat	21 (12.0)	52 (29.7)	102 (58.3)		
Meat products	77 (44.0)	64 (36.6)	34 (19.4)		
Fish	12 (06.9)	34 (19.4)	129 (73.7)		
Sea products	26 (14.9)	38 (21.7)	111 (63.4)		
Milk	41 (23.4)	56 (32.0)	78 (44.5)		
dairy products	62 (35.4)	47 (26.9)	66 (37.7)		

From this study it comes out that 77.1% of hypertensive patients regular consumed animal proteins. The foods consumed up to 3 times per week are fish (73.7%), red meat (60.0%), white meat (58.3%), sea products (63.4%) and eggs (51.4%) (Table 7).

Table 8. *Distribution of Hypertensive Patients Based on Consumption of Fats and Oils*

Fats and oils	Never n (frequency)	Rarely (1 - 2 times/ week) n (frequency)	>3times/ week n (frequency)	Regular consumption (%)	Irregular consumption (%)
Crude palm oil	55 (31.4)	41 (23.4)	79 (45.1)	26.3%	73.7%
Refine palm oil	26 (14.9)	37 (21.1)	112 (64.0)		
Cotton oil	137 (78.3)	19 (10.9)	19 (10.8)		
Soya oil	110 (62.9)	25 (14.3)	40 (22.8)		
Maize oil	168 (96.0)	07 (4.0)	00 (0.0)		
Olive oil	101 (57.7)	26 (14.9)	48 (27.4)		
Butter	113 (64.6)	30 (17.1)	32 (18.3)		
Margarine	92 (52.6)	29 (16.6)	54 (30.8)		

In our study, only 26.3% of hypertensive patients consumed fats and oils. The fats and oils most used by patients 3 times/weeks are refined palm oil (64.0%), crude palm oil (45.1%) and margarine (30.8%) (Table 8).

Concerning spices, the study revealed that 82.9% of hypertensive patients consumed spices in their diet like onion (*Allium cepa*) (85.7%) and garlic (*Allium sativum*) (81.7%) (Table 9).

Table 9. *Distribution of Hypertensive Patients Based on Consumption of Spices*

Spices	Never n (frequency)	Rarely (1 - 2 times/ week) n (frequency)	>3times/ week n (frequency)	Regular consumption (%)	Irregular consumption (%)
Onion	07 (04.0)	18 (10.3)	150 (85.7)	82.9%	17.1%
Garlic	08 (04.6)	24 (13.7)	143 (81.7)		
White pepper	35 (20.0)	36 (20.6)	104 (59.4)		
Ginger	30 (17.1)	72 (41.1)	73 (41.7)		
Djangsang	24 (13.7)	46 (26.3)	105 (60.0)		
Chili pepper	31 (17.7)	58 (33.1)	86 (49.1)		

Table 10. *Distribution of Hypertensive Patients Based on Consumption of Pasties and Sugary Foods*

Pastries and sugary foods	Never n (frequency)	Rarely (1 - 2 times/ week) n (frequency)	>3times/ week n (frequency)	Regular consumption (%)	Irregular consumption (%)
Cakes	85 (48.6)	38 (21.7)	52 (29.7)	17.7%	82.3%
Donuts	61 (34.9)	32 (18.3)	82 (46.8)		
Pizza	148 (84.6)	15 (08.6)	12 (06.8)		
Hamburger	136 (77.7)	22 (12.6)	17 (09.7)		
Hot dog	149 (85.1)	14 (08.0)	12 (06.8)		
Chocolate	83 (47.4)	48 (27.4)	44 (25.1)		
Ice cream	136 (77.7)	17 (09.7)	22 (12.6)		
Chips	119 (68.0)	32 (18.3)	24 (13.7)		
Confit peanuts	116 (66.3)	44 (25.1)	15 (08.6)		
Candies	137 (78.3)	26 (14.9)	12 (06.8)		

The consumption of pastries and sugary foods showed that 17.7% of hypertensive patients regular eat pastries and sugary foods particularly donuts (46.8%), cakes (29.7%) and chocolate (25.1%) (Table 10).

Distribution of Hypertensive Patients According to the Consumption of Foods Rich in Micronutrients

The study revealed that 44.6% of patients regularly consumed food rich in potassium, 43.4% consumed food rich in magnesium, 80% consumed food rich in sodium and 39.4% consumed food rich in calcium (Table 11).

Table 11. *Distribution of Hypertensive Patients Based on Consumption of Food Rich in Micronutrients Important for Hypertension*

Food rich in micronutrients important for hypertension	Foods rich in Potassium (%)	Foods rich in Magnesium (%)	Foods rich in Calcium (%)	Foods rich in Sodium (%)
Regular consumption	78 (44.6)	76 (43.4)	69 (39.4)	140 (80.0)
Irregular consumption	97 (55.4)	99 (56.6)	106 (60.6)	35 (20.0)

Evaluation of Food Consumption Score

The evaluation of global quality of diet showed that 82.1% of patients have a limit Food consumption score (21.5<FCS<35) (Table 12).

Table 12. *Distribution of Hypertensive Patients According to Food Consumption Score (FCS)*

Food Consumption Score (FCS)	n (frequency)
Acceptable (adequate quality and quantity): FCS>35	07 (04.0)
Limit (inadequate quality and adequate quantity): 21.5<FCS<35	08 (04.6)
Poor (inadequate quality and quantity) : FCS<21.5	35 (20.0)
Total	30 (17.1)

DISCUSSION

The studies of Raschke V, Cheema B[11] showed that actually, the dietary choices are oriented towards the abandonment of traditional foods habits and an increase of consumption of food known as "*Western*," are controlled by several factors that seem to join together the majority of the countries in the process of development such as Cameroon. The results of the dietary habits showed that 42% of hypertensive patients regularly consumed fruits, 56.2% consumed vegetables, 29.4% consumed tubers, 80.4% consumed cereals, 54% consumed legumes, 73.6% consumed animal proteins, 76.2% consumed fats, 61.5% consumed spices, and 83.4% consumed sweet foods. In our population of study, tastes could be justified by the standardized choices of available and attractive food rich in energy (sugar, fat).[12]

Several studies established an association between a food rich in lipids or simple sugars, or salt, and high blood pressure,[13] whereas food rich in plants and fruits would be associated with a weak prevalence of hypertension.[14]

In addition to the great availability of these Western or industrial foods and their attraction, the food choices could be justified by the economic environment. Thus, the people of the socio-economic low-level, with the limited resources, would choose food rich in energy, rich in manufactured cereals, sugars and rich in fat, in order to save money.[15]

The results of the consumption of food rich in micronutrients showed that 45.9% of hypertensive patients have a regular consumption of foods rich in potassium (fruits: like banana, avocado papaw and local vegetables like zoom (*Solanum Nigrum*), folong (*Amaranthus hybridus*), and ndole (*Vernonia amygdalina*)). This could explain the prevalence of the diastolic blood pressure which is 52.9%.

Epidemiological studies have established a negative relation between low potassium intake and tension level. Clinical trials showed that a diet rich in potassium attenuates the increase in the blood pressure induced by the sodium loading; while a low potassium diet induces the opposite effect.[16] This effect of the increase in the potassium intake can result from various mechanisms: increase in the natriuresis with sodium depletion, reduction of the pressure to circulating noradrenalin, reduction in the renin activity, vasodilatation. The consumption of food rich in potassium is better than the drug supplementation.[17]

According to the literature, persons who can have hypertension are those at which diet is poor or insufficient in cal-

cium. We have also those particularly elder people at which intestinal absorption and/or the renal reabsorption of calcium are decreased.[17] The study revealed that 36.6% of patients regularly consumed foods rich in calcium. This result could explain the frequency of patients having a personal history of pains to the legs (38.3%). These results are similar to those of[17] which showed that the ingestion of food calcium was less to the hypertensive patients than the normotensive patients. It also showed that calcium intake higher than 800 mg/day is associated with the reducing of the particular pains and hypertension.

The study also revealed that 43.4% of hypertensive patient regularly consumed foods rich in magnesium (Folong, okok, ndolè, avocado, banana, groundnuts, plantain, milk, white pepper and rice) and 56.6% irregularly. The irregular consumption of magnesium, could explain the appearance of pains (hyperalgy) and muscular cramps in study population (38.3%). Experimental works suggested that an insufficient food magnesium could be associated with HBP, and some studies noted an improvement of hypertension after magnesium supplementation; particularly if the diet is rich in salt.[18] The explicative mechanism is the role of magnesium in the regulation of cellular physiology. However, it should be noted that the daily consumption of 350 or 400 mg of magnesium would have a favorable effect on hypertension.

In this study we noted that 77.1% of hypertensive patients consumed animal proteins, and 26.3% consumed fats. Many studies established the link between hypertension, consumption of fat and animal proteins. These studies revealed that excessive consumption increased the blood viscosity, bad cholesterol level and the systolic blood pressure.[19]

Moreover, 38.3% of hypertensive patients were obese and 30.3% were overweight. This could be explained by the excessive consumption of fats and animal proteins by patients associated with being sedentary because 50% of subjects did not do a physical activity. Studies of,[19] defined the hypercholesterolemia like a too high cholesterol rate in blood. Consumption of animal proteins, fats, certain plant proteins (groundnuts) leads to a deposit of cholesterol excess on the arterial walls, forming atherosclerosis plates, which thicken with the time.[20]

Contrary to being sedentary, the regular physical exercise leads to decrease blood pressure and cardiovascular risk, and that remains valid at any age.[21] These beneficial effects are primarily related to the fall of the blood pressure by the weight loss.[17] It would involve also a significant fall of the left ventricular mass.[22]

It is also shown in the study that 80% of the patients consume foods rich in sodium (amount of sodium for food); this could be explained by a constant rise in tensional parameters of patients. Other studies showed that the response of the blood pressure to variations of the sodium intake is heterogeneous and has a family character. Indeed, certain patients known as "salt – sensitive" have increased blood pressure with an increased salt intake thus leading to a higher risk of cerebrovascular and coronary heart diseases.[22]

According to,[23] if the blood contains many salt, the kidneys retain more water and excrete some lesser quantities. The increased blood volume which results from it leads to an increase in the blood pressure which leads to hypertension. A modest reduction of the sodium intake (6 g NaCl/day) can facilitate control of tension to the hypertensive patient in order to reduce the number of antihypertensive drugs. The Results of the food consumption score (FCS) that 82.3% of hypertensive patients have a limit food consumption score (FCS) which according to[24] shows that diet is inadequate quality; this result will be able to explain the prevalence of obesity (38.3%) and overweight (30.3%) observed in the population of study.

CONCLUSION

This study revealed that patients suffering from hypertension consume all groups of foods. Most foods consumed are rich in sodium. In addition, the quality diet of these patients is inadequate. This study could contribute to defining a new strategy in the management of hypertension focused on nutritional habits particularly on the reduction of food rich in sodium and promotion of foods rich in magnesium, potassium, and calcium.

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The authors express their grateful thanks to the administrative staff of the district Deido hospital and to all the participants to the survey.

AVAILABILITY OF DATA AND MATERIALS

The primary data and materials of this study are available in Deido district hospital. Official registration is required to access the database *via* secretariat of Director. The datasets analyzed during the study are available from the corresponding author.

AUTHORS' CONTRIBUTIONS

NBCF and MMP designed the study protocol and wrote the first manuscript draft. NBCF led the statistical analyses and contributed to the manuscript drafting. NEVB and medical personnel of hospital contributed to data collection. NBCF and MMP critically contributed to the analysis, discussion and interpretation of the data and NEVB, NBCF and MMP contributed to data interpretation and the writing of the manuscript. All authors reviewed and approved the final manuscript draft.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted according to the principles of the Declaration of Helsinki and approved by the Deido District Hospital (Registry number N°960/AV/MINSANTE/DRSPL/SSDD/HDD). Participation to the study was voluntary and written informed consent was obtained from each participant.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Original Research

Anti-Inflammatory Properties of a Colored Avocado Seed Extract

Deepti Dabas, PhD[1*]; Gregory R. Ziegler, PhD[1]; Joshua D. Lambert, PhD[1,2]

[1]*Department of Food Science, The Pennsylvania State University, University Park, PA 16802, USA*
[2]*Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, University Park, PA 16802, USA*

[*]***Corresponding author***
Deepti Dabas, PhD
Department of Food Science, The Pennsylvania State University, University Park, PA 16802, USA; Tel. 2695487646; E-mail: dabasd@gmail.com

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ABSTRACT

Objective: The anti-inflammatory potential of colored avocado seed extract (CASE) was explored based on the ethnobotanical use of avocado seed for inflammatory diseases.

Introduction: Chronic inflammation contributes to many diseases including cancer, cardiovascular diseases, and arthritis. Avocado seeds have been used ethnobotanically for their anti-inflammatory properties.

Methods: Lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophage cells were treated with CASE for 24 h, after which the pro-inflammatory cytokines, Interleukin -6 (IL-6), tumor necrosis factor-α (TNF-α) and IL-1β (Interleukin -1β) were measured along with NO production. Effect of CASE on Cyclooxygenase -2 (COX-2) and prostaglandin E2 (PGE2) was also observed. We also studied if CASE would inhibit the activity of secreted Phospholipase A2 (PLA_2).

Results: Treatment of LPS-stimulated RAW264.7 cells with CASE for 24 h reduced the production of pro-inflammatory cytokines, IL-6, TNF-α, and IL-1β. NO production was reduced in a dose-dependent manner, and the reduction was associated with a decrease in the protein expression of inducible nitric oxide synthase (iNOS). Whereas PGE_2 production was significantly reduced by CASE, no change in the protein expression of Cyclooxygenase -2 (COX-2) was observed. CASE did inhibit the activity of purified secreted PLA_2 (IC50=36 μg/mL). Kinetic analysis indicated that the inhibition was non-competitive with respect to substrate concentration. Nuclear translocation of nuclear factor ϰB (NF-κB) to the nucleus was reduced by treatment with CASE, and this inhibition may underlie the effects of CASE on iNOS and cytokine expression.

Conclusion: These results suggest that the CASE may represent a source of anti-inflammatory compounds which can be exploited as functional food ingredients or as lead compounds for pharmaceutical development.

Keywords
Avocado seed; Colored avocado seed extract; Anti-inflammatory; *Persea Americana*; Cytokines; Phospholipase A2; Nitric oxide synthase; Cyclooxygenase-2.

Abbreviations
CASE: Colored Avocado Seed Extract; LPS: Lipopolysaccharide; iNOS: inducible Nitric Oxide Synthase; COX-2: Cyclooxygenase -2; PAC: Procyanidin; PLA2: Phospholipase A2; IL-6: Interleukin-6; Il-1β, TNF-α: Tumor Necrosis Factor-α; JNK: c-Jun N-terminal Kinase; MAPK: Mitogen Activated Protein Kinase; NF-κB: Nuclear Factor κB; NO: Nitrous Oxide.

