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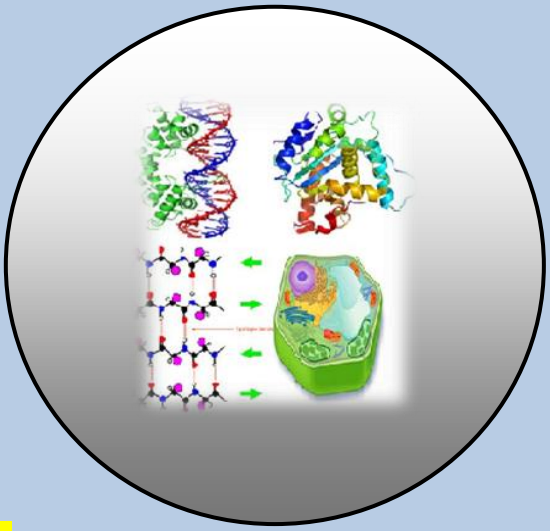

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Pre-Treatment with *Phyllanthus amarus* Stalls Dyslipidemia and Uterine Toxicity in Monosodium Glutamate Administered Rats

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ABSTRACT

Studies has implicated intake of monosodium glutamate (MSG) in fibroid but reports on correlation between dyslipidemia and fibroid induced with intake of MSG are inconsistent. Thisstudy investigates the risk of dyslipidemia in MSG intake and the therapeutic effect of administration of Phyllanthus amarus. Forty adult female wistar rats randomize to 5 groups of 8 rats each were used. MSG was administered continuously to groups 2-5 at 200 mg/Kg body weight for 28 days. After 28 days,blood was withdrawn from rats in groups 1, 2 and 3after anaesthetizing with diethylether. They were then sacrificed and the uterus, kidney and liver excised. MSG administrations were then discontinued in the remaining groups of rats and P. amarus leaves extract was administered daily to group 4. Treatments were continued for the next 28 days after which the rats were sacrificed. Total cholesterol (TC), Low density lipoprotein (LDL-c), High density lipoprotein (HDL-c), triglyceride, arherogenic (AI) and coronary risk index (CRI), glucose and total protein were then determined in the serum. The organs were also weighed and the uterus was used for histopathology studies. Results indicate that serum cholesterol, LDL-c, triglycerides, API, CRI and glucose were raised with MSG administration whereas; HDL-C was reduced. Co-administration of P. amarus with MSG prevented these alterations. Withdrawal of MSG without treatment and administration of P. amarus after MSG administration also reduced the cholesterol, triglyceride, LDL, AI and CRI. Although the final body weights and uterine weight ratio were increased with MSG administration and co administration of P. amarus with MSG prevented these alterations, no observable difference was observed in the histology of the uterine tissues.

The study suggests that MSG administration is positively correlated with dyslipidemia and uterine toxicity. Results also show that co-administration of P. amarus with MSG may offer protection against these metabolic changes.
Key words: Dyslipidemia, Phyllantus amarus, Monosodium Glutamate, Fibroid and Smooth Muscle Cell.

INTRODUCTION

Most food addictives acts either as preservatives or enhancer of palatability. One of such food addictive is MSG and is sold in most open market stalls globally. Monosodium-L-glutamate (MSG) is a sodium salt of glutamic acid, which is used worldwide as a flavor enhancer. L-gluatmic acid an amino acid component of monosodium glutamate has a long history of use in foods as a flavor enhancer (FAO/WHO, 1974, Obaseki-Ebor, *etal*., 2003). The safety of MSG's usage has generated much controversy locally and globally. In Nigeria, most communities and individuals often use MSG as a bleaching agent for the removal of stains from clothes (Olugbenga et al, 2014). There is a growing apprehension that its bleaching properties could be harmful or injurious to the body or worse still inducing terminal disease in consumers when ingested as a flavor enhancer in food.

Of particular interest to this study are reports linking MSG intake to smooth muscle cell proliferations. Diniz*et al*., (2005) reported that MSG caused infertility in male wistar rats by inducing testicular hemorrhage, degeneration and alteration of sperm cell population and morphology. Andrew Oluba *et al*., (2011) reported that high dosage of MSG was able to cause hypertrophy in the fallopian tube. Another study by Obochi et al (2009) reported that MSG increases level of estrogen in experimental rats. High amount of estrogen than progesterone has been reported to cause proliferation of cells of the uterus and even cause uterine cancer (Lilyan*et al*., 2012). Although studies has linked fibroid development with increase estrogen level, reports on correlation between fibroid development, alterations in lipid profile and MSG intake are rather inconsistent. Ikonomidou and Turski (1995), Eskes, (1998) and Rodriguez *et al*., (1998) reported that monosodium Glutamate (MSG) causes reduction in the secretion of growth hormones, leading to stuntedgrowth and irreversibility in obesity. These changes were essentially attributed to accumulation of excess fats in adipose tissue arising from high cholesterol levels leading to cardiovascular diseases and endocrinological disorder. Another reports by Bosetti et al (2001) and Sadlonova*et al*. (2008) noted that estrogen levels are inversely related to cholesterol levels. The reports further stated that as fibroids are estrogen-related tumors, an inverse association between hyperlipidemia and the risk for fibroids should be observed.

