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Comparative Study on the *in vivo* antioxidant properties of *Moringa oleifera* and *Camellia sinensis* on MSG-induced oxidative-stressed rats

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Abstract
Introduction: The production of reactive oxygen species (ROS) is usually implicated in oxidative stress which is widely reported to be induced by monosodium glutamate (MSG), a known flavour enhancer commonly used as food seasoning. Oxidative stress is known to play key role in the development of various disease conditions. The *in vivo* antioxidant properties of both *Moringa oleifera* and *Camellia sinensis* were both investigated in MSG- induced oxidatively stressed rats.
Methods: *Moringa oleifera* leaves were obtained from the local market while popular branded *Camellia sinensis* was purchased from a supermarket in Enugu, Nigeria. The dried plant materials were powdered using a clean electric blender. Hundred grams (100 g) of dried, ground sample of both samples were extracted using the maceration method. The extracts were evaporated to dryness using a rotary evaporator and stored at 4°C until use. Antioxidant properties of both plant samples were also determined. Twenty male wistar rats were divided into five groups of four rats each. The rats were administered with 0.6mg/kg body weight (b.w) dose of MSG solution for 14 days to induce oxidative stress, the control group was given distilled water. Subsequent treatment with MO, GT and combination of both extracts for a period of 28 days was carried out. One group was left untreated. The antioxidant levels and lipid profile of the rats were investigated.
Results: The antioxidant properties of the sample extracts showed the presence of flavonoid (135.14 ± 5.20 and 208.24 ± 14.38), and phenolics (62.85 ± 1.70 and 91.68 ± 0.22) for both *Moringa oleifera* and *Camellia sinensis* respectively. The plant samples both showed *in vivo* and *in vitro* 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 reversed the histopathological damage that occurred in MSG-induced oxidative stressed rats.
Conclusion: This study confirmed the usefulness of the medicinal plants: *Camellia sinensis*, *Moringa oleifera* and their combination in alleviating common medical conditions through the antioxidant properties.

Keywords: *Moringa oleifera*, *Camellia sinensis*, *in vivo*, monosodium glutamate oxidative stress, antioxidants

Introduction

The significance of medicinal plants to the human race cannot be overemphasized. Many of the modern medicines like aspirin are produced indirectly from medicinal plants [1]. Hence, plants are used directly as medicines by a majority of culture throughout the world and Nigeria is not left out in the use of medicinal plants for the treatment of various ailments. The medicinal effects of plants are due to metabolites especially secondary compounds produced by plant species and these plant metabolites include primary and secondary metabolites. The primary metabolites are organic compounds produced in the plant kingdom, they have metabolic functions essential for plant growth and development, and they are produced in every plant [2]. The primary metabolites in essence include carbohydrates, amino acids, nucleotides, fatty acids, steroids and lipids [3].

The secondary metabolites on the other hand are also organic compounds produced in plant kingdom and they do not have apparent functions involved in plant growth and development, they are produced in different plant families, in specific groups of plant families or in specific tissues, cells or developmental stages throughout plant development. The secondary metabolites include terpenoids, nitrogen metabolite (including non-protein amino acids, amines, cyanogenic glycosides, glucosinolates, and alkaloids) and phenolics [3].

The need for the use of plants to treat human disease could be attributed to increase in human population, 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 (MO)} and *Camellia sinensis* {Green tea (GT)} are two of such medicinal plants gaining popularities for their reported health benefits in recent years [4, 5].

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 tropic and subtropical countries where it is mostly cultivated [6]. The medicinal properties of the plant's edible parts have been recognized by both the Ayurvedic and Unani systems of medicine in India [6]. Several Reports showed that moringa presented in-vitro

oxidative properties [7] showed the strong *in vitro* antioxidant properties of the methanolic extract of Moringa edible parts. This antioxidant activity of Moringa extracts is due to the presence of various bioactive compounds such as chlorogenic acid, rutin, quercetin and glucoside. Also, the extract of Moringa leaves and other parts have been shown to have potent antioxidant action *in vivo* [7, 8, 9].

Green tea has been associated with lowering the risk of cancer, lowering the risk of coronary heart disease and improvement of oral health [10]. It has been found to have antimicrobial health benefits and antioxidant properties [11]. There are also suggestions that tea extracts offer protection against bone loss [10], body weight control, anti-hypertensive properties, solar ultraviolet protection, neuroprotective properties and anti-fibrotic properties [11]. Tea 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^-, H_2O_2, $^{\cdot}OH$) 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, immune suppression and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease [13, 14].

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 [15].

Monosodium glutamate (MSG) popularly known as AJI-NOMOTO is the sodium salt of glutamic acid [16]. MSG contains 78% of glutamic acid, 22% of sodium and water [17]. As food additive, MSG is described and listed on food labels as a “flavouring” or “hydrolysed vegetable protein”.