INTRODUCTION

Avocado (*Persea Americana, Lauraceae*) is an important tropical crop that is rich in unsaturated fatty acids, fiber, vitamins B and E, and other nutrients. The Hass avocado is the most important variety grown commercially. The seed of the Hass avocado accounts for approximately 16-20 % of the total weight of the avocado fruit and is considered a low-value waste product. In 2017, the total avocado shipped into the USA was 994,340 metric tons, including domestic production of 91,660 metric tons.[1] Most of this imported from Mexico, the world's largest grower of avocado seed. The amount of seed generated in the USA in 2016 was 159,100 metric tons.[1]

Ethno-pharmacological studies of the Aztec and Maya cultures have reported the use of decoctions of avocado seeds for the treatment of mycotic and parasitic infections, diabetes, inflammation, and gastrointestinal irregularity. Our previous review highlights multiple potential applications of avocado seeds including anti-inflammatory effects.[2]

Avocado seeds are rich in polyphenols and contain a large number of different classes of phytochemicals. The seed has higher polyphenol content and greater antioxidant activity than the pulp.[3-5] Wang et al (2010) have reported the presence of catechin, epicatechin, and A- and B-type procyanidin (PAC) dimers – hexamers in the seed. The seeds have also been reported to contain phytosterols, triterpenes, fatty acids, furanoic acids, and abscisic acid.[5] Among the compounds identified in CASE are perseitol, abscisic acid, epicatechin/catechin, PAC B2 and salidroside.[6] Melgar et al. (2018) identified many polyphenolics from the hydroethanolic extract of seed, including isorhamnetin-glucuronide, catechin, epicatechin, trans-3-O-Caffeoylquinic acid, B-type PAC dimer and trimer, cis 3-O-caffeoylquinic acid, and cis-3-p-coumarouylquinic acid. These authors compared the polyphenolic content of seeds and peels and found the peels to have 3-fold higher polyphenolic content but only around twice the antioxidant activity. The extracts also displayed bactericidal and fungicidal characteristics.[7]

We have previously reported that the avocado seed when crushed, a stable orange color develops and we have investigated the potential use of colored avocado seed extract (CASE) as a food additive.[8] The principal colored compound in CASE has been identified as a novel glycosylated benzotropolone-containing polyphenol.[9] A large number of studies have demonstrated the potential anti-inflammatory activities of benzotropolone-containing natural products such as theaflavins from black tea. Based on these previous studies on the anti-inflammatory activity of benzotropolones, the potential usefulness of a CASE as a food additive, and the general lack of studies on the anti-inflammatory activity of avocado seed extracts, an investigation of the potential anti-inflammatory activity of this new extract was warranted. We hypothesized that CASE would exhibit dose-dependent inflammation inhibitory activity *in vitro*. In the present study, we examined the effect of the colored avocado seed extract (CASE) on LPS-induced inflammatory responses of RAW264.7 murine macrophages.

MATERIAL AND METHODS

Reagents

Ripened avocado (*Persea Americana*, Hass variety) were sourced locally and stored at 4 °C until use. The ELISA for PGE_2 was obtained from Cayman (Ann Arbor, MI, USA). ELISAs IL-6, Il-1β, TNF-α ELISA kits were obtained from R&D systems (Minneapolis, MN, USA). Lipopolysaccharide and Greiss reagent was obtained from Sigma (St. Louis, MO, USA). The phospholipase A2 enzyme assay was purchased from Invitrogen (Carlsbad, CA). Antibodies against JNK, MAPK, phospho MAPK, NF-κB, iNOS, COX2, and β actin were purchased from Cell Signaling (Danvers, MA). All other reagents were of the highest grade commercially available.

Preparation of CASE

CASE was prepared as previously described8. In brief, avocado seeds were separated from the fruit, washed and peeled. Seeds were ground in 0.7 volume of deionized (DI) water using a Waring Blender. The resulting paste (pH 6.4) was incubated at 24°C for 35-min. The colored paste was transferred to a beaker, an equal volume of methanol added, and the mixture sonicated for 20 min, an additional 2 volume of methanol was added, and the mixture centrifuged at 1200 × g for 10 min. Methanol was removed using a rotary evaporator and the water removed by freeze-drying. Stock solutions (200 mg/mL) were prepared in dimethyl sulfoxide and stored at -80°C.

Cell Culture and Viability

RAW264.7 cells were maintained in log phase growth with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C under a humidified CO_2: air (5:95) atmosphere.

The effect of CASE on cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In brief, cells were seeded (104 cells/well) in 96 well plates and allowed to attach overnight. The cells were co-treated with 1 µg/mL LPS and CASE for 24 h. After CASE treatment, cells were combined with MTT and absorbance read at 540 nm. The viability of treated cells was normalized to LPS-only treated controls.

Modulation of Inflammatory Cytokines

RAW264.7 cells were seeded (104 cells/well) in 96 well plates and allowed to attach overnight. Cells were then co-treated with CASE and 1 µg/mL LPS for 24 h in serum complete medium IL-6, IL-1β and TNF-α levels in the medium were determined by ELISA as per manufacturer's instructions. The levels of these cytokines were compared to unstimulated cells and LPS stimulated control cells.

NO Production

RAW264.7 cells were plated and stimulated as above. The quantity of nitrite in the culture medium of CASE-treated cells was measured after 24 h as an indicator of NO production using Griess reagent. Briefly, 50 µl of cell culture medium was mixed with 50 µl of Griess reagent, the mixture was incubated at room temperature for 20 min and the absorbance at 540 nm was measured. The values were expressed as a percentage of LPS-only treated cells.

Prostaglandin E_2 Production

PGE_2 levels in media of CASE/LPS co-treated and LPS-stimulated RAW264.7 cells were measured using a PGE_2 EIA monoclonal ELISA kit (Cayman Co., Ann Arbor, MI, USA).

Phospholipase A_2 Inhibition Assay

Inhibition of PLA_2 was examined using a commercially available fluorometric enzyme method. Buffered PLA_2 solution (4 U/ well, pH 8.9) and CASE were combined in a 96-well plate. A fluorogenic PLA_2 substrate (Red/Green BODIPY PC-A2, 1.5 µM) was dispensed to each well to start the reaction. After incubation at room temperature in the dark for 10 min, fluorescence was determined at λex=485 nm and λem=538 nm (Fluoroskan Ascent FL, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Kinetic analysis of inhibition by PLA_2 was carried out similarly but with the following modification–the concentration of CASE was held constant while the substrate concentration (0.5-4 µM) was varied.

Western Blot

Preparation of whole cell lysate: RAW264.7 cells (10^6) were seeded in 75 cm^2 flasks for 36 h. The media was replaced with media containing 1 µg/mL LPS and CASE at 5 or 6 µg/mL and incubated for 24 h. The cells were washed with PBS, scraped off and centrifuged at 1200 × g. The cells pellet was combined with lysis buffer(25 mM 3-(N-morpholino) propanesulfonic acid, 2 mM Ethylenediaminetetraacetic acid (EDTA), 10% glycerol, 0.5% Nonidet P-40 and 0.02% sodium azide) containing 1:100 phosphatase inhibitor I, phosphatase inhibitor II and a protease inhibitor. The samples were mixed and disrupted by freeze-thawing.

Preparation of nuclear lysate: The cells were treated as above and scraped and centrifuged at 800 × g for 10 min at 4 °C. The cell pellet was suspended in buffer A (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol (DTT), 0.1% Nonidet P40), incubated on ice for 10-min and centrifuged at 12,000 × g for 2-min at 4 °C. The pellet was re-suspended in buffer B (10mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 0.4 M NaCl, 0.5 mM DTT. 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 25% glycerol). The tubes were then vortexed and incubated on ice for 15-min with mixing every 5-min. They were then centrifuged at 10,000 × g for 10-min at 4 °C. The supernatant was removed and used as nuclear fraction.

Immuno blots: Protein (60 µg total or 20 µg for nuclear extract) was resolved by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes. After blocking for 1 h with blocking buffer (Li-Cor, Lincoln, NE), the membrane was incubated with respective primary antibody overnight. The bands were visualized using a fluorescent-conjugated secondary antibody using a Li-cor Odyssey Infrared system (Lincoln, NE).

Data Analysis

For kinetic analysis of PLA_2, Michaelis Menten plots were generated using Graph Pad Prism (San Diego, CA, USA), and the maximum velocity (V-max), Michaelis Menten constant (Km), and mode of inhibition were determined from those plots. All other data were analyzed by one-way analysis of variance (ANOVA) with Dunnett's post-test. *p*-values <0.05 were considered as statistically significant. Data are presented as the mean±SD unless otherwise specified.

RESULTS

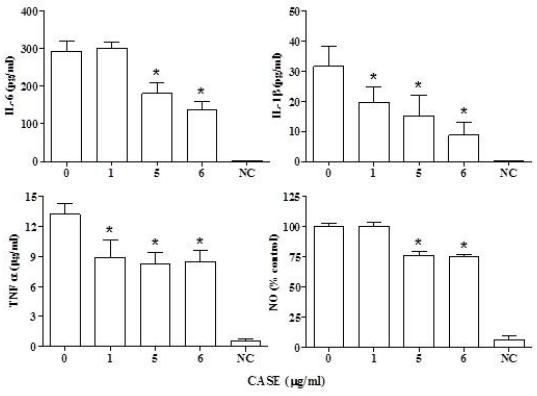
Cytotoxicity

The viability of RAW264.7 cells was determined after co-incubation with LPS and CASE for 24 h. At concentrations of CASE less than or equal to 6 µg/mL, cell viability was greater than 80% (data are not shown). Hence, only concentrations up to 6 µg/mL were used for further experiments.

Inhibition of Pro-Inflammatory Cytokine and Nitric Oxide Production by CASE

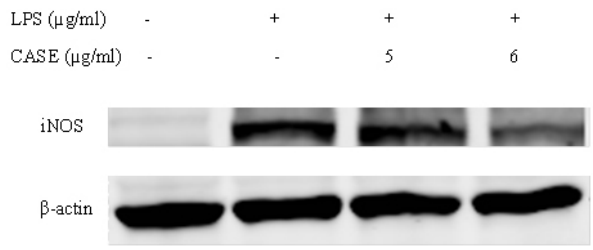
The levels of IL-6, IL-1β and TNF-α were measured in the media of LPS-stimulated RAW264.7 macrophages after co-treatment with CASE for 24 h. A dose-dependent reduction in the concentration of IL-6 was observed with a significant reduction at concentrations greater than 5µg/mL(Figure 1). The levels of IL-1β and TNF-α were significantly reduced by treatment with all the concentrations of CASE (Figure 1).

Figure 1. *Effect of CASE on the Production of Inflammatory Mediators by RAW264.7 Cells Stimulated with LPS. Cytokine and NO Levels were Measured in the Supernatant of Cells Treated with CASE. Mean±SD of Three Independent Experiments. 'NC' Indicates Non-LPS Stimulated Control*



NO production by LPS-stimulated RAW264.7 cells co-treated with CASE was assessed using the Greiss reagent. CASE inhibited the formation of nitrite in a concentration-dependent manner with a significant reduction at concentrations greater than 5 µg/mL (Figure 1). Western blot analysis showed that CASE dose-dependently reduced the protein expression of iNOS in LPS-stimulated RAW264.7 cells after 24 h treatment (Figure 2).

Figure 2. *Effect of CASE on the Protein Expression of iNOS in LPS-Stimulated RAW264.7 Cells. Cells were Treated with CASE for 24 h. Figure is Representative of Three Independent Experiments*



Inhibition of PGE_2 Production by CASE

PGE_2 production by RAW264.7 cells was assessed after co-incubation with 1 µg/mL LPS and CASE for 24 h. It was observed that the PGE_2 production was significantly reduced by treatment with 6µg/mL CASE compared to LPS-stimulated controls (Figure 3a). Western blot analysis of COX-2 showed that CASE did not affect its expression (results not shown).

Inhibition of PLA_2 Activity by CASE

CASE dose-dependently inhibited the activity of secreted PLA_2 in a cell-free system. The IC_{50} of CASE was 36 µg/mL (Figure 3b).

Kinetic analysis showed that CASE significantly reduced the V-max but had no significant effect on Km of PLA_2 (Figure 3c; Table 1). These results suggest that CASE inhibits PLA_2 in a non-competitive manner with respect to substrate concentration.

Figure 3. *(a) The effect of CASE on the Production of PGE2 in RAW264.7 Cells Stimulated with LPS for 24 h. Mean±SD of Three Independent Experiments. (b) The Effect of the CASE on the Activity of Pure Secreted PLA2. Symbols Represent Mean±SD (n=5) (c) Kinetic Analysis of CASE Inhibition of Secreted PLA2. Concentrations of CASE Used were (●) nil, (■) 36 µg/mL and (▲) 72 µg/mL. Inhibitory kinetics were Determined by Using Michelis-Menton Analysis.*

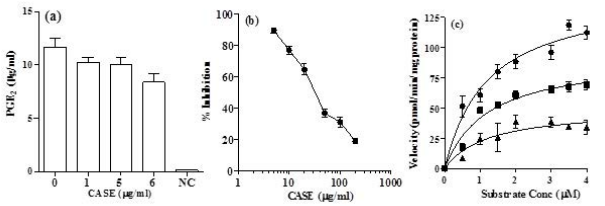


Table 1. *Kinetic Analysis of Inhibition of Secreted PLA2 by CASE**

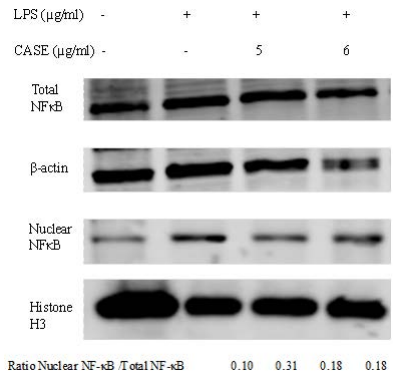
CASE (µg/mL)	0	36	72
Km (µM substrate)	1.2±0.1[a]	1.2±0.1[a]	1.2±0.3[a]
Vmax (pmol/min/mg protein)	145.8±15.6[a]	92.6±3.9[b]	49.5±3.9[c]

*Values in the same row not sharing a common superscript letter are significantly different ($p < 0.05$).

Change in the Expression of NF-κB by CASE

The nuclear localization of NF-κB is a critical signal in LPS-induced inflammation. Co-incubation with CASE reduced nuclear levels of NF-κB, but had no effect on total NF-κB expression (Figure 4).

Figure 4. *Effect of CASE on Nuclear Translocation of NF-κB in LPS-Stimulated RAW264.7 Cells. Nuclear and Total Levels NF-κB of were Determined by Western Blot .β-actin and Histone H3 were Used as Protein Loading Controls for Total and Nuclear Protein Respectively. Representative Results of Two Independent Experiments.*



DISCUSSION

This study was carried out to assess the anti-inflammatory effects of CASE in LPS-stimulated RAW264.7 murine macrophage cell line. On stimulation by LPS, macrophages produce pro-inflammatory cytokines, eicosanoids and NO. These inflammatory mediators play a key role in the progression of inflammatory diseases. Treatment with CASE reduced production of TNF-α, IL-1β, and IL-6.IL-1 β induces the expression of COX-2 and iNOS. TNF-α binds to different receptors than IL-1β, the post-receptor events are similar, and both activate a similar portfolio of genes. IL-6 also triggers the formation of other inflammatory markers including eicosanoids and NO, and as a result, there is growing interest in developing anti-IL-6 agents.[10] Inhibition of these cytokines by CASE indicates that the formation of downstream inflammatory products may also be inhibited.

CASE inhibited LPS-induced NO production. NO is a key inflammatory mediator and inhibition of NO production has an anti-inflammatory effect. CASE reduced the expression of iNOS providing a mechanism of the observed decrease of NO. The promoter region of iNOS gene contains several binding sites for transcriptional factors such as NF-κB and AP-1 as well as other proteins. LPS-stimulated induction of iNOS is mediated through NF-κB.[11] CASE inhibited the formation of PGE_2, a pro-inflammatory eicosanoid, but did not affect COX-2, its biosynthetic enzyme. CASE also inhibited the activity of secreted PLA_2. CASE-mediated inhibition of PGE_2 production may result from inhibition of PLA_2. Alternatively, it could be the result of inhibition of COX-2 activity. PLA_2 catalyzes the release of arachidonic acid from phospholipids. Arachidonic acid is then metabolized through the COX pathway to prostaglandins

and through the LOX pathway to leukotrienes.COX-2 is a known drug target with clinically useful inhibitors, whereas inhibitors of PLA_2 are currently sought as potentially useful anti-inflammatory agents.[12] Cytosolic PLA_2 ($cPLA_2$) is upregulated due to stimulation with LPS; however, secreted PLA_2 was used in this study.

The nuclear translocation of NF-κB was reduced by treatment with CASE. NF-κB is a transcription factor, which in the resting state is bound *via* non-covalent interactions to inhibitor of Kappa B (IκB) and sequestered in the cytoplasm. Treatment with LPS causes phosphorylation of IκB and subsequently it degrades thus allowing NF-κB to enter nucleus and induce gene expression. NF-κB is involved in regulating many aspects of cellular function, including immune response. The expression of inflammatory cytokines, adhesion molecules, angiogenic factors COX-2 and iNOS are all regulated by NF-κB.[13] NF-κB therefore represents a possible pathway through which the CASE may exert its anti-inflammatory effects. Modulation of NF-κB signaling may account for the effects of CASE on cytokine production and iNOS expression. The effects on PGE_2 and PLA_2, however, are likely due to an alternative mechanism, since COX-2 expression was unchanged by CASE.