Herbs are effective substances of the olden ages known to be good for cure of some diseases. One of the long present and which has a long history of usage by the folk because of its rich medicinal values is *Phyllanthus amarus.* It has been reported to possess antiinflammatory, antihepatotoxic, analgesic, hypotensive, diuretic, antispasmodic, antiviral, antibacterial, anticarcinogenic and hypoglycemic properties (Hannah, 2008). *Phyllanthus amarus* is widely distributed in all tropical regions of the planet. It is a common pantropical weed that grows well in moist, shady and sunny places. In Nigeria the plant it is known as Eyin Olobe in Yoruba, Enyikwonwa and Ngwu in Ibo, Oyomokeso Amanke Edem in Efik and Geeron-Tsuntsaayee (bird's millet) in Hausa (Mortaon, 1981).

The present study is carried out to investigate whether MSG intake is able to alter serum lipid profile, disrupt uterine morphology and cause tissue toxicity to some selected organs. Furthermore, the study focused on the assessment of how administration of *P. amarus* could impact upon these MSG induced metabolic alterations in animals continuously treated with exposed daily to the extract.

MATERIAL AND METHODS

Chemical and drug

Synthetic monosodium glutamate (MSG) was obtained from Sigma Chemical Company Japan. A solution of MSG was prepared by dissolving 10 grams of MSG in 500mils distilled water.

Plant collection, authentication and Preparation of extract

Fresh leaves of *phyllanthus amarus* were obtained from a local garden in Ikenne town, Ogun state, Nigeria in June 2014. The plant was identified at the herbarium of Plant Biology Department, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria where a voucher number (O14/P1124) was assigned. Thereafter, a voucher sample was deposited at the herbarium. The leaves were shade dried to constant weight for 2 weeks. The dried samples were then pulverized with an electric blender, weighed and kept air-tight prior to extraction. For the extraction, 200 g of the powdered samples was soaked in 2litres of absolute ethanol for 72 hours. The mixture obtained was then filtered (with Whatman No. 1 filter paper). The filtrate was concentrated using a rotary evaporator and dried by evaporation. The dried product was weighed to give 22.1 g corresponding to the yield of 4.3%. The dried sample was reconstituted in water at the required dosage for administration.

Experimental design

Forty (40) adult female Wister rats weighing between 160 and 200 g were obtained from University of Ibadan The rats were kept in metallic cages in a well ventilated room maintained at a temperature of 25 ± 2◦C with a 12-h light–dark cycle for one week toacclimatize. They were allowed free access to food and water *adlibitum.* The protocol of the experiment was in conformity with the National Research Council (1999) and was approved by the Animal and Human Health Ethics Committee, College of Health Sciences, Olabisi Onabanbjo University, Ago-Iwoye. The animals were thereafter randomized into five groups of 8 rats each. Group 1 (normal control) rats received only distilled water; Group 2 (Test control) rats were administered with monosodium glutamate (200 mg/kg body weight) for 28 days; group 3 (Test group 1*)* rats were simultaneously treated with MSG (200 mg/kg bodyweight) and *Phyllanthus amarus* extract (100 mg/kg body weight) for 28 days; Group 4 (Test group II*)* rats were first given MSG (200 mg/kg body weight) for 28 days and then thereafter co-administered MSG with *Phyllanthus amarus* extracts for the next 28days; Group 5 (Self recovery) rats were administered MSG (200mg/kg body weight) for 28days. Thereafter MSG was withdrawn for the next 28days. All administrations were done orally as a single dose daily using oral in tubator. The rats were weighed at the commencement and at the end of the study. After the last weights of the rats were taken, the feed was withdrawn overnight. Each rat was then anaesthetized in a closed chamber containing cotton wool soaked with diethyl ether. Blood was then withdrawn into plain bottles by cardiac puncture and spinned with a centrifuge. The resulting serum was carefully aspirated with Pasteur pipette into sample bottles and used for biochemical analysis.