Chronic administration of MSG (4mg/kg body weight and above) as well as sub-chronic administration (0.6-1.6mg/kg body weight) has been reported to induce oxidative stress in experimental rats [18, 19].

Materials and method

Sample Collection and Preparation

Moringa oleifera (MO) leaves were obtained from Ogbete Main Market in Enugu while popular 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 Conservation and Development Program (BDCP), Nsukka. The dried plant materials were powdered using an electrical blender. The extraction was done at room temperature.

Hundred grams (100g) of dried, ground sample materials were soaked in methanol (1L of 80%) for 5days 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 till use [20].

Animal Experiment

Twenty male Wistar strain rats with mean weight of 114.27±14.59 g were used for the experiment. The 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, they 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 0.6mg/kg body weight for 14 days. The MSG administration was discontinued after 14 days.

Group II: no extract was administered.

Group III: The leaf extract of MO was administered orally at the dose of 250mg/ kg body weight for 28 days.

Group IV: The leaf extract of GT was administered orally at the dose of 250mg /kg body weight for 28 days.

Group V: The leaf extract of MO and GT was administered orally at the dose of 250mg /kg body weight for 28 days. The dose was formulated by equal weight of MO and GT in ratio 1:1.

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.

At the end of 28 days treatment, the animals were sacrificed by euthanizing them (rendering unconscious, with chloroform) and dissected. The liver was harvested for further studies.

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 malondialdehyde (MDA) level [21].

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 nitrite concentration was determined by extrapolation from sodium nitrite standard graph [22].

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.* [23]
Catalase activity (C.A) was assayed calorimetrically at 620 nm and expressed as Unit/g tissue as described by Sinha [24].

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 [25].
Triglycerides (TG), was determined by enzymatic hydrolysis with subsequent determination of the liberated glycerol by Boeringer Mannheim GPO-PAP Kit [26] and high-density lipoprotein (HDL) was determined by Friedewald *et al.*, method [27]. Low-density-lipoprotein (LDL) was obtained by calculation: LDL = TC - (VLDL + HDL); where VLDL = TG/5 [27].

Results

Table 1: Result of Nitric Oxide Level between the Groups

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

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: Effect of Moringa leaf and Green Tea Extracts on Lipid Peroxidation

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

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: 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 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

Table 4: Effect of Moringa and Green Tea Extracts and MSG on Lipid Profile

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.

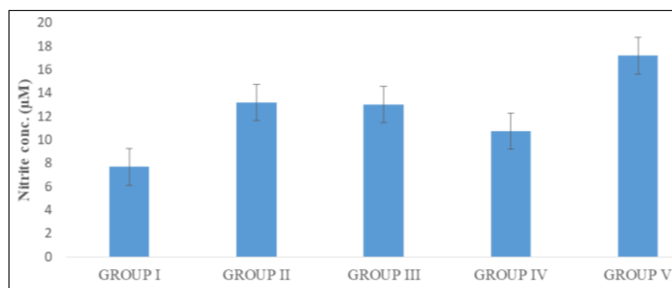
Table 5: Effect of MSG, Moringa and Green Tea Extracts on Body Weight (g)

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

Means 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

Discussion

Table1presents the result of 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).
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.



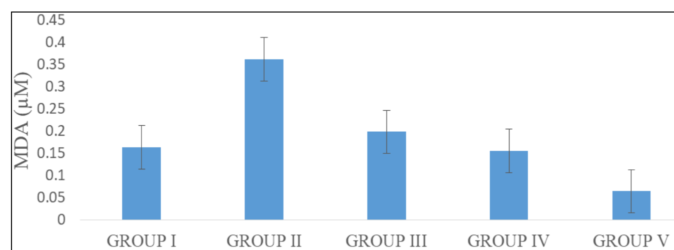
GROUP I=Control, GROUP II=MSG, GROUP III=MSG+MO, GROUP IV=MSG+GT, GROUP V=MSG+MO+GT

Fig 1: Levels of NO in treated rats

The result of the lipid peroxidation assay (Tab. 2) showed that Group II (0.362 ± 0.115μM) particularly demonstrated the highest 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.

MSG 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 [28, 29].

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 test groups (Figure 2). This is similar to the findings of Farombi and Onyema, [29] and Egbuonu *et al.*, [30] 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 [31] while green tea was also reported to reduce MDA level in stress induced oxidative damage [32]. 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.