CONCLUSION

This study characterized the *in vitro* anti-inflammatory effects of a colored extract obtained from avocado seed. The extract was found to reduce the production of pro-inflammatory mediators by stimulated macrophage cells. Anti-inflammatory medications such as NSAIDs and steroids are commonly used to treat various diseases. CASE represents a potential source for novel anti-inflammatory compounds that can be developed as a functional food ingredient or as pharmaceuticals.

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CONFLICTS OF INTEREST

The authors Dabas, Ziegler, and Lambert have applied for a US patent on the colored compound described in this manuscript. Authors Ziegler and Lambert have an equity interest in Persea Naturals limited liability company (LLC) that has licensed related technology from Pennsylvania State University, PA, USA.

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In Vitro-Evaluation of the Antioxidant Properties of *Moringa Oleifera* and *Camelia Sinensis* Leaves

Moses Babatunde Ayoola, MSc; Nonso C. Ejiofor, MSc*; Ikechukwu Edwin Ezeagu, PhD

Department of Medical Biochemistry, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria

Corresponding author
Nonso C. Ejiofor, MSc
Department of Medical Biochemistry, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria; E-mail: bianate@yahoo.ca

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ABSTRACT

Introduction
Moringa oleifera and *Camellia sinensis* are edible plants widely distributed in parts of South East of Nigeria. Both are used as a beverage and medicinal plant in the treatment of several ailments by alternative medical practitioners. The in vitro-antioxidant properties of both *Moringa oleifera* and *Camelia sinensis* leaves were investigated in the current study..

Method
Phytochemical analysis was carried out to evaluate the flavonoids and total phenolic contents of the plants crude extracts. Also, reducing power ability and nitric oxide scavenging activity of the plants were also determined in order to ascertain their antioxidant capacities to eliminate free radicals, and attenuate oxidative stress.

Results
Both plant samples demonstrated antioxidant properties. The samples were rich in flavonoid (135.14±5.20 and 208.24±14.38), and total phenolics (62.85±1.70 and 91.68±0.22) for both *Moringa oleifera* and *Camelia sinensis* respectively. The plant samples also showed nitric oxide scavenging and reducing power ability. *Camelia sinensis* appears to be a better antioxidant plant.

Conclusion
Moringa oleifera and *Camelia sinensis* contains substantial amount of antioxidant substances which could warrant their utilization in alternative medicine. However, further research works are needed to properly ascertain and harness these bioactive agents present in the plants.

Keywords
Moringa oleifera; *Camellia sinensis*; Phytochemical; In vitro; Antioxidants; Oxidative stress.

INTRODUCTION

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mentioned the use of plants in treatment of various human ailments. According to Namita et al[1] traditional system of medicine has been found to have many utilities in treatment of various diseases. The need for the use of plants to treat human disease could be attributed to several factors including, inadequate supply of drugs and high cost of treatment associated with conventional medical practices. Side effects coupled with drug resistance encountered with synthetic drugs also necessitated the need for alternative therapy. The affordability of herbals has also drawn the attraction towards their use. *Moringa oleifera* (*Moringa* (MO)) and *Camelia Sinensis* (green tea (GT)) are two of such medicinal plants gaining popularities for their reported claims on health benefits in recent years.[2]

Moringa oleifera is one of the most widely distributed species of the *Moringaceae* family throughout the World, especially in Asian countries, having a remarkable range of pharmacological properties in addition to significant nutritional value. *Moringa oleifera* is a highly valued plant in tropical and subtropical countries where it is mostly cultivated.[2] The medicinal properties of the plant's edible parts have been recognized by both the Ayurvedic and Unani systems of medicine in India.[3]

Camelia Sinensis (Green tea) has been associated with lowering the risk of cancer, lowering the risk of coronary heart disease

and improvement of oral health.[4] It has been found to have antimicrobial health benefits and antioxidant properties.[5] There are also suggestions that tea extracts offer protection against bone loss,[4] body weight control, anti-hypertensive properties, solar ultraviolet protection, neuroprotective properties and anti-fibrotic properties.[5] Therefore, tea provides a very interesting beverage with potential for a variety of medicinal uses and health-promoting benefits.

The use of traditional medicine is widespread, and plants have more potential for natural antioxidants that may serve as leads for the development of novel drugs. Thus, medicinal plant research is a search for cheaper and more readily available alternative treatment as conventional therapy is becoming expensive.[3]

Members of the genus *Moringa* and *Camellia* are known to possess very strong biological activities and thus widely used in ethnobotany medicine and pharmacy. However, information on possible adverse effects of these plants on human health are scarce. Therefore, the study of medicinal plants helps to understand the possible adverse effects of these plant species and protect humans and animals from natural poisons.

Oxidative stress has been defined as a disturbance in the balance between the production of reactive oxygen species (ROS) or free radicals and antioxidant defenses.[6]

Many metabolic reactions depend on oxygen[7]–a primary oxidant in metabolic reactions designed to obtain energy from the oxidation of a variety of organic molecules——which is also used as substrate by numerous enzymes each with differing substrate affinities for oxygen and involved in a wide range of metabolic processes such as metabolism of amines, prostaglandins, purines, steroids, and amino acids.[8] ROS are generated a by-product of normal aerobic metabolism, but their level increases under stress which proves to be a basic health hazard.[9] These ROS include superoxide (O_2-), hydroxyl radical (HO.) and non-radical molecules like hydrogen peroxide (H_2O_2). Nitric oxide (NO) is another abundant reactive radical that acts as an important oxidative biological signal in a large variety of diverse physiological processes, including smooth muscle relaxation, neurotransmission, and immune regulation.[10]

The mitochondrion is the major cell organelle responsible for ROS production.[11] In order to protect the cell and organs systems of the body against reactive oxygen species, humans and other organisms have evolved a highly sophisticated and complex antioxidant protection system which involves a variety of components that are both endogenous and exogenous in origin that function interactively and synergistically to neutralize free radicals.[12,13]

The common antioxidant enzymes are superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase which catalyze free radical scavenging reactions. The nutrient derived antioxidants are ascorbic acid (vitamin C), vitamin E, carotenoids, and other low molecular weight compounds such as glutathione and lipoic acid as well as numerous other antioxidant phytochemicals present in a wide variety of plant foods.[13,14]

ROS can be beneficial, as they are used by the immune system as a way to attack and kill pathogens.[12,15] Short-term oxidative stress may also be important in prevention of aging by induction of a process named mitohormesis.[16]

During the past decade, there has been a growing interest in the medical implications of free radicals. As a result of aerobic life, organisms must deal with the continuous generation of reactive oxygen species (O_2-, H_2O_2, •OH) as by-products of metabolism and defend itself against the harm that these can do to cellular macromolecules.[12] Free radicals produced as a result of normal biochemical reactions in the body are implicated in contributing to a number of medical conditions such as cancer, aging, diabetes, atherosclerosis, immuno-supression and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease.[17,18]

MATERIALS AND METHODS

Sample Collection and Preparation

Moringa oleifera (MO) leaves were obtained from ogbete main market in Enugu, Nigeria while popularly branded *Camellia Sinensis*, green tea (GT) from Qualitea, Ceylon Ltd., Sri Lanka was purchased from a supermarket in Enugu. *Moringa* leaf was identified and authenticated by Mr. Alfred Ozioko of Bio resources Development and Conservation Program (BDCP), Nsukka. Both samples were dried at room temperature and powdered by using an electrical blender.

100 g of dried, ground sample materials were soaked in 80% methanol (1L) for 5 days separately. The soaked material was stirred every 18 hours using a sterilized glass rod. The final extracts were passed through Whatman filter paper No.1. The filtrates obtained were concentrated under vacuum to dryness using a rotary evaporator at 40° C. The resulting fine powders obtained were weighed (MO=6.44 mg and GT=8.15 mg), dissolved in 300 ml of distilled water separately and stored at 4° C till use.

Assays and Principles

Total flavonoids were determined using the colorimetric assay developed by Zhishen et al.[19] An aliquot (1 ml) of extract (concentration 1 mg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled H_2O. Into the flask, 0.3 ml 5% $NaNO_2$ was added and 5 min later 0.3 ml 10% $AlCl_3$ was added. After 6 min, 2 ml of 1M NaOH solution was added and the total volume was made up to 10 ml with distilled H_2O. The solution was well mixed and the absorbance was measured at 510 nm against the control that had been prepared in the same manner only with replacing the extract with distilled water. Total flavonoids content was expressed as mg rutin equivalents (RE) per g of dry extract.

Total phenols were determined by Folin-Ciocalteu reagent method.[20] A dilute extract of each plant extract (0.5 ml of 1 mg/ml) or gallic acid (standard phenolic compound) was mixed with FolinCiocalteu reagent (2.5 ml, 1:10 diluted with distilled wa-

ter) and aqueous $NaHCO_3$ (2 ml, 7.5%). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 6.25, 12.5, 25, 50, 100 mg/L (µg/ml) solutions of gallic acid (GA) in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

The reducing power of extracts of MO and GT was determined according to the method of Oyaizu.[21] Various concentrations of extracts (6.25, 12.5, 25, 50,100 mg/ml of distilled water) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide $[K_3Fe(CN)_6]$ (1%, w/v), and then the mixture was incubated at 50° C for 20 min. Afterwards, 2.5 ml of trichloroacetic acid (10%, w/v) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of upper-layer solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%, w/v), and the absorbance was measured at 700 nm. Ascorbic acid (AA) was used as standard antioxidant. Higher absorbance of the reaction mixture indicated greater reducing power.

Nitric oxide was generated from sodium nitroprusside and measured by the Griess Illosvoy reaction. Sodium nitroprusside in aqueous solution at physiological pH (7.2) spontaneously generates nitric oxide,[21,22] which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide.[22] Two ml Sodium nitroprusside (10 mM) in 0.5 ml phosphate-buffered saline (pH 7.4) was mixed with 0.5 ml of different concentrations of the extracts dissolved in the suitable solvent systems (distilled water) and incubated at 25° C for 150 min. The samples from the above were reacted with Greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediaminedihydrochloride) in ratio 1:1. After incubation at room temperature for 30 mins, the absorbance of the chromaphore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamme was read at 540 nm. Control experiment without the test compounds but with equivalent amount of buffer was conducted in an identical manner.

Calculated the % inhibition by formula and plot graph in comparison.

Formula:

$$\% \text{ inhibition} = \frac{([\text{O.D of Control - O.D of Test}])}{([\text{O.D of Control}])} \times 100$$

RESULTS

Phytochemical analysis of *Moringa oleifera* (MO) and green tea (GT) shows that total flavonoid concentration in both plants is 135.14±5.20 mg/g and 208.24±14.38 mg/g rutin equivalent (RE) respectively (Table 1). The total phenolic concentration in MO and GT extracts are 62.85±1.70 mg/g and 91.68±0.22 mg/g Gallic acid equivalent (GA Eq) respectively (Table 1). Administration of monosodium glutamate (MSG).

Table 1. *Flavonoid and Total Phenolics Concentration of MO and GT (mg/g)*

Sample	MO	GT
Flavonoids	135.14 ± 5.20	208.24 ± 14.38
Total Phenolics	62.85 ± 1.70	91.68 ± 0.22

Table 2 presents the result of reducing power assay for the plants compared to ascorbic acid (AA), a known antioxidant used as standard. The result showed dose-dependent reduction ability with a maximum absorbance of 0.629 and 1.195 for *Moringa* and green tea respectively, at a concentration of 100 µg of the extracts.

Table 2. *Dose-dependent Reducing Power of Moringa, Green Tea and Ascorbic Acid*

Concentration (µg/ml)	MO	GT	AA
6.25	0.120 ± 0.043	0.263 ± 0.032	0.421 ± 0.003
12.50	0.143 ±0.031	0.547 ± 0.021	0.532 ± 0.059
25.00	0.225 ± 0.010	0.771 ± 0.057	0.620 ± 0.013
50.00	0.324 ± 0.111	1.188 ± 0.011	0.651 ± 0.004
100.00	0.629 ± 0.022	1.195 ± 0.067	0.738 ± 0.023

Means±SD of three independent determinations, difference is not significant ($p>0.05$)

The result of the nitric oxide generation and Scavenging assay is presented in Table 3. A marked decrease in the absorbance at various concentrations of the extracts as against the control shows a good nitric oxide scavenging activity. GT appears to have a better percentage (61.82%) inhibition of nitric oxide at lower concentration (6.25 µg/ml) compared to MO (61.57%) which requires a higher concentration of the extract (25 µg/ml).

Table 3. *Nitric Oxide Generation and Scavenging Activity of Moringa and Green Tea (% inhibition)*

Concentration (µg/ml)	MO	GT
6.25	59.52	61.82
12.50	46.18	62.88
25.00	61.57	51.14
50.00	54.92	45.08
100.00	50.44	39.28

DISCUSSION

Flavonoids and Total Phenolics

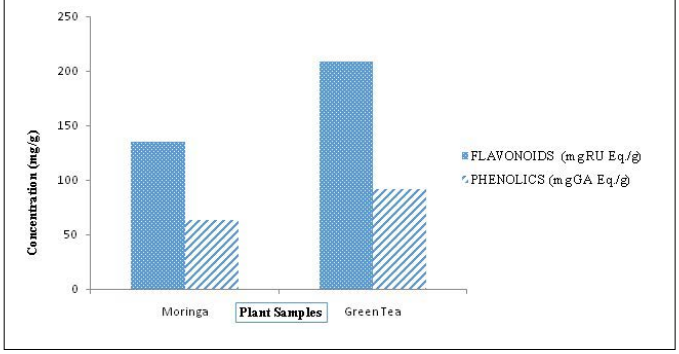
Over the past years, numerous experimental and epidemiological studies have shown that a wide variety of phytochemicals such as flavonoids, phenolics, catechins are able to slow down oxidative stress-induced damage leading to large number of diseases.[14] Natural products mainly from the plant kingdom among which we have *Moringa oleifera* and *Camellia sinensis*, offer a wide range of biologically active compounds that act as natural antioxidants with potential in drug discovery and development.[14,19] Flavonoid and phenolic compounds are known for their potent antioxidant properties.

Various values of total flavonoid have been reported for both *Moringa* and Green tea. Tariq and Reyaz,[23] reported a value of 14 mg/g for *Camellia sinensis* while Nor Qhairul Izzreen, and Mohd Fadzelly,[24] reported a value of 20.9 mg/g for *Camellia sinensis*. A similar study in Nigeria[25] reported total flavonoids in green tea as 10.0 mg/g. However, the total flavonoid content obtained in this study though higher [(135.14±5.20 and 208.24 ± 14.38 mg/g Rutin Equivalent (RU Eq)], falls within the range of 16.02-233.68 mg RU Eq/g reported by Stankovic et al.[26] The total flavonoid concentration in *Moringa* is observed to be less than that in *Camellia sinensis* though the difference is not significant ($p<0.05$). Values ranging from 65.38±2.37 mg/g to 645.00±0.10 mg /g have been reported for total flavonoid in methanolic extracts of *Moringa*.[27] The results obtained in this study have shown that both *Moriga* and Green tea leaves are good source of total flavonoid.

The total phenolic concentration obtained in this study for Green tea extract is comparable to the findings of Nor QhairulIzzreen and Mohd Fadzelly,[24] However, higher values have also been reported by other researchers.[23,25,28]

As with the total flavonoid, the total phenolic concentration [mg/g Gallic acid equivalent (GA Eq)] in *Moringa* (62.85±1.70 mg/g GA Eq) is less than that in *Camellia sinensis* (91.68±0.22 mg/g GA Eq). The total phenolic value obtained for *Moringa* in this study is at the same level with that reported by Woranan et al.[29] Higher total phenolic values of 216.45±4.64 mg GA Eq/g and 541.00±0.02 mg GA Eq/g have also been reported for Moringa (Figure 1).[27,30]

Figure 1. *Comparison of Flavonoids and Total Phenolics in Test Extracts*

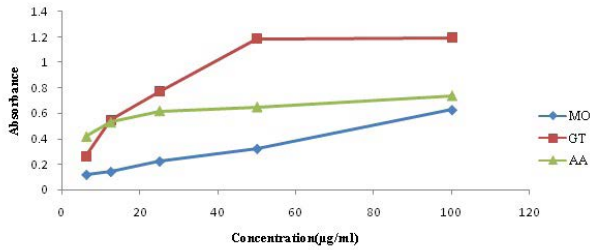


Reducing Power

Table 2 presents the result of reducing power assay for the plant extracts compared to ascorbic acid (AA), a known antioxidant used as standard. The result showed dose-dependent reduction ability with a maximum absorbance of 0.629 and 1.195 for *Moringa* and Green tea respectively, at a concentration of 100 µg of the extracts.