The rats were then sacrificed and organs of interest (uterus, kidney and liver) excised.

Histopathology study

The dissected uterus was immersion-fixed in bouins fluid over night at room temperature after which the tissues were transferred to ascending grades of alcohol for dehydration. The tissues were cleared with two changes of xylene for one and half hours each, transferred into two changes of molten paraffin wax I and II for one and half hour each and wax- III for overnight in an oven at 65^0C for infiltration.

The tissue was then processed according to the method previously described (Solomon *et al*., 2010). The tissue block was serially sectioned at 6µm thickness using microtome. Strips of sections were gently lowered into the surface of a warm water bath at 40°C. The floated sections were mounted on egg albumin coated microscopic slides, and put in an oven maintained at 60°C for 30 minutes to fix the tissue firmly on the slide. The slides were dewaxed with two changes of xylene and hydrated with decreasing alcohol concentration and then immersed in water for 5 minutes. The sectioned tissues were then stained regressively with Ehrlich's hematoxylin and counter stained with Eosin. After staining with eosin, tissues were washed in tap water and dehydrated by rinsing in increasing concentration of alcohol and then xylene-I. They were then placed in xylene-II until mounting. Finally, a drop of mountant DPX (A mixture of Distyrene, a Plasticizer, and Xylene) was placed on top of the sections and the cover slip was applied.

Biochemical assay

Initial and the final body weight of all the rats were measured using a Mettler weighing balance (Mettler Toledo Type BD6000, Greifensee, Switzerland). Serum was prepared from the collected blood samples by centrifugation and used for analysis. Fasting blood glucose was measured according to method adopted previously by Miwa *et al*. [1972] using a glucose kit (enzymatic method) (Sigma). Serum protein determination was by the method of Lowry et al [1951]. Triglyceride was determined using enzymatic colorimetric kits [Wahlefeld, 1974]. Both Total cholesterol and HDL-C were estimated according to the method described by Stein [1986]. From the results, LDL cholesterol was calculated according to Friedewal *et al*. [1972]. According to the method, LDL can be calculated as follows:

LDL = Total cholesterol-HDL-TG/5

Atherogenic index was calculated from serum HDL and cholesterol levels using the equation previously reported by Gillies *et al*. [1986].

Atherogenic index =

$$\frac{\text{serum cholesterol level- serum HDL level}}{\text{Serum HDL level}}$$

Coronary risk index was obtained by the method of Alladi and Khada [1989].

$$\text{Coronary risk index} = \frac{\text{Total Cholesterol}}{\text{HDL-Chollesterol}}$$

Statistics

All data were expressed as mean ±SEM. One-way analysis of variance (ANOVA) was used to analyze the data. Comparisons between the groups were made at a two-sided alpha level of 0.05. $p < 0.05$ was considered statistically significant.

RESULTS

Table 1.Effect of treatment on serum lipid profile.

Group	Treatment	Total Cholesterol (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	Triglycerides (mg/dL)
(Normal control)	Distilled water	87.03 ± 5.89[a]	45.91 ± 3.63[a]	20.63 ± 4.79[a]	102.42 ± 2.11[a]
(Test control)	MSG (200 mg/Kg)	114.41 ± 9.95[b]	24.59 ± 5.57[b]	54.98±7.39[b]	174.18 ± 16.19[b]
(Test group 1)	Co administration of MSG 200 mg/Kg) + *P. amarus (100mg/Kg)*	94.03 ± 3.01[a]	40.44± 3.08[a]	27.79 ± 3.80[a]	130.32 ± 9.84[a,c]
(Test group 2)	MSG 200 mg/Kg) followed by *P. amarus (100mg/Kg)*	113.87± 8.70[b]	35.45 ± 5.59[ab]	54.09±11.00[b]	151.63 ± 15.31[b]
(Self recovery)	MSG (200 mg/Kg) followed by self- recovery process.	99.74 ± 1.38[a,b]	39.50 ± 2.98[ab]	44.14±3.92[b]	130.51 ± 9.78[bc]

Note

- Result is expressed as Mean ± Standard Error of mean
- n (number of samples used) = 8
- Mean with different superscript indicates p< 0.05 at 95% confidence using one way analysis of variance (ANOVA)9

Table 2.Effect of treatment on atherogenic risk index (AI) and coronary risk index (CRI).