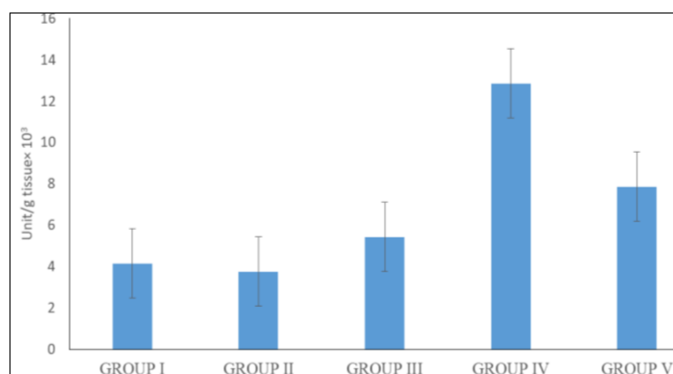


Group I=Control, GROUP II=MSG, GROUP III=MSG+MO, GROUP IV=MSG+GT, GROUP V=MSG+MO+GT

Fig 2: Lipid Peroxidation Levels of Treated Rats

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 × 10^3 unit/g tissue), followed by Group V (7.86± 4.82 × 10^3 unit/g tissue) treated with mixture of MO and GT. The least activity was shown in the untreated Group II (3.75 ± 1.28 × 10^3 unit/g tissue).

Catalase is an important cellular antioxidant enzyme found in the peroxisomes of most aerobic cells and defend the cells against oxidative stress. It serves to protect the cell from toxic effects of high concentrations of hydrogen peroxide by catalysing its decomposition into molecular oxygen and water [33]. 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, however, it is not significant. 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 streptozotocin induced oxidative stress [34]. Moringa improved the activity of the enzyme, although, not significant. This is contrary to the report by Sharida *et al.,* [31] 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.



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

Fig 3: Catalase Activity of Experimental Rats

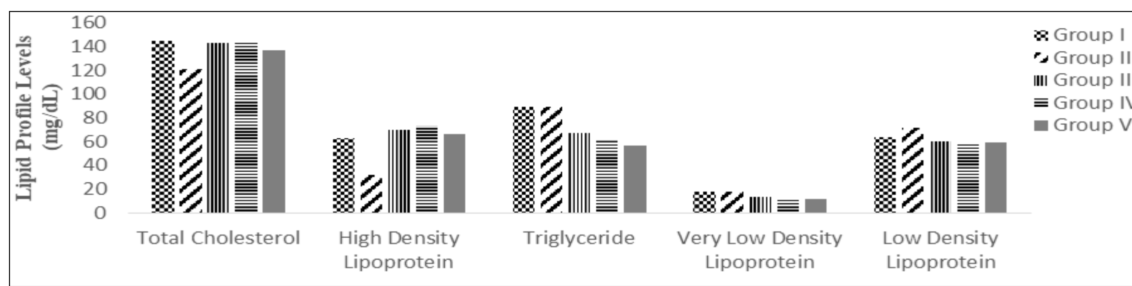
A significant decrease was observed in both HDL level in untreated Group II and TG level in Group V. TG was markedly increased in Group I and II (Table 4). Though there was an increase in LDL level of untreated Group II, it was not significant from other groups.

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 comparable 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 [35]. It appears to be comparable 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 [36, 37].



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

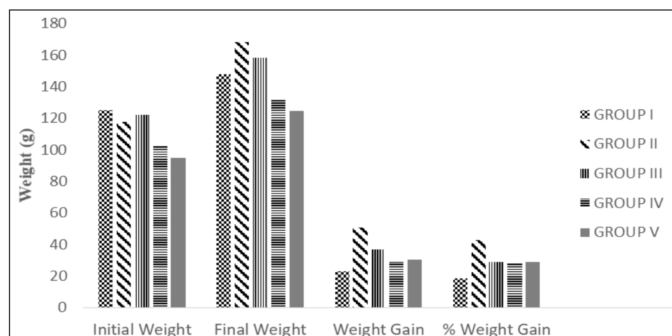
Fig 4: Comparative Lipid Profile in the Groups

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).

Several reports on potential link between monosodium glutamate and body weight have been conflicting. Various studies [38, 39, 40] reported a significantly reduced body weights at 40 days after MSG administration in rats, while Tawfik and Al-Bashir [41, 42], 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 [43]. MSG has been reported to induce obesity which is characterised by a marked gain in weight [44, 45].

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 attenuate the effect of the increased weight caused by MSG (Fig. 5). All the treatments showed promise in modulating the gain in weight though to a lesser extent by moringa extract. Green tea has been reported to cause a significant decrease in body weight [45, 46].



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

Fig 5: Effects of the MSG Administration and Extracts on Weight Change

Conclusion

This study confirmed the usefulness of the medicinal plants: *Camellia sinensis*, *Moringa oleifera* and their combination in alleviating common medical conditions through the antioxidant properties. The production of reactive oxygen species (ROS) is usually implicated in oxidative stress which is widely reported to be induced by monosodium glutamate (MSG) a known flavour enhancer commonly used as food seasoning. Levels of natural enzyme antioxidants are usually affected in condition of oxidative stress and are thus used as its biomarkers. In this study MSG caused the induction of oxidative stress as noted in the increase in the malondialdehyde (MDA) in the lipid peroxidation assay and decrease in catalase activity; these markers were duly attenuated by the sample extracts. A marked increase in weight also signifies a deleterious effect of MSG which wasattenuated by the methanolic extracts of green tea, moringa and their combination.

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