The reducing power of a substance or compound is related to its electron transfer ability and may therefore serve as a significant indicator of its antioxidant activity.[31] The reducing ability of the methanolic extracts of *Moringa* and Green tea was measured by the transformation of Fe_3+ to Fe_2+ in the presence of the extract at 700 nm where increased absorbance of the reaction mixture indicates increased reducing power. Dose-dependent reduction ability was observed in the test extracts (Figure 2). This trend was similarly observed by Koruthu et al,[21] and Nurul et al[32] for *Moringa* and Tariq and Reyaz,[33] for Green tea. The reducing power of green tea is higher than that of *Moringa*, although both *Moringa* and Green tea compare favorably with ascorbic acid (a standard antioxidant). However, Green tea has higher reducing power than ascorbic acid contrary to Tariq and Reyaz,[33] while *Moringa* and ascorbic acid shows comparable reducing ability similar to the findings of Ogbunuga for et al.[34] Thus, both plants may be a good source of antioxidants (Figure 2).

Figure 2. *Reducing Activity of Plant Extracts Compared to Ascorbic Acid*

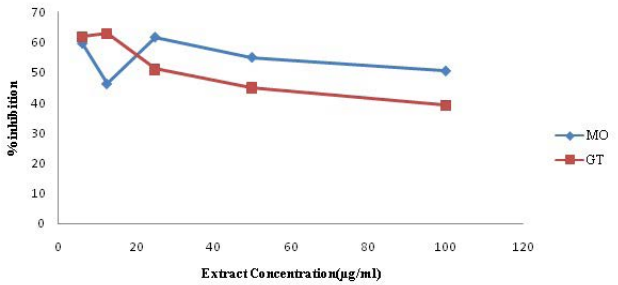


Nitric Oxide Scavenging Activity

The result of the nitric oxide generation and Scavenging activity is presented in Table 3. GT appears to have a better nitric oxide scavenging activity (61.82% inhibition) at lower concentration (6.25 µg/ml), compared to MO (61.57% inhibition) which requires a higher concentration of the extract (25 µg/ml).

Nitric oxide (NO) is an important bioregulatory molecule, which has a number of physiological effects including control of blood pressure, neural signal transduction, platelet function, antimicrobial and antitumor activity.[35,36] It is a diatomic free radical that is extremely short-lived in biological systems.[37] NO also shows toxic property after reaction with oxygen and superoxide radicals and is said to be involved in a number of important human diseases.[38] The reaction products are able to cause much cellular damage. Thus, the unregulated production of nitric oxide can cause nitrosative stress, leading to damages of proteins/DNA and to cell injury and death.[39] NO scavenging capacity is determined by the decrease in the absorbance at 540 nm, induced by antioxidants. The result obtained in the *in vitro* NO assay suggests that the plant extracts have the capacity to scavenge NO as shown by high percentage (%) inhibition.[40] Moringa showed a stronger tendency, based on the percentage of NO inhibited at various concentration compared to green tea. The results are comparable to the work of Jagetia et al (Figure 3).[35]

Figure 3. *Effect of the Extracts on the in vitro Nitric Oxide Assay*



CONCLUSION

This study confirmed the usefulness of the medicinal plants: *Camellia sinensis*, and *Moringa oleifera*. The present study showed that Moringa, and green tea plants have substantial amounts of flavonoids and total phenols which could play significant roles in the antioxidant properties of both plants.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Kritika Shakya, MS[1]; Patricia Rayas-Duarte, PhD[2,3]; Zorba Hernandes, PhD[3]; Sanjeewa Gamagedara, PhD[4]; Kanika Bhargava, PhD[1*]

[1]*Department of Human Environmental Sciences, University of Central Oklahoma, Edmond, Oklahoma 73034, USA*
[2]*Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma 74078, USA*
[3]*Robert M Kerr Food & Agriculture Products Center, Oklahoma State University, Stillwater, Oklahoma 74078, USA*
[4]*Department of Chemistry, University of Central Oklahoma, Edmond, Oklahoma 73034, USA*

[*]***Corresponding author***
Kanika Bhargava, PhD
Associate Professor, Department of Human Environmental Sciences, University of Central Oklahoma, Edmond, Oklahoma 73034, USA; Tel. 405-974-5556; Fax: 405-974-3850; E-mail: kbhargava@uco.edu

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ABSTRACT

Objective: The objective of the study is to formulate, optimize, and perform a sensory acceptability study on chickpea fortified finger millet tortillas.

Introduction: Researches have shown potential health benefits of finger millet in many health conditions due to its nutritional content. However, the absence of gluten in finger millet flour prevents binding properties required to formulate tortillas.

Methods: We developed a gluten free flour composition of tortilla consisting of chickpea fortified finger millet flour (30% w/w chickpea flour). We further optimized it with 2% sugar, 4% of glycerin and 15% of starch (rice, potato, and tapioca) to enhance functional and sensory properties.

Results: The results showed that there was no significant difference in chemical and nutritional content of tortillas with different starches but some physical differences were observed. The descriptive sensory analysis was conducted that eliminated tortillas with tapioca starch due to least likeability. The sensory acceptability study showed that overall likability was slightly higher for tortillas with potato starch in comparison to rice starch which correlated with higher scores for taste, texture, and aroma of the tortillas with potato starch. On the other hand, the appearance of the rice starch was preferred in comparison to tortillas with potato starch which correlated with the smooth and spreadable characteristics of rice starch.

Conclusion: The results indicated that incorporation of potato starch results in the formulation of chickpea fortified finger millet tortillas with acceptable textural and sensory properties which would be a gluten-free, nutrient-dense alternative to traditional tortillas.

Keywords
Finger millet; Chickpea; Celiac disease; Tortilla; Starch; Gluten-free; *Cicer arietinum*; *Eleusina coracana L.*

INTRODUCTION

Millets are a good source of phytochemicals, micronutrients, and essential amino acids except for lysine and threonine. There have been studies that have shown potential health benefits in many conditions such as diabetes, cardiovascular disease, aging, cancer, and celiac disease.[1] There are six types of most common and important millets, amongst them finger millet has the highest amount of calcium, potassium, sodium, dietary fiber, and iron.[2] Millets are ancient grain that had been cultivated as early as 2700 BC in China, continue to be stapled in India, and eastern and central Africa while in developed countries millets are used as cattle feed.

The nutrition composition of millets is comparable to other cereals and they are superior source in terms of dietary fiber, minerals, B-vitamins, starch properties and physiological action.[2] Studies were conducted on 76 varieties of finger millet from all over the world for years and the nutritional composition of finger millet determined; it consists of 73 to 82% of carbohydrate, 4 to 8% of protein, 1 to 4.5% of lipid, 200 to 450 mg calcium, 5 to 15 mg iron, 0.4 to 4 mg B-vitamins, 3 to 12% crude fiber and seven

essential amino acids.[3] The carbohydrate composition of finger millet consists of 15 to 20% of dietary fiber and 2 to 4.5% free sugar and mostly starch consisting of amylose, amylopectin, and other starch fractions.[2,4] Finger millet is also a very good source of micronutrients especially calcium and iron. According to Gopalan, it has 344 mg% of calcium, 3.9 mg% of iron and 283 mg% of phosphorus in comparison to other cereal grains and millet.[5]

Finger millets are very versatile grain that can be used in many different types of foods and processes including fermented, germinated, puffed, milled and baked or cooked into food products. Traditionally finger millet was considered poor man's food and used to make staples like unleavened bread, porridge, finger millet balls, and some non-alcoholic as well as alcoholic drinks. In the last two decades, the properties of finger millet have been in the limelight which has contributed to a renewed interest and commercial products in the market. There are nearly 40 processed foods that have been documented most of which are in India.[3]

Chickpea (*Cicer arietinum* L.) is a Leguminosae family legume that originated in Asia, it contains high amount of protein (23-27%) and lipids (5.8-6.2%) compared to other legumes.[6] Chickpea is high in lysine which makes it an excellent protein enhance in tortillas with finger millet which is a deficit in lysine. The combination on finger millet flour and chickpea would be a perfect match to fulfill the requirement of all the basic macro and micronutrients. The struggle in the formulation of the tortilla is the absence of gluten in these flour. However, the specific amino acids content of chickpea has characteristic of high foam expansion and stability in comparison to other legumes which is beneficial in gluten-free product development.[7]

The growing trend of ethnic food in the United States has peaked the demand of tortillas; making the tortilla industry the fastest growing sector in the baking industry in U.S. Tortillas were commonly made with wheat and/or corn flour; the budding tortilla industry opens up opportunities to explore various legumes and millets as alternative ingredients to the gluten-free landscape. However, studies for optimization of tortilla made of millet and chickpea are limited. We aim to study the formulation and optimization of finger millet tortillas fortified with chickpea flour that could be an alternative to a traditional flour tortilla.

MATERIAL AND METHODS

Finger millet (*Eleusina coracana L.*) flour and chickpea (*Cicer arietinum*) was procured from Swad Food Products (Skokie, IL, USA). Starches (rice, potato, and tapioca) and glycerin were procured from Ingredion, Inc., (Westchester, IL, USA) and Plant Guru (Plainfield, NJ, USA), respectively. All other ingredients were procured from a local market in Edmond, Oklahoma.

Optimization of the Tortilla Flour

Chickpea is high in protein specifically lysine which makes it an excellent fortifying legume to compliment finger millet which is deficient in lysine. Moreover, according to Bazzi et al, the specific amino acids content of chickpea has characteristic of high foam expansion and stability in comparison to other legumes which is beneficial in gluten-free product development.[8] The combination on finger millet flour and chickpea would not just fulfill the requirement of all the macro and micronutrients but also aid in stability of gluten-free tortillas. The chickpeas were grinded and both flours (finger millet and chickpea) were passed through 60 mesh size (250 microns) to obtain uniform particle size flour. The flours were then combined at the ratio of 70:30 w/w to obtain a protein content of 12.1 g per 100 g flour; 2% of sugar was added to enhance flavor and 4% glycerin to aid in stability. Other ingredients included starch, baking powder, and olive oil. A preliminary experiment was conducted to determine the optimum amount of starch to use in tortillas based on their binding effect of the dough and final tortilla. Four percentage (5, 10 15 and 20%) levels of starch were used. The tests performed were physical appearance and rollability of the tortillas. Tortillas with 5% and 10% starch did not improve their physical appearance and rollability while tortillas with 15% and 20% had similar physical characteristics. Therefore, 15% of starch was selected to keep the quantity of starch low and to help in the binding of the dough.

Processing of Chickpea Fortified Finger Millet Tortilla

A hot-press tortilla-making process was used to make tortillas. Dry ingredients (finger millet flour, chickpea flour, starch, baking powder, sugar, hand salt) were mixed for 1 minute and 30 s on speed 1 in a KitchenAid mixer (KitchenAid, St. Joseph, MI, USA). Olive oil and glycerin were added and mixed for 45 s at speed 1. The sides were scraped down with a spatula and the ingredients mixed further for another 45 s at speed 2 until no clumps are visible. Warm water (38 °C) was slowly added while mixing at speed 1 and increasing to speed 3 for a total mixing time of 1 minute and 30 s. The dough was kneaded for 30 s with a hook in the mixer, rested for 10 min in a plastic container with a lid to retain moisture. The dough was divided into 60 g balls and stored in a plastic container with a lid until ready to bake.

A tortilla maker (CPP-200 International Chef™ Stainless Steel, Cuisinart Kitchen Appliances, Stamford, CT, USA) was used. The dough balls were pressed for 6 s and baked in for 1 min and 30 s at 204 °C. Tortillas were cooled on a cooling rack for 2 minutes, stored in a re-sealable plastic bag for 2 h before analysis. The tortillas were processed in triplicate. The cooking time was determined by a preliminary experiment using at 204 °C for 80, 90 and 100 s. The evaluation included color, texture and sensory evaluation.

Physio-Chemical and Nutritional Quality of Flours

Moisture content and ash of finger millet and chickpea flour was determined using American Association of Cereal Chemists (AACC) approved method 44-15.02 and 08-01, respectively. Protein content were determined according to the Association of Official Analytical Chemists (AOCS) method, Ba 4d-90.

Physio-Chemical Characteristics of Tortillas

A 6-inch liquid-crystal display (LCD) digital caliper was used to determine the diameter and thickness of baked tortillas; diameter was the average two perpendicular measurements of 3 baked tor-

tillas and thickness was the average of a stack of 3 tortillas. The weight 3 tortillas were recorded using an analytical balance and the average reported. Moisture content and ash of the tortillas was determined using AACC approved methods 44-15.02 and 08-01, respectively. The color of the tortillas was determined using Hunter Lab MiniSacn XE Plus (Reston, VA, USA) and L, a, b values were reported. Calcium analysis of the baked tortillas was done using flame atomic absorption spectrometry method described by Bazzi, Kreuz, & Fischer[8] and pH of the tortillas was determined using a pH meter model pH-009 (I) pen type that had been calibrated against standard buffers 7 and 4.

Texture Evaluation

Stretchability and extensibility of the tortillas were evaluated on three replicates of 10 baked tortillas each using a TA-XT2i textural analyzer (Texture Technologies Corp., Hamilton, MA, USA/Stable Micro Systems, Godalming, Surrey, UK).

Stretchability Test

A 60 mm TA-108 Tortilla Fixture and a 20 mm TA-108 acrylic rounded edge probe were used. The test settings included 20 g force, the test speed of 1.70 mm/s; distance 30 mm total before returning to its original position.

Extensibility Test

TA-96 tensile grips were used and the test settings were 5 g force, test speed of 1 mm/s and 25 cm distance. Samples were cut using a stainless steel dog bone template with an average length of 60 mm and samples obtained secured with the tensile grips. The tortilla pieces were pulled up vertically and the maximum peak force values and distance values were recorded.

Nutritional Analysis

Nutritional analysis of the tortillas was performed using Genesis R&D software (ESHA Research, Salem, OR, USA; version 9.12.1.0) at the University of Central Oklahoma.

Sensory Evaluation

Sensory evaluation of the tortillas was conducted at the University of Central Oklahoma in two independent evaluations (duplicate). A descriptive analysis of three sample tortillas (T-Rice, T-Tapioca, and T-Potato) was done by trained panelists consisting of dietetic interns and graduate students, the result of the analysis lead to the elimination of the T-Tapioca treatment. The remaining two samples (T-Rice and T-Potato) were taken further into additional testing and sensory acceptance study. Sensory acceptance study was conducted by students and staff. Institutional review board's (IRB) approval was granted for all stages of this study through the University of Central Oklahoma.

Descriptive Analysis

The dietetic interns and food science graduate students were selected as candidate subjects by personal interview and questionnaire. All candidates had experience working with foods. A tortilla descriptive analysis was evaluated by 8 trained panelists. A modified Spectrum™ method was used.[9] Briefly, flavor (sweet, salty, nutty, bitter, doughy) and texture (roughness, tearability, hardness, fracturability, grittiness) attributes were studied. The panelists were trained in two sessions; in the first four-hour training session the attributes and references on taste, texture, odor, and appearance of the tortillas were defined. Attributes developed by experience in a previous study of the research group of sorghum flour in the research group was referred from a similar study on sorghum flour which accounted for all the characteristics of the finger millet flour and a 15-point numbered absolute scale was used to score perceived intensity. In the second session, the attributes and references were analyzed and refined with standard compounds. The final session, sampling the tortillas were conducted the next day to eliminate panelist fatigue and were provided with three samples of tortillas on a white paper plate with random three-digit numeric codes assigned. The sensory sessions were conducted at 22-24°C, the panelists were provided with unsalted crackers and distilled water to cleanse their palate after each sample in separated booths for all the sessions.