Group	Treatment	AI	CRI
1 (Normal control)	Distilled water	0.90±0.01[a]	1.90± 0.02[a]
2 (Test control)	MSG (200 mg/Kg)	3.65±0.05[b]	4.65±0.02[,b]
3 (Test group 1)	Co administration of MSG 200 mg/Kg) + *P. amarus (100mg/Kg)*	1.45 ±0.03[c]	2.33 ± 0.02[C]
4 (Test group 2)	MSG 200 mg/Kg) followed by *P. amarus (100mg/Kg)*	3.77±0.03[d]	4.47 ± 0.03[b]
5 (Self recovery)	MSG (200 mg/Kg) followed by self-recovery process.	1.60±0.02[d]	1.60 ± 0.03[a]

Note

Result is expressed as Mean ± Standard Error of mean

N (number of samples used) = 8

Mean with different superscript indicates p < 0.05 at 95% confidence using one way analysis of variance (ANOVA).

Table 3.Effect of treatment on fasting blood glucose and total protein concentration.

Group	Treatment	FBS (mg/dL)	Total Protein (mg/Dl
1 (Normal control)	Distilled water	82.00±0.93^{a}	8.89 ± 0.49^{a}
2 (Test control)	MSG (200 mg/Kg)	112.00±3.86^{b}	9.55 ± 2.79^{a}
3 (Test group 1)	Co administration of MSG 200 mg/Kg) + *P. amarus (100mg/Kg)*	86.50 ± 0.81^{a}	9.03 ± 0.86^{a}
4 (Test group 2)	MSG 200 mg/Kg) followed by *P. amarus (100mg/Kg)*	97.67 ± 1.65^{c}	9.46 ± 3.00^{a}
5 (Self recovery group)	MSG (200 mg/Kg) followed by self- recovery process.	90.00 ± 1.29^{c}	9.69 ± 0.94^{a}

Note

- Result is expressed as Mean ± Standard Error of mean
- n (number of samples used) = 8
- Mean with different superscript indicates $p < 0.05$ at 95% confidence using one way analysis of variance (ANOVA)

Table4.Effect of treatment on final body and organ weight.

Groups	Final body Weight (g)	Organ Weight			Organ to body weight ratio (%)		
		Uterus	Kidney	Liver	Uterus	Kidney	Liver
1	**165.83 ± 2.71^{a}**	**0.332 ± 0.023^{a}**	**1.221± 0.050^{a}**	**4.812± 0.35^{a}**	**0.200± 0.003^{a}**	**0.736± 0.022^{a}**	**2.902± 0.76^{a}**
2	**196.67 ±8.82^{b}**	**0.427 ± 0.035^{b}**	**1.321 ± 0.024^{b}**	**4.897±0.26^{a}**	**0.267± 0.052^{b}**	**0.672± 0.004^{a}**	**2.490± 0.14^{a}**
3	**165.00 ± 4.28^{a}**	**0.344 ± 0.046^{acd}**	**1.238 ± 0.072^{a}**	**4.472± 0.27^{a}**	**0.187± 0.004^{a}**	**0.635± 0.021^{a}**	**2.806± 0.35^{a}**
4	**194.17 ± 6.38^{b}**	**0.420± 0.034^{b}**	**1.411 ± 0.260b**	**4.98 ± 0.13^{a}**	**0.216± 0.002^{a}**	**0.727± 0.058^{a}**	**2.565± 0.10^{a}**
5	**175.00 ± 6.19^{ab}**	**0.303±0.036^{ad}**	**1.28 ±0.065^{ab}**	**4.20 ± 0.26^{a}**	**0.173± 0.002^{a}**	**0.731± 0.013^{a}**	**2.40±0.18^{a}**

Note

- Result is expressed as Mean ± Standard Error of mean
- n (number of samples used) = 8
- Mean with different superscript indicates $p < 0.05$ at 95% confidence using one way analysis of variance (ANOVA).