Consumer Acceptance Study

The consumer acceptance study was conducted at the University of Central Oklahoma with fifty (50) untrained panelists and included survey questions on demographics, education, and consumption of tortillas. Tortillas with rice starch and potato starch were the samples in the study. The sample tortillas were placed on a white paper with a three-digit numeric code assigned to each. Samples were given to the panelist one at a time to eliminate bias; unsalted cracker and distilled water were provided to cleanse their palate between tastings. A 9-point hedonic scale ballot was provided to score each sample. The 9-point hedonic scale displayed degrees of like and dislike (1, extremely dislike; 9 extremely like). The attributes tested were appearance, aroma, texture, tenderness, taste, and overall likability. The study was conducted at 22-24 °C temperature room in separated booths.

Statistical Analysis

All the analyses (except nutritional analysis) were conducted in triplicates. Means and standard deviations of all samples were reported for color, moisture, nutrition, and sensory evaluations. One-way ANOVA was performed using the general linear model procedure to identify significant difference ($p<0.05$) among the samples followed by Tukey's test. All statistical analyses were carried out using SPSS (SPSS 20.0, IBM Crop, Armonk, NY, USA) and microsoft excel 2016 MSO, version 16.0.6001.1078.

RESULTS AND DISCUSSION

Physical and Chemical Measurements

Moisture, ash, protein, and calcium content of finger millet flour and chickpea flour: The average moisture, ash and protein content of finger millet flour, chickpea flour, potato starch, and rice starch were reported in table 1 the results agree with those of Ozer et al who reported chickpea containing 17.55-23.32% of

protein, 2.54-3.41% of ash and 6.39-10.57% of moisture.[10] The results are in agreement with previous reports of finger millet showing approximately 7% protein, 1.7-4.13% ash and 13.2% of moisture.[2,11] The slight variation comparing to literature reports could be explained in part by different varieties of the grains used. There was no significant difference in moisture content of the flours but there were significant differences in ash and protein content. The ash of chickpea flour was higher than finger millet flour which shows there is a higher quantity of minerals in chickpea flour in comparison to finger millet flour. The data for potato starch and rice starch was obtained from their manufacturer. The calcium analysis using flame atomic absorption (FAA) showed that finger millet has 43.553 mg of calcium per 100 g and chickpea has 14.167 mg per 100 g.

Table 1. *Comparison of Moisture, Ash and Protein Content Results of Finger Millet Flour and Chickpea Flour*[*]

Sample	Moisture (%)	Ash (%)	Protein (%)
Finger millet flour	10.7±0.09[a]	1.83±0.13[a]	5.2
Chickpea flour	10.3±0.11[a]	2.41±0.31[b]	19.7
Potato Starch	4%	0.21%	<0.1%
Rice Starch	12%	0.24%	0.43%

[*]Means±standard deviation with different superscripts within columns indicate significant differences among treatments (p<0.05).

Weight, diameter, thickness and bake-off moisture percentage of Tortillas: Table 2 shows the weight, diameter, and thickness of finger millet tortillas made with rice and potato starches. There was a significant difference in weight and diameter but no significant differences in thickness of the tortillas. The weight of the tortillas is indirectly proportional to the bake-off moisture % of the tortillas. The bake-off moisture % of T-Rice tortilla containing rice starch is higher than T-Potato tortilla made with potato starch which shows that more moisture was baked off in the process of making the tortilla in comparison to T-Potato. The moisture bake-off from the tortilla is also a characteristic of starch, it shows that potato starch absorbs and holds more water than rice starch. The potato starch has larger, irregular granules and higher content of phosphate group in comparison to rice starch which has been reported supporting in higher swelling power without disintegration.[12,13]

The diameter and the thickness of the tortillas are indirectly proportional; higher diameter co-relates to lower thickness due to the spreadability of the tortillas. T-Rice had higher spreadability with higher diameter and lower thickness compared to the T-Potato (Table 2). This is in part explained by the small mean diameter of rice starch and unique spreadable characteristics which is valuable in food as well as pharmaceutical applications.[14]

Moisture content, ash and pH: Average moisture content, ash, and pH of two chickpea fortified finger millet tortilla sample (Table 2). There were no significant differences in moisture content, ash or pH of the sample tortilla. It is interesting that the moisture content of the final tortillas was similar despite showing significant differences in bake-off values. In these formulations, the type of starch used did not affect moisture, ash, and pH of the tortillas.

Color: Table 3 shows the average 'L', 'a', and 'b' values which were significantly different in both the samples. The tortillas T-Potato made with potato starch were lighter in color in comparison to T-Rice made with rice starch with a 'L' value of 57.1. The values of 'a' were higher in T-Rice which indicates that it has more redness and values of 'b' T-Potato were higher indicating more yellow color. According to Singh et al, the higher phosphate monoester content in potato starch results in pastes with higher light transmittance whereas higher phospholipids in cereal starch (rice) results in pastes with lower transmittance.[11] According to recent study on gluten free rice flour tortillas, L^* value of chapati increased with increase in rice flour concentration from 0% to 20%.[15] The transmittance properties of the starches explain the lighter color of tortillas with potato starch in comparison to tortillas with rice starch. According to Yang, Hattori, Kawaguchi & Takahashi, Maillard reaction occurs between potato starch and

Table 2. *Comparison of Weight, Thickness, Diameter, Moisture, Ash, and Ph Results of Chickpea Fortified Finger Millet Tortillas with Different Starches*[*]

Starch type	Weight (g)	Thickness (mm)	Diameter (mm)	Bake-off moisture (%)	Moisture %	Ash %	pH
T-Rice[1]	46.50±0.39[a]	3.28±0.36[a]	144±2[a]	33.5	26.4±0.09[a]	2.86±0.07[a]	6.48±0.08[a]
T-Potato[2]	49.25±0.94[b]	3.61±0.09[a]	134±3[b]	28.0	26.6±0.04[a]	2.86±0.03[a]	6.48±0.02[a]

*Means (n=3)±standard deviation with different superscripts within columns indicate significant differences among treatments (p<0.05).

[1]Chickpea fortified finger millet tortilla with rice starch.

[2]Chickpea fortified finger millet tortilla with potato starch.

Table 3. *Comparison of Color and Texture (Extensibility and Stretchability) Results of Chickpea Fortified Finger Millet Tortillas with Different Starches*[*]

Sample	Color			Bake-off moisture (%)		Ash %	
	L	a	B	Force (g)	Distance (mm)	Force (g)	Distance (mm)
T-Rice[1]	52.56±0.99[a]	8.32± 0.15[a]	16.64±0.33[a]	8.32± 0.15[a]	16.64±0.33[a]	424.6±38.31[a]	3.92±0.75[a]
T-Potato[2]	57.09±1.41[b]	7.82±0.39[b]	17.62±0.83[b]	7.82±0.39[b]	17.62±0.83[b]	372.14±53.77[b]	3.82±1.09[a]

*Means±standard deviation with different superscripts within columns indicate significant differences among treatments (p<0.05).

[1]Chickpea fortified finger millet tortilla with rice starch.

[2]Chickpea fortified finger millet tortilla with potato starch.

lysine resulting in higher yellowness, which explains the yellower color of tortillas with potato starch in comparison to tortillas with rice starch.[15]

Texture (stretchability and extensibility): T-Rice tortillas were firmer but had an insignificant difference in distance meaning they had similar extensibility. According to Frenholz, a higher force indicates greater stretchability, the higher force on T-Rice suggests that it has the higher stretchability in comparison to T-Potato tortillas with potato starch. However, Frenholz also states that gluten-network in wheat tortillas creates flexibility so, stretchability test may not be a good indicator for a gluten-free tortilla due to the absence of gluten-network.[16]

Table 3 shows the average force and distance of the tortillas testing its extensibility. There was significantly different for force and distance with the lowest value of 1184.93 g. According to Suhendro, a low force value and longer distance of extension indicate soft and extensible tortillas whereas higher force value and shorter rupture distance indicates hard and brittle tortillas.[17] T-Rice made with rice has the low force and long distance whereas T-Potato made with potato has the high force and short distance making indicating T-Rice being softer and more extensible than T-Potato.

Nutrition Analysis

There was no significant difference in nutritional facts of the tortillas since the only difference in the formulation was the use of different starch which had similar properties. The composition of starches is very similar consisting of polymers and minor compound however the physio-chemical properties and functional characteristics are subjected to an aqueous system, biological origin and annealing.[11,14]

Sensory Analysis

Descriptive analysis: Table 4 shows the descriptive analysis for flavor, the only significant difference was in sweetness and doughy after taste of the tortillas. The tortillas with potato starch were the sweetest compared to the tortilla with tapioca starch which has an average score of 1.4 (least sweet comparable to 0.47 or sucrose solution). The doughy profile was high (5.5 scores comparable to butter roll) for tortillas with potato starch while the scores were similar for tortillas with rice or tapioca starch. Overall the highest acceptance scores were observed on tortillas with potato starch compared to those with rice or tapioca starch.

In attributes of texture (Table 4), there was no significant difference across the parameters both in hand and mouth feel the texture. But the scores for roughness and tearability were slightly higher for a tortilla with potato starch which correlates with the physicochemical texture data that indicated it is firmer in comparison to a tortilla with rice starch.

The shape is the only attribute that has significant difference in context of appearance of the tortillas (Table 5). The tortillas with rice starch were rounder than other tortillas with a high score of 14.1. The data indicates that tortillas with rice starch and tortillas with tapioca starch were rounder and smoother than tortilla with potato starch.

In odor and overall likability (Table 5), there was no significant differences among the three types of tortillas, but the scores indicated that tortillas with tapioca starch, tortillas with rice starch and tortillas with potato starch had least sweet and musty odor respectively. The panelists preferred tortillas with potato starch with an overall likability score of 11.1 and disliked tortillas with tapioca starch with the least score of 6.9. Due to

Table 4. *Comparison of Flavor and Texture Attributes in Description Analysis of Chickpea Fortified Finger Millet Tortillas with Different Starches*[*]

	Flavor					Texture				
Sample	Sweet[I]	Salty[II]	Nutty[III]	Bitter[IV]	Doughy[V]	Roughness[I]	Tearability[II]	Hardness[III]	Fracturability[IV]	Grittiness[V]
T-Rice[1]	1.6±0.74[a]	2.3±0.71[a]	6.5±2.62[a]	1.3±0.71[a]	2.9±.099[a]	5.5±1.07[a]	12.3±2.38[a]	8.6±3.96[a]	7.3±3.20[a]	5.6±3.99[a]
T-Potato[2]	2.6±0.92[b]	2.6±1.51[a]	6.1±3.18[a]	1.0 ±0.00[a]	5.5 ±2.56[b]	6.1±2.90[a]	12.5±2.14[a]	5.8±1.66[a]	5.9±2.85[a]	3.1±1.45[a]
T-TAPIOCA[3]	1.4±0.52[a]	1.8±0.89[a]	5.6±2.20[a]	2.0±1.77[a]	3.0±1.69[a]	5.4±1.19[a]	12.4±1.77[a]	7.9±3.39[a]	6.4±2.19[a]	5.0±3.07[a]

[*]Means±standard deviation with different superscripts within columns indicate significant differences among treatments ($p<0.05$).

1. Chickpea fortified finger millet tortilla with rice starch.

2. Chickpea fortified finger millet tortilla with potato starch.

3. Chickpea fortified finger millet tortilla with tapioca starch.

Flavor:

I. Sweet intensity was evaluated on a scale from 1 (not detectable) to 15 (extremely sweet)

II. Salty intensity was evaluated on a scale from 1 (not detectable) to 15 (extremely salty)

III. Nutty intensity was evaluated on a scale from 1 (not detectable) to 15 (extremely nutty)

IV. Bitter intensity was evaluated on a scale from 1 (not detectable) to 15 (extremely bitter)

V. Doughy intensity was evaluated on a scale from 1 (not detectable) to 15 (extremely doughy)

Texture:

I. Roughness intensity was evaluated on a scale from 1 (not detectable) to 15 (extremely rough)

II. Tearability intensity was evaluated on a scale from 1 (easily pulled apart) to 15 (extremely hard to pull apart)

III. Hardness intensity was evaluated on a scale from 1 (extremely easy to bite down) to 15 (extremely hard to bite down)

IV. Fracturability intensity was evaluated on a scale from 1 (extremely easy break) to 15 (extremely hard to break)

V. Grittiness intensity was evaluated on a scale from 1 (absence of gritty particles) to 15 (extremely presence of gritty particles)

Table 5. *Comparison of Appearance Attributes, Odor and Overall Likability in Description Analysis of Chickpea Fortified Finger Millet Tortillas with Different Starches*[*]

Sample	Appearance			Odor		
	Evenness of the color[I]	Shape[II]	Surface[III]	Sweet[I]	Musty[II]	Overall likability[III]
T-Rice[1]	9.8±3.73[a]	14.1±0.35[a]	6.0±2.97[a]	2.0±1.07[a]	6.8±2.36[a]	9.3±2.76[a]
T-Potato[2]	8.3±3.77[a]	8.6±3.02[b]	6.5±3.33[a]	2.1±1.46[a]	5.8±3.69[a]	11.1±3.72[a]
T-TAPIOCA[3]	11.3±2.12[a]	12.5±2.5[a]	5.6±2.06[a]	1.8±1.49[a]	7.3±2.76[a]	6.9±4.32[a]

[*]Means±standard deviation with different superscripts within columns indicate significant differences among treatments (p<0.05).

1. Chickpea fortified finger millet tortilla with rice starch.

2. Chickpea fortified finger millet tortilla with potato starch.

3. Chickpea fortified finger millet tortilla with tapioca starch.

I. Evenness of the color intensity was evaluated on a scale from 1 (very even) to 15 (extremely uneven)

II. Shape intensity was evaluated on a scale from 1 (not round) to 15 (perfectly round)

III. Surface intensity was evaluated on a scale from 1 (presence of blistering) to 15 (absence of blistering)

IV. Sweet intensity was evaluated on a scale from 1 (not detectable) to 15 (extremely sweet)

V. Musty intensity was evaluated on a scale from 1 (not detectable) to 15 (extremely musty)

VI. Overall likability intensity was evaluated on a scale from 1 (extremely dislike) to 15 (extremely like)

low score tortillas with tapioca was eliminated from the experiment and tortillas with rice and potato starch continued for testing and sensory acceptability study.

Consumer study: Table 6 shows the average scores from the consumer acceptability test. Appearance is the only attribute that has significant differences with a score of 6.3 for a tortilla with rice starch and 5.6 for a tortilla with potato starch. The overall likability score for a tortilla with potato starch were slightly higher in comparison to a tortilla with rice starch which correlates with the higher score in taste, aroma and texture. In contrast, the appearance and the tenderness score were low for a tortilla with potato starch which correlates with it being smaller, thicker and tougher tortillas from physicochemical testing. According to Wani et al, rice starch has bland taste, smooth, creamy and spreadable characteristics which correspond with lower scores in taste but higher scores in appearance and tenderness of the tortillas with rice starch.[14]

Table 6. *Comparison of Scores from Consumer Acceptance Study of Chickpea Fortified Finger Millet Tortillas with Different Starches*[*]

Sample	Overall likeability	Appearance	Texture	Tenderness	Aroma	Taste
T-Rice[1]	6.0±1.77[a]	6.3±1.51[a]	5.8±1.68[a]	6.0±1.76[a]	5.9±1.39[a]	5.9±1.82[a]
T-Potato[2]	6.3±1.7[a]	5.6±1.70[b]	6.0±1.75[a]	5.9±1.93[a]	6.1±1.51[a]	6.0±1.87[a]

[*]Means ± standard deviation with different superscripts within columns indicate significant differences among treatments (p<0.05).

1. Chickpea fortified finger millet tortilla with rice starch

2. Chickpea fortified finger millet tortilla with potato starch

Physical, chemical, textural and sensory testing showed differences between tortillas with different starches. The tortillas with rice starch were better in texture and appearance whereas the overall acceptability was higher for tortillas with potato starch due to its flavor neglecting the textural characteristics. The results indicated that incorporation of potato/rice starches may result in the formulation of chickpea fortified finger millet tortillas with acceptable textural and sensory properties which would be a gluten-free, nutrient-dense alternative to traditional tortillas for people with celiac disease and a potential medicinal food for people with diabetes.

The increasing prevalence of obesity and overweight are linked to several health conditions (diabetes, cardiovascular disease, etc.), along with the growing incidences of food allergies are of major concern globally. These health conditions are not only talking toll in the health but also the economy of the people. The change in lifestyle and diet are one of the few measures to reduce risk and manage these conditions. The availability of healthier food choices and the awareness of functional ingredients are of utmost importance. Finger millet is an ancient millet grain that has superior nutritional values and has shown to aid in many health conditions. Formulation of food incorporating finger millet could provide alternative and boost healthier diet leading to better health.

However, the textures of the tortillas with potato starch was not ideal and comparable to commercial tortillas. Further research should include hydrocolloids and emulsifiers (sodium stearoyl lactylate, diacetyl tartaric acid ester of mono- and diglycerides (DATEM), and others) to improve the overall quality of the tortillas. The shelf- life of the tortillas has not been studied in this research, so further research is required in the field of the shelf- life along with research on the effect of high protein flour composition as an alternative of gluten.

CONCLUSION

Nutrient-dense gluten-free chickpea fortified finger millet tortillas optimized with rice and potato starch was analyzed for physical, chemical, textural and sensory properties. The results indicated that incorporation of potato/rice starches may result in the formulation of chickpea fortified finger millet tortillas with acceptable textural and sensory properties producing a gluten-free, nutrient-dense alternative to traditional tortillas for people with celiac disease and a potential medicinal food for people with diabetes. The overall acceptability was higher for tortillas with potato starch due to its flavor but there is room for improvement of the texture of tortillas with potato starch. Further research should include hydrocolloids and emulsifiers to improve overall texture quality and shelf life of the tortillas.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

Dr. Bhargava reports in addition, Dr. Bhargava has a patent composition and process for making millet-based flour useable informed food products pending to the University of Central Oklahoma.

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Organo-Protective Effect of *Moringa oleifera* (Moringa) and *Camellia sinensis* (Green Tea) against Histopathological Damage in Monosodium Glutamate-induced Oxidative-Stressed Rats

Ikechukwu E. Ezeagu, PhD[1]; Moses B. Ayoola, MSc[2]; Chukwunonso C. Ejiofor, MSc[1*]; Peter Achukwu, PhD[3]

[1]*Department of Medical Biochemistry, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria*
[2]*Department of Basic Sciences, Faculty of Veterinary Medicine, Mississippi State University, Mississippi, USA*
[3]*Department of Medical Laboratory Science, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria*

[*]***Corresponding author***
Chukwunonso C. Ejiofor, MSc
Department of Medical Biochemistry, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria; E-mail: bianate@yahoo.ca

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ABSTRACT

Background

Moringa oleifera and *Camellia sinensis* are widely consumed edible plants in parts of South East, Nigeria. It is used as a beverage and also used in the treatment of several ailments by alternative medical practitioners because of its medicinal properties medicinal plant. The histopathological damage repair properties of *Moringa oleifera* and *Camelia sinensis* on the brain, liver and kidney organs were investigated on monosodium glutamate (MSG)-induced oxidative-stressed rats.

Method

Twenty male Wister rats were divided into five groups of four rats each. Rats were administered with 0.6 mg/g body weight (b.w) dose of MSG solution for 14 days to induce oxidative stress. Control group was given distilled water. A subsequent treatment with MO, GT and their mixture for a period of 28 days was carried out. One group was left untreated. On day 28 of the treatment regime, animals were sacrificed and necessary organs (brain, liver and kidney) harvested. These organs were processed according to paraffin wax embedding technique, stained with haematoxylin and eosin for light microscopy.

Results

The plant samples both showed in vivo nitric oxide scavenging properties. The plant samples also presented an increased catalase enzyme activity and the lipid profile analyses showed no significant difference at 95% level of probability. Both plants resulted in reversing the damage that occurred in MSG-induced oxidative stressed rats. This was particularly true for both the kidney and liver.

Conclusion

The study provides a pharmacological basis for the traditional use of *Camellia sinensis* and *Moringa oleifera* extracts in alleviating common medical conditions.

Keywords

Moringa oleifera; *Camellia sinensis*; Histopathology; Monosodium glutamate oxidative stress; Damage.

INTRODUCTION

Over the years, plant materials have been sources of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. Traditional system of medicine has been found to have many utilities in treatment of various diseases.[1] The need for the use of plants to treat human disease could be attributed to the increase in human population (more people are seeking for alternative cheaper source of medication), inadequate supply of drug and high cost of treatment. Side effect coupled with drug resistance encountered with synthetic drugs also necessitated the need for alternative therapy. The affordability of herbals has also drawn the attraction towards their use. *Moringa oleifera* (Moringa) and *Camellia sinensis* (Green

Tea) are two of such medicinal plants gaining popularity for their reported health benefits in recent years.[1,2]

MO is one of the most widely distributed species of the Moringaceae family throughout the World, especially in Asian countries, having a remarkable range of pharmacological properties in addition to significant nutritional value. MO is a highly valued plant in tropic and subtropical countries where it is mostly cultivated.[3] The medicinal properties of the plant's edible parts have been recognized by both the Ayurvedic and Unani systems of medicine in India.[4]

GT has been associated with lowering the risk of cancer, lowering the risk of coronary heart disease and improvement of oral health.[5] It has been found to have antimicrobial health benefits and antioxidant properties.[6] There are also suggestions that GT extracts offer protection against bone loss,[5] body weight control, anti-hypertensive properties, solar ultraviolet protection, neuroprotective properties and anti-fibrotic properties.[6] GT therefore provides a very interesting beverage with potential for a variety of medicinal uses and health promoting benefits.

During the past decade, there has been a growing interest in the medical implications of free radicals. As a result of aerobic life, organisms must deal with the continuous generation of reactive oxygen species (O_2-, H2O2, •OH) as by-products of metabolism and defend itself against the harm that these can do to cellular macromolecules.[7] Free radicals produced as a result of normal biochemical reactions in the body are implicated in contributing to a number of medical conditions such as cancer, aging, diabetes, atherosclerosis, immune suppression and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease.[8,9] Oxidative stress has been defined as a disturbance in the balance between the production of reactive oxygen species (ROS) or free radicals and antioxidant defences.[10]

Monosodium glutamate (MSG) popularly known as AJINOMOTO is the sodium salt of glutamic acid.[11] MSG contains 78% of glutamic acid, 22% of sodium and water.[12] As food additive, MSG is described and listed on food labels as a "flavouring" or "hydrolysed vegetable protein".

Chronic administration of MSG (4 mg/kg body weight and above) as well as sub-chronic administration (0.6-1.6 mg/kg body weight) has been reported to induce oxidative stress in experimental rats.[13,14]

MATERIALS AND METHODS

Sample Collection and Preparation

Ripened MO leaves were obtained from Ogbete Main Market in Enugu while popular branded GT from Qualitea, Ceylon Ltd, Sri Lanka was purchased from a supermarket in Enugu. Moringa leaf was identified and authenticated by Mr. Alfred Ozioko of bioresources Conservation and Development Program (BDCP), Nsukka. The dried plant materials were powdered using an electrical blender. The extraction was done at room temperature.

A hundred grams (100 g) of dried, ground sample materials were soaked in 80% methanol (1 l) for 5 days separately. The soaked material was stirred every 18 h using a sterilized glass rod. The final extracts were passed through Whatman filter paper No. 1. The filtrates obtained were concentrated under vacuum to dryness using a rotary evaporator at 40 °C. The resulting fine powders obtained were weighed (MO=6.437 mg and GT=8.146 mg), dissolved in 300 mL of distilled water separately and stored at 4 °C until use.[15]

Animal Experiment

Twenty male Wistar strain rats with mean weight of 114±15 g were used for the experiment. Rats were divided into five groups containing four rats each. They were fed with standard laboratory diet and water for a period of one week for acclimatization. At the end of the acclimatization period, rats were divided into five groups as shown below

Group I (Control): maintained on only standard laboratory diet.
Groups II, III, IV and V: were given water solution of monosodium glutamate (MSG) at the dose of 0.6 mg/kg body weight for 14 days. The MSG administration was discontinued after 14 days.
Group II: MSG only.
Group III: MSG+leaf extract of MO administered orally at the dose of 250 mg/kg body weight for 28 days.
Group IV: MSG+leaf extract of GT was administered orally at the dose of 250 mg/kg body weight for 28 days.
Group V: MSG+leaf extract of MO and GT was administered orally at the dose of 250 mg/kg body weight for 28 days. The dose was formulated by weight of MO and GT in a ratio 1:1.

At the end of 28 days treatment, animals were sacrificed by euthanizing them (rendering unconscious, with chloroform) and dissected. The liver, kidney and brain tissues were harvested for histology studies. These organs were fixed in 40% formaldehyde solution. The standard paraffin process method was used for tissue processing, while the haematoxylin and eosin staining method was employed.

The stained sections were evaluated and interpreted by an independent histopathologist.

Blood Sample Collection

Blood sample collection was done using the periorbital or posterior-orbital method. This requires orbital venous plexus bleeding. The animal was scruffed with thumb and the forefinger of the non-dominant hand and the skin around the eye was pulled taut. A capillary was inserted into the medial canthus of the eye (30 degree angle to the nose). Slight thumb pressure was enough to puncture the tissue and enter the plexus/sinus. Once broken, blood collection takes place. As soon as the required volume of blood was collected from plexus, the capillary tube is gently removed and wiped with sterile cotton. Bleeding was stopped by applying gentle finger pressure.

Preparation of Homogenate

A portion of liver was weighed, perfused with saline and homogenized in chilled potassium chloride (1.17%) using a homogenizer. The homogenates were centrifuged at 800 g for 5 min to separate the nuclear debris. The resulting supernatant was centrifuged at 10,500 g for 20 min to get the post mitochondrial supernatant which was used to assay catalase (CAT) activity and malonyldialdehyde (MDA) level.[16]

Assays

Nitric Oxide Level (*in vivo*): the indirect determination of NO involving the spectrophotometric measurement of its stable decomposition product nitrite (NO_2^-) was used. The Griess reaction is a two-step diazotization reaction in which the NO-derived nitrosating agent, dinitrogen trioxide (N_2O_3) generated from the acid-catalyzed formation of nitrous acid from nitrite (or autoxidation of NO) reacts with sulfanilamide to produce a diazonium ion which is then coupled to N-(1-napthyl)ethylenediamine to form a chromophoric product that absorbs strongly at 540 nm. A known volume of premixed Griess reagent is added to equal volume of serum, incubated for 10 min and the absorbance of each sample determined at 540 nm. The nitrite concentration was determined by extrapolation from sodium nitrite standard graph.[17]

Lipid Peroxidation: The extent of lipid peroxidation in the tissue was determined by measuring the amounts of malondialdehyde (MDA) produced following the method of Ohkawa et al[18] 0.2 ml of liver homogenate, 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 8% TBA were added. The volume of the mixture is made up to 4 ml with distilled water and then heated at 95 °C on a water bath for 60 min using glass balls as condenser. After incubation the tubes were cooled to room temperature and final volume was made to 5 ml in each tube. Five ml of butanol/pyridine (15:1) mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer was taken and its optical density was measured at 532 nm against an appropriate blank without the sample. The level of lipid peroxides can be expressed as n moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of 1.56X105 M^{-1} cm^{-1} i.e.,

$$\text{Malondialdehyde concentration (M)} = \frac{\text{(Absorbance at 532 nm)}}{\text{(1.56X105)}}$$

Catalase activity (CA) was assayed colorimetrically at 620 nm and expressed as unit/g tissue as described by Sinha.[19] The reaction mixture (1.5 ml) contained: 0.01 M pH 7.0 phosphate buffer (1.0 ml), liver homogenate (0.1 ml) and 2 M H_2O_2 (0.4 ml). The reaction was stopped by the addition of dichromate-acetic acid reagent (2.0 ml of 5% potassium dichromate and glacial acid mixed in 1:3 ratios) and the unit value was interpolated from the standard graph.

Lipid Profile Assay: Serum was drawn for lipid assessment after 12 hours overnight fast. The lipid profile was determined as follows: total cholesterol (TC) was determined by enzymatic colorimetric method.[20] Triglycerides (TG), was determined by enzymatic hydrolysis with subsequent determination of the liberated glycerol by Boeringer Mannheim GPO-PAP Kit[21] and high-density lipoprotein (HDL) was determined by Friedewald et al., method.[22] Low-density-lipoprotein (LDL) was obtained by calculation: LDL=TC- (VLDL+HDL); where VLDL=TG/5.[22]

Weight Change: The weights of the experimental rats were measured and recorded at day 0, day 14 and day 42 of the experiment with the aid of weighing balance.

RESULTS

Results from the table below (Table 1) show the *in vivo* nitric oxide assay between the groups and the descriptive statistics for the groups. Higher values of nitrite concentration in experimental Groups (II-V) as compared to the control (Group I) suggest that the experimental animals were oxidatively stressed after the administration of monosodium glutamate (MSG).

Table 1. *Result of Nitric Oxide Level between the Groups*

Group	Nitrite Conc. ± SD
I (Control)	7.681 ± 1.372a
II	13.172 ± 4.146ab
III	13.008 ± 1.038ab
IV	10.733 ± 1.202ab
V	17.172 ± 8.6107b

Mean with the same letters are not significant at p<0.05, Group I=Control, Group II=MSG, Group III=MSG+MO, Group IV=MSG+GT, Group V=MSG+MO+GT

Table 2 presents the result of the lipid peroxidation assay for each group with Group II (0.362±0.115 µM) particularly demonstrating the high oxidative stressed state caused by the administration of monosodium glutamate. Significant lower value in Group V (0.065±0.022 µM) suggests that there might be synergistic antioxidant effect mixing the two plants together.

Table 2. *Effect of Moringa Leaf and Green Tea Extracts on Lipid Peroxidation*

Group	MDA ± SD (µM/)
I (Control)	0.164± 0.042a
II	0.362 ± 0.115b
III	0.199 ± 0.032a
IV	0.156 ± 0.027a
V	0.065 ± 0.022c

Mean with the same letters are not significant at p<0.05, Group I=Control, Group II=MSG, Group III=MSG+MO, Group IV=MSG+GT, Group V=MSG+MO+GT

Table 3 shows the result of catalase activities between the groups to estimate the effect of the extracts on the antioxidant enzyme activity. Group IV treated with GT has a significant level of catalase activity (12.85±0.92×103 unit/g tissue), followed

by Group V (7.86±4.82×103 unit/g tissue) treated with mixture of MO and GT. The least activity was shown in the untreated Group II (3.75±1.28×103 unit/g tissue).

Table 3. *Effect of Moringa leaf and Green Tea Extracts on Catalase Activity*

Group	Catalase (unit/g tissue)× 10^3
I (Control)	4.15 ± 1.04a
II	3.75 ± 1.28a
III	5.43 ± 2.03a
IV	12.85± 0.92b
V	7.86 ± 4.82ab

Mean with the same letters are not significant at p<0.05, Group I=Control, Group II=MSG, Group III=MSG+MO, Group IV=MSG+GT, Group V=MSG+MO+GT

Table 4 present various results of lipid profile parameters of all the groups after treatment with both MSG and extracts. A significant decrease was observed in both HDL level in untreated Group II and TG level in Group V. TG was markedly increased in Group I and II. Though there was an increase in LDL level of untreated Group II, it was not significant from other groups.

Table 4. *Effect of Moringa leaf and Green Tea Extracts on Catalase Activity*

Group	TC	HDL (mg/dL)	TG	VLDL	LDL
I (Control)	144.9 ± 8.3[a]	63.0 ± 5.7[a]	89.5 ± 0.7[a]	17.9 ± 2.7[a]	64.0 ± 11.3[a]
II	121.4 ± 2.8[a]	32.0 ± 5.7[b]	89.5 ± 10.6[a]	17.9 ± 2.1[a]	71.5 ± 10.6[a]
III	142.9 ± 1.27[a]	69.5 ± 3.5[a]	67.0 ± 9.9[ab]	13.4 ± 2.0[a]	60.0 ± 2.8[a]
IV	144.8 ± 5.9[a]	73 ± 1.4[a]	61.5 ± 2.1[ab]	12.3 ± 0.4[a]	59.5 ± 4.9[a]
V	137.4 ± 14.1[a]	66.5 ± 2.1[a]	57.0 ± 7.1[b]	11.4 ± 1.4[a]	59.5 ± 10.6[a]
Normal Range	140-200	35-75	36-165	15-52	60-140

Mean with the same letters in the same column are not significantly different from each other at p<0.05
Group I=Control, Group II=MSG, Group III=MSG+MO, Group IV=MSG+GT, Group V=MSG+MO+GT
TC=Total Cholesterol, HDL= High Density Lipoprotein, TG= Triglyceride, VLDL= Very Low Density Lipoprotein and LDL= Low Density Lipoprotein.

Table 5 presents the data recorded for the various weight measured before and after treatment. The weight gain and the percentage (%) gain was also recorded. Significant weight gain was observed in Group II (50.38±4.79 g) given only MSG as compared to Control, Group I (22.85±8.15 g) and extracts-treated Group III-V (36.53±11.44; 28.98±10.97; 20.12±3.01).

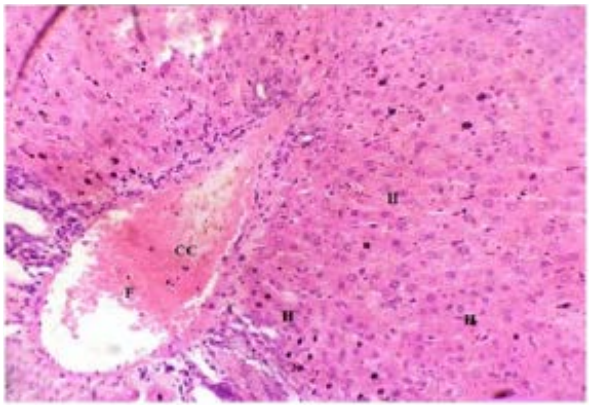
Table 5. *Effect of MSG, Moringa and Green Tea Extracts on Body Weight (g)*

Group	Initial Weight	Final Weight	Weight Gain	% Gain
I (Control)	124.78 ± 16.25[a]	147.63 ± 13.98[ab]	22.85 ± 8.15[a]	18.31
II	117.78 ± 14.51[a]	168.15± 14.32[b]	50.38± 4.79[b]	42.77
III	121.85 ± 0.98[a]	158.36 ± 12.28[b]	36.53 ± 11.44[ab]	28.98
IV	102.30 ± 12.70[a]	131.28 ± 10.59[a]	28.98 ±10.97[a]	28.33
V	104.65 ± 12.50[a]	124.77± 10.13[a]	20.12 ± 3.01[a]	28.79

Mean with the same letters in the same column are not significantly different from each other at p<0.05
Group I=Control, Group II=MSG, Group III=MSG+MO, Group IV=MSG+GT, Group V=MSG+MO+GT

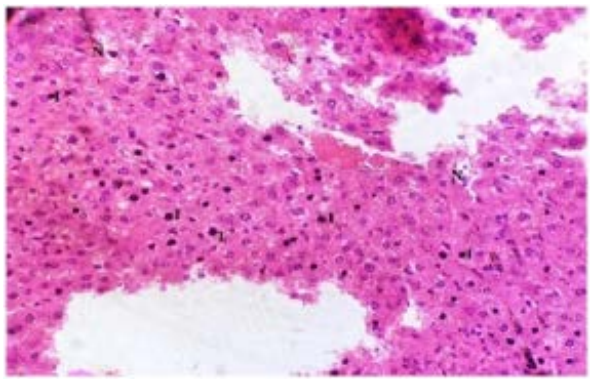
Histology Study: Liver

Plate 1. *A Section of Liver Tissue of (Group I) Normal Rats. Note: Normal Hepatocytes (H), Normal Central Canal (CC) and Frank RBC (F)*



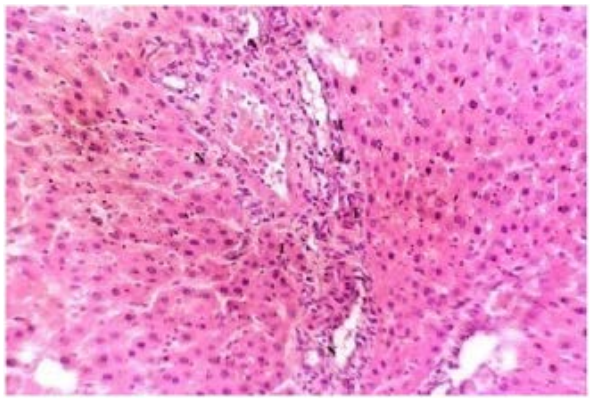
Stain: H/E.. *Mag: ×200*

Plate 2. *A Section of Untreated Liver Tissue of (Group II) Rat Following Liver Damage by MSG. Note the Necrotic (N) and Inflammatory (I) Cells*



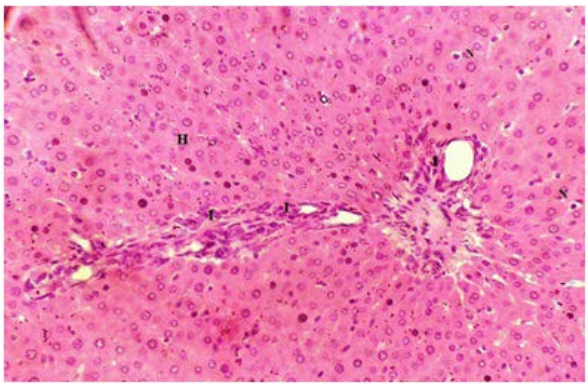
Stain: H/E.. *Mag: ×200*

Plate 3. *A Section of Liver Tissue of (Group III) Rat Treated with 250 mg/kg b.w MO Following Liver Damage by MSG. Note: Inflammatory Cells (I), Mild Necrotic Cells (N) Showing Recovery from Damage*



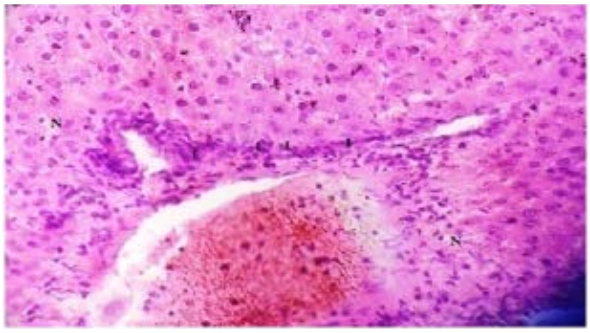
Stain: H/E.. *Mag: ×200*

Plate 4. *A Section of Liver Tissue of (Group IV) Rat Treated with 250 mg/kg b.w GT Following Liver Damage by MSG. Note: Infiltration of Inflammatory Cells (I) at the Perivascular Area. The Hepatocytes Appeared to be Normal (H) with Mild Necrosis (N)*



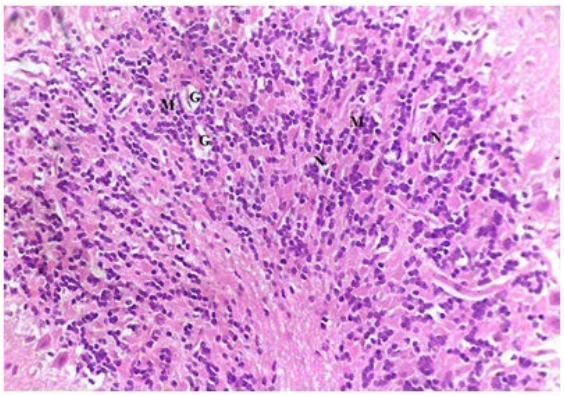
Stain: H/E.. *Mag: ×200*

Plate 5. *A Section of Liver Tissue of (Group V) Rat Treated with 250mg/kg b.w MO & GT (1:1) Following Liver Damage by MSG. Note: Inflammatory Cells (I) at the Perivascular Area. Minimal necrotic Cells (N) Showing Recovery from Damage Due to MSG Treatment*



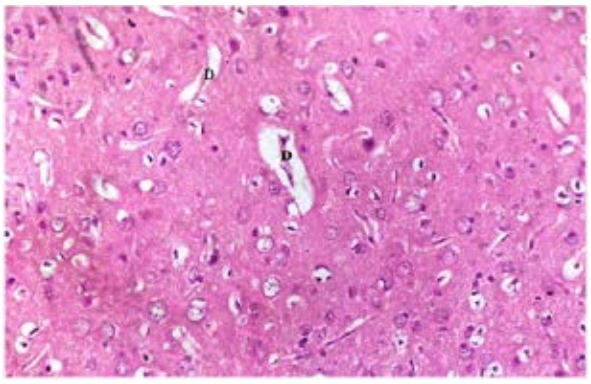
Stain: H/E.. *Mag: ×200*

Plate 7. *Brain Section of (Group II) Rat Treated with MSG. Predominant Mononuclear Cells Demonstrated (M). Necrotic Focci were Also Shown (N) with Decreased Glial Cells (G)*



Stain: H/E.. *Mag: ×200*

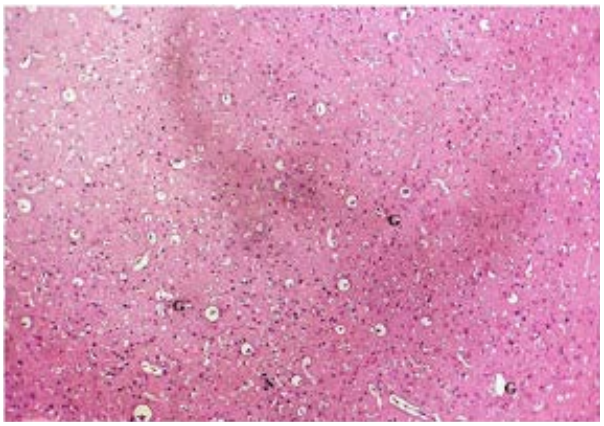
Plate 8. *Section of Brain Tissue of (Group III) Rat Treated with Dose of 250 mg/kg b.w MO After Damage by MSG. Note: Damaged Fibres of Glial Cells (G)*



Stain: H/E.. *Mag: ×200*

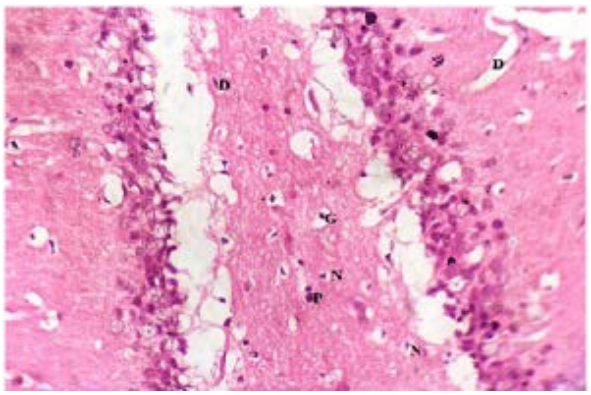
Histology Study: Brain

Plate 6. *A Cross Section of Brain tissue of (Group I) Normal Rat. Note: Normal Glial Cells(G), Normal Neurons (N)*



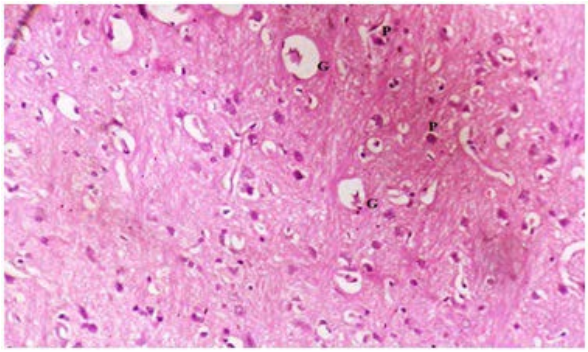
Stain: H/E.. *Mag: ×200*

Plate 9. *Section of Brain Tissue of (Group IV) Rat Treated with Dose of 250 mg/kg b.w of GT Following Brain Damage by MSG. Note: Damaged Fibres of Glial Cells (G)*



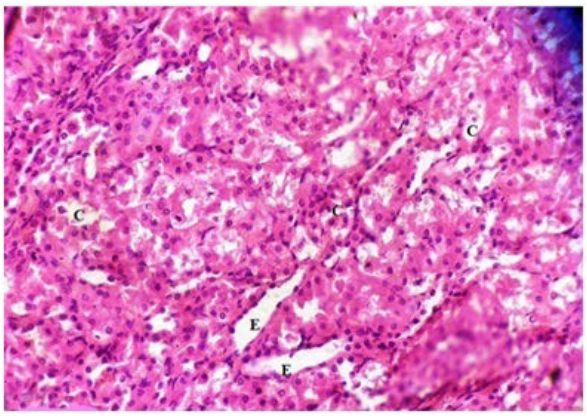
Stain: H/E.. *Mag: ×200*

Plate 10. *Section of Brain of (Group V) Rat Treated with Dose of 250 mg/kg b.w of Mixture of MO&GT (1:1) Following Brain Damage by MSG. Note: Plaques (P) and Shrunken Glial Cells (G)*



Stain: H/E.. *Mag: ×200*

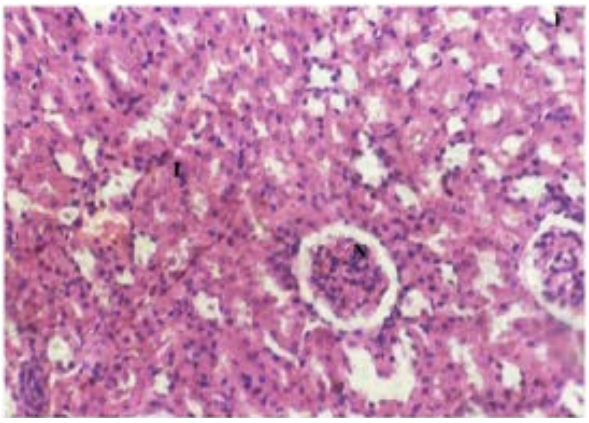
Plate 13. *Section of (Group III) Rat Kidney Treated with 250 mg/kg b.w MO Following Damage by MSG. Numerous Tubular Casts (C), Tubular Erosion (E) are shown*



Stain: H/E.. *Mag: ×200*

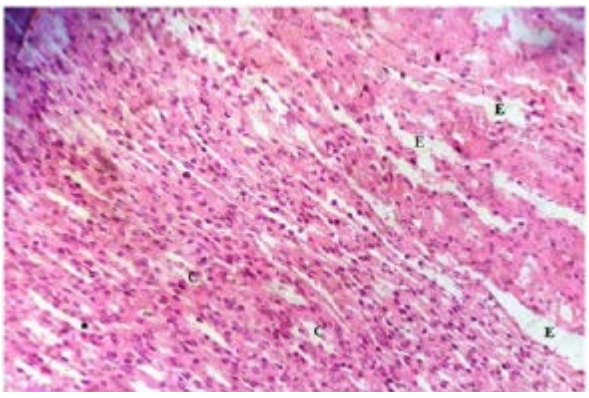
Histology Study: Kidney

Plate 11. *Section of Kidney of (Group I) Normal Rats. Note: Inflammatory Cells (I) and Normal Renal Cells (N)*



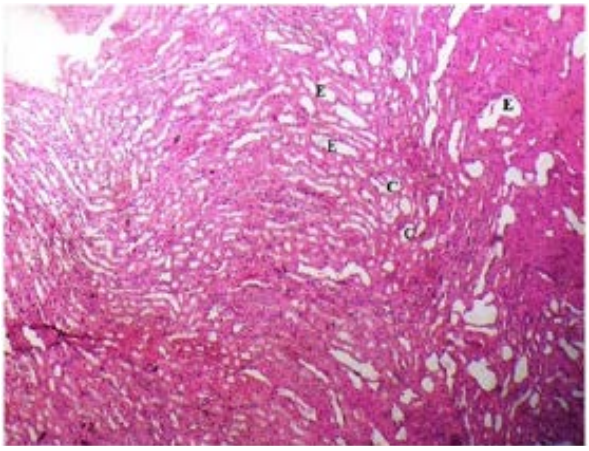
Stain: H/E.. *Mag: ×200*

Plate 14. *Section of Medulla of (Group IV) Rat Kidney Treated with 250 mg/kg b.w GT Following MSG Treatment. Note: Tubular Casts (C) and Tubular Erosion (E)*



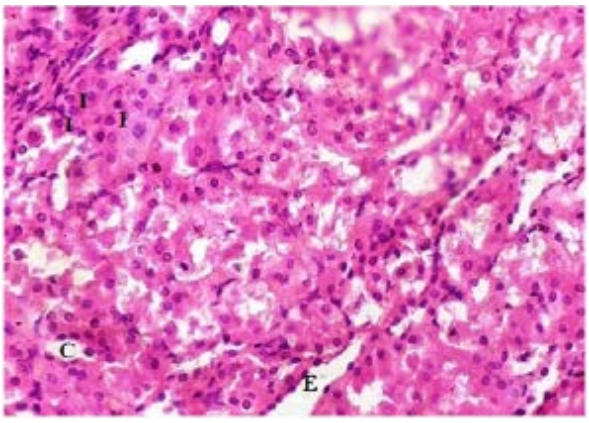
Stain: H/E.. *Mag: ×200*

Plate 12. *Medullary Section of (Group II) Rat Kidney Following Treatment with MSG. Note: Distribution of Eroded Tubules (E) and Tubular Casts {C}*



Stain: H/E.. *Mag: ×200*

Plate 15. *Section of Medulla of (Group V) Rat Kidney Treated with 250 mg/kg b.w MO&GT (1:1). Note: Inflammatory Cells (I), Tubular Casts (C) and Tubular Erosion (E)*

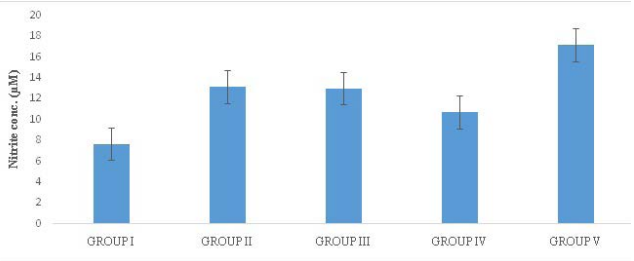


Stain: H/E.. *Mag: ×200*

DISCUSSION

In the *in vivo* NO assay, there was a marked increase in NO level in terms of nitrite concentration (μM) in Group II-IV (13.172±4.146; 13.008±1.038 and 10.733±1.202) and a significant increase in Group V (17.172±8.6107) compared to the Control, Group I (7.681±1.372) (Figure 1). The result suggests that MSG induced oxidative stress in Group II-V.

Figure 1. *Levels of NO in Treated Rats*

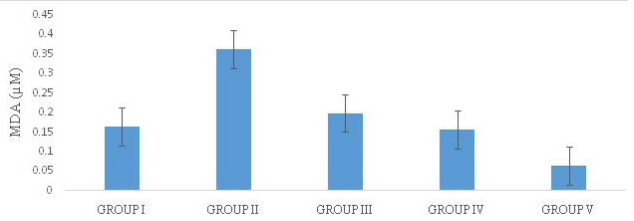


Group I=Control, Group II-V=MSG

Lipid Peroxidation Extent

Monosodium glutamate is one of the world's most widely used food additives, it has been known to be a flavour enhancer in West African and Asian diets, and has been used to induce oxidative damage in animal model as a marked change in oxidative stress markers like lipid peroxidation and enzyme activity were observed.[23,24]

Figure 2. *Lipid Peroxidation Levels of Treated Rats*



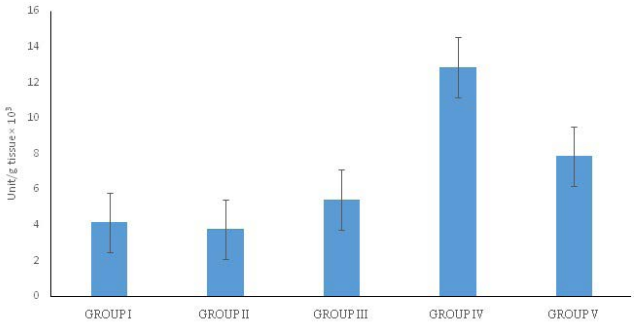
Group I=Control, Group II=MSG, Group III=MSG+MO, Group IV=MSG+GT, Group V=MSG+MO+GT

Lipid peroxidation is a well-established mechanism of cellular injury and therefore used as an indicator of oxidative stress which is characterised by an increase in malondialdehyde concentration (MDA). In this study, the concentration of MDA was markedly increased in the MSG-treated group which is significantly different (p<0.05) from the control group and the other groups. This is similar to the findings of Farombi and Onyema,[24] and Egbuonu et al[25] in which an increase in MDA concentration after MSG administration reported. The result of the Lipid Peroxidation assay therefore shows the oxidative stress effect caused by MSG administration. Moringa extracts administered to experimental rats has been reported to significantly reduce MDA levels in acetaminophen induced oxidative stress[26] while green tea was also reported to reduce MDA level in stress induced oxidative damage.[27] These previous reports are similar to the results in this study. The extracts of MO and GT significantly (p<0.05) lowered the MDA level (μM/) in Group III (0.199±0.032), Group IV (0.156±0.027) and Group V (0.065±0.022) compared to untreated group II (0.065±0.022). A possible synergistic effect of both extracts was observed in Group V with the lowest MDA level (Figure 2).

Catalase Level

Catalase is an important cellular antioxidant enzyme that defends against oxidative stress found in the peroxisomes of most aerobic cells. It serves to protect the cell from toxic effects of high concentrations of hydrogen peroxide by catalysing its decomposition into molecular oxygen and water.[28] Thus, estimation of catalase activity is a good indicator of oxidative stress in experimental animals. In this study, a reduction in catalase activity was observed in the MSG-treated group as against the control which is not significant however. This may be due to the inhibition of the activity of this enzyme as a result of the presence of high-level of reactive oxygen species and other toxic metabolites. Green tea was observed to significantly improve the catalase activity. Similar effect was observed in streptozotoc in induced oxidative stress.[29] Moringa improved the activity of the enzyme though not significant. This is contrary to the report by Sharida et al[26] in which the increase in catalase activity caused by moringa extract was significant (p<0.05). These suggest that the extracts have good antioxidant properties with green tea probably stimulating more production of catalase enzyme (Figure 3).

Figure 3. *Catalase Activity of Experimental Rats*



Group I=Control, Group II=MSG, Group III=MSG+MO, Group IV=MSG+GT, Group V=MSG+MO+GT

Lipid Profile Parameters

As shown in Table 4, there is no significant difference (p<0.05) in the total cholesterol (TC) of all the groups after the treatment. There was a significant (p<0.05) decrease in high density lipoprotein (HDL) of MSG-treated group (32 mg/dL) as compared to control (63 mg/dL). Triglyceride (TG) and very low density lipoprotein (VLDL) were found to be comaparble in both Control (89.5 and 17.9 mg/dL) and MSG-treated Group II (89.5 and 17.9). The low density lipoprotein (LDL) in Control (64 mg/dL) is lower compare to 71.5 mg/dL in MSG-treated. This suggests that MSG could be a risk factor in cardiovascular disease (CVD)

as it raised the level of LDL (bad cholesterol) and decreased HDL (good cholesterol).

The treatment with leaf extract of MO, GT and their mixture showed an increase in TC in treated group III-V (137.4-142.9 mg/dL) as compared to untreated group (121.4 mg/dL). There was also increment in HDL of extract treated groups: III-V, (66.5-73 mg/dL) compared to untreated Group II (32 mg/dL). LDL (59.5-60 mg/dL), TG (57-67 mg/dL) and VLDL (11.4-13.4 mg/dL) all showed a similar decrease pattern in extract treated Group III-V compared to untreated Group which have 71.5, 89.5 and 17.9 mg/dL for LDL, TG and VLDL respectively.

This suggest that leaf extracts have potentials to lower the risk developing of CVD as it lowered the LDL and TG/VLDL and increased HDL.

This study confirmed GT anti-lipidermic property.[30] It appears to be compare to MO and their combination because it was able to raise the HDL level highest (73 mg/dL). MO shows the least tendency in treatment of CVD though its extract has been reported to have significant cholesterol lowering action (Figure 4).[31,32]

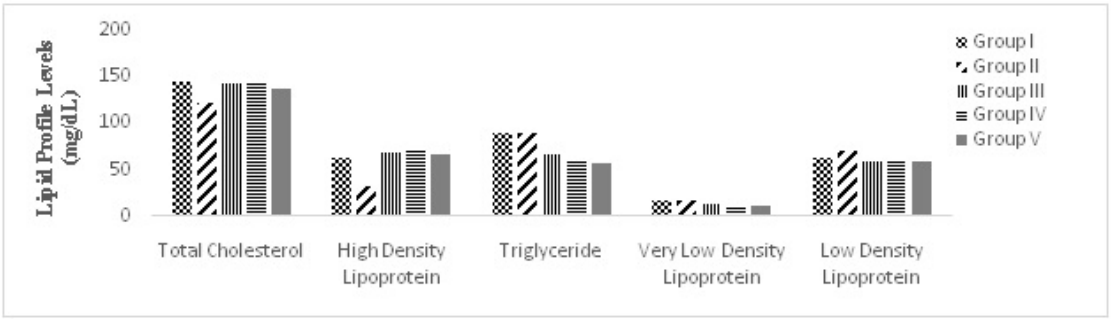
Weight Change

Several reports on potential link between monosodium glutamate and body weight have been conflicting. Several studies[33-35] reported a significantly reduced body weights at 40 days after MSG administration in rats, while Tawfik MS et al[36] and Bhattacharya S[37] reported weight gain.

In this study, there was significant ($p<0.05$) weight gain in the untreated Group II (50.38±4.79 g) compared to the control, Group I (22.85±8.15) this marked gain in weight observed may be attributed to the monosodium glutamate administered which could induce an increase in energy intake.[38] MSG has been reported to induce obesity which is characterised by a marked gain in weight.[39,40]

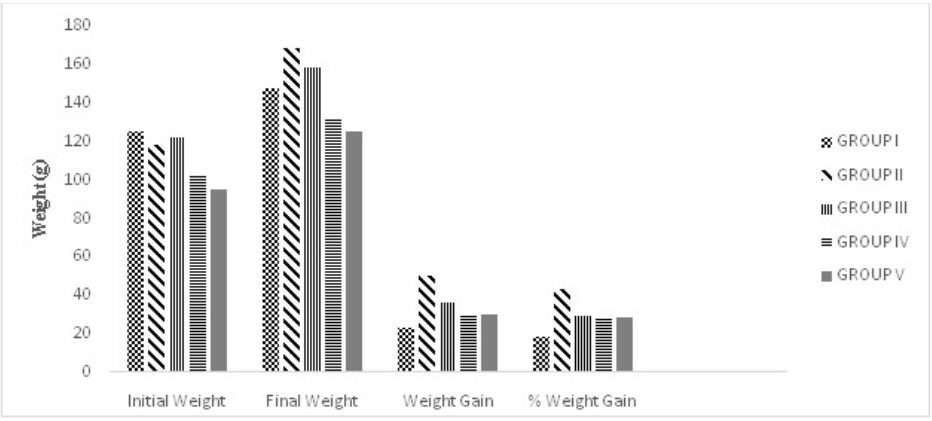
Weight gain in Group II is comparable to the moringa treated group (III=36.53±11.44 g) with the green tea extract (Group IV=28.98±10.97 g) and its combination with moringa (Group V=20.12±3.01) appears to modulate the effect of the increased weight caused by MSG. All the treatments showed promise in modulating the gain in weight though to a lesser extent by moringa extract. Green tea particularly has been reported to cause a significant decrease in body weight (Figure 5).[40,41]

Figure 4. *Comparative Lipid Profile in the Groups*



Group I=Control, Group II=MSG, Group III=MSG+MO, Group IV=MSG+GT, Group V=MSG+MO+GT

Figure 5. *Effects of the MSG Administration and Extracts on Weight Change*



Group I=Control, Group II=MSG, Group III=MSG+MO, Group IV=MSG+GT, Group V=MSG+MO+GT

Results of the histology revealed that MSG induced damage to the liver, brain and kidney (Plates: 2-5, 7-10 and 11-15). This MSG-induced organ damage is consistent with earlier studies reported by.[24,36] In an earlier study, it was observed that infant mice, on account of poorly developed blood brain barrier, showed neurological lesion even when MSG was given in lower dose.[42]

From the results, tissue sections of the normal control rats (Plates 1, 6 and11) showed normal histological structures of the liver, brain and kidney. The effects observed in both the liver (Plate 2-5) and kidneys (12-15) of treated rats could have occurred because these organs are involved in the metabolism of glutamate. The result from this study showed that MSG induced marked histopathological alterations in the liver, brain and kidney tissues of MSG-treated rats. Presences of necrotic cells, eroded tubules, and inflammatory cells are all evidences of histological damage as a result of MSG administration.

The liver of the untreated oxidatively stressed group II (Plate 2) shows high presence of necrotic cells (N) and inflammatory cells (I) within the architecture of the tissue. This MSG induced liver injury corroborates earlier studies[43,44] which reported MSG induced liver injury.

Histological results of the effects of MSG on the brain tissue (Plates 6-10) showed presence of brain necrosis due to consumption of MSG. The brain section of the untreated group II (Plate 4.7) shows presence of necrotic foci (N) and constricted glial cells (G). A report on histological and biochemical effects of monosodium glutamate on the frontal lobe of adult Wister rats45 corroborated this brain necrotic property of MSG, another report46 also posted similar results.

The kidney damage (Plates 12-15) was characterized with increased tubular casts (C) and eroded tubules (E). Presence of inflammatory cells (I) were also seen. Similar studies showed that MSG could cause kidney damage.[11,47-52] In the present study, many renal tubules of the rat kidneys showed marked degenerative lesions under the effect of MSG. This is expected since the renal tubules are particularly sensitive to toxic assault. Renal tubules have high oxygen consumption and vulnerable enzyme systems, and have complicated transport mechanisms that may easily be damaged by such toxins. Also, the tubules come in contact with toxic chemicals during their excretion and elimination by the kidney.[53]

In a study on the effects of methanolic extract of MO roots on the histology of kidney and liver of guinea pigs concluded that methanolic extracts of MO roots was found to distort the histo-architecture of both liver and kidney of guinea pigs.[54] These effects seem to be time and dose-dependent.

As the rats were treated with plant extracts, results showed that the damaged brain tissues were reversed and tend to normalize. The brain histology of the treated Groups III-V (plates 8-10) showed fewer damaged glial cells (G and D). The Group V treated with mixture of both extracts had the best recovery rate and indicate a better synergistic effect of both plants. This is the first reported study on the effect of MO, GT or the synergistic effect of both plants on the brain histology of MSG induced oxidative stressed rats.

It was observed that as the MSG-induced oxidative stressed groups (III-V) were treated with plant extracts; the damaged tissues architecture of both kidney and liver were restored and tends to normalise. Histology of the treated rat groups showed reduced levels of tubular casts, eroded tubules and fewer inflammatory cells. The level of necrotic cells was also reduced. When compared among the groups, it was seen that both plants cannot be easily differentiated in terms of how well the tissues were repaired. The two plants could be said to have exerted the same level of healing property. The synergistic effect of both plant extracts seems to have a better restorative result showing very fewer necrotic cells and inflammatory cells on the liver of rats treated with mixture of plants extracts. Also, the kidney of rats treated with combination of MO and GT extracts seemed to have better recovery when compared with rats treated with separate plant extract.

A study showed that MO extract accelerated recovery of hepatic cells after intoxicated with 3 g acetaminophen/kg body weight.[26] It was revealed that animals pre-treated with MO were able to prevent further damage by acetaminophen intoxication.[55] A study on the therapeutic potential of MO extracts against acetaminophen-induced hepatotoxicity in rats corroborated the ability of MO to reverse histological damage caused by acetaminophen-induced hepatotoxicity.[56]

Studies by Gad SB et al,[57] Othman M[58] and El-Fattah LI[59] all gave credence to the tissue repairing property of green tea as well.

CONCLUSION

This study provided a pharmacological insight for the potential use of GT, MO extracts and their combination in alleviating common medical conditions through the antioxidant properties and raises the possibility of its potential clinical usefulness. However, further studies into its pharmocotoxicity would be necessary before clinical recommendations could be made.

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