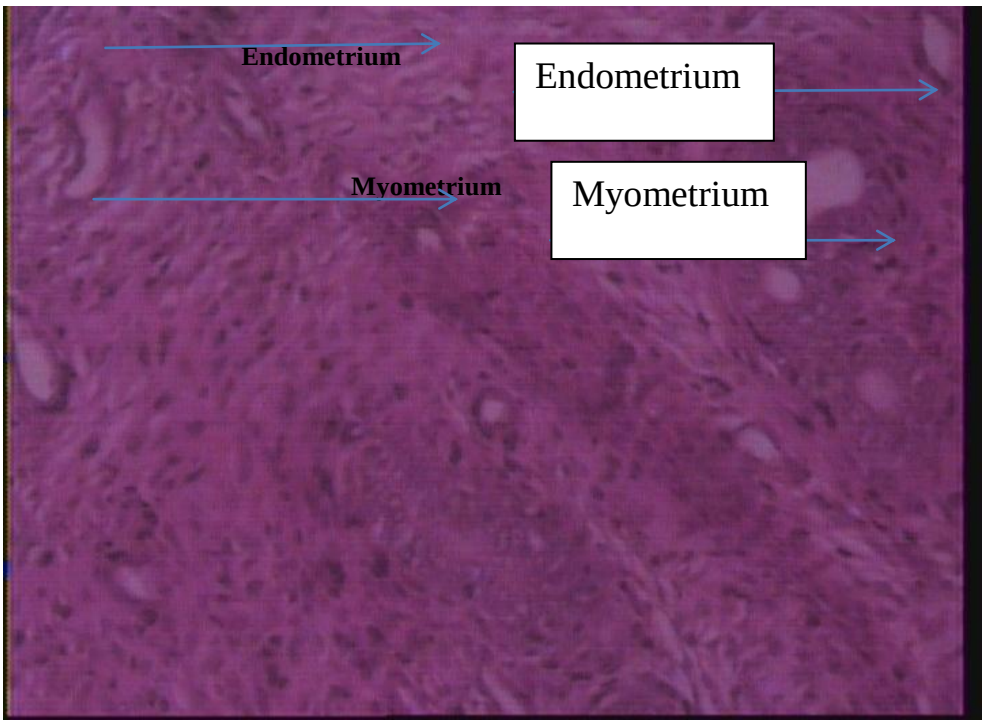

Figure 1. Photomicrographs of hematoxylin and eosin stained uterum cross-section of control ratsshowing a normal histological picture. Magnification x 400.

Figure 2. Photomicrographs of hematoxylin and eosin stained uterum cross-section of rats administered with MSG showing a normal histological picture. Magnification x 400.

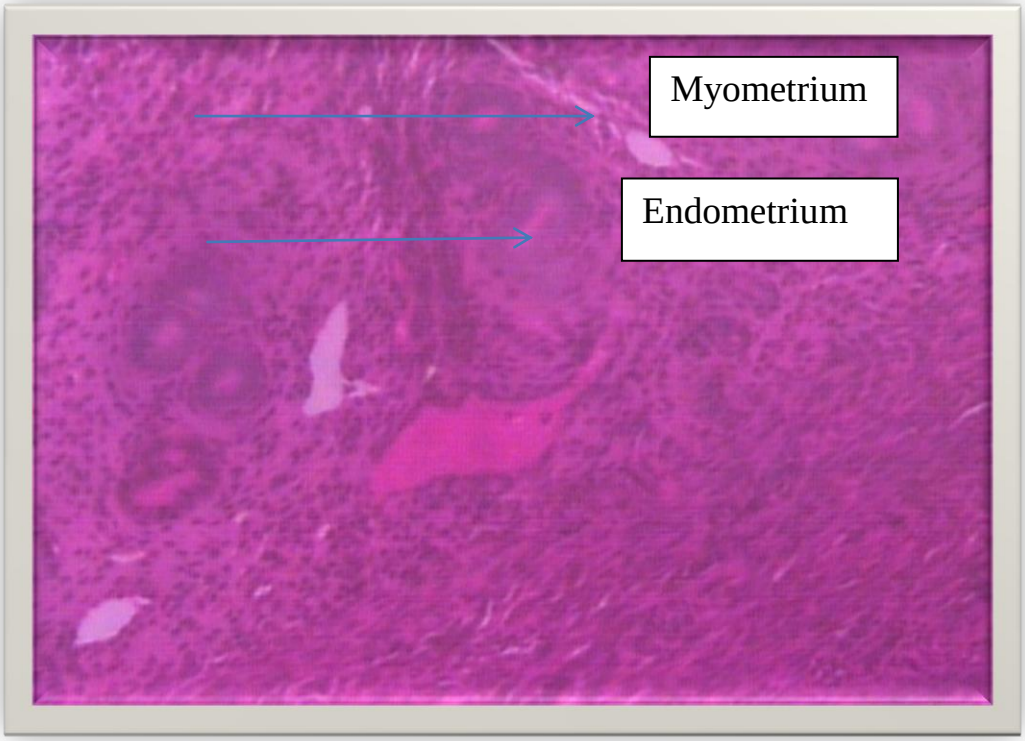

Figure 3.Photomicrographs of hematoxylin and eosin stained uterum cross-section of rats simultaneously treated with *P. amarus* and MSG showing a normal histological picture of a uterine tissue. Magnification x 400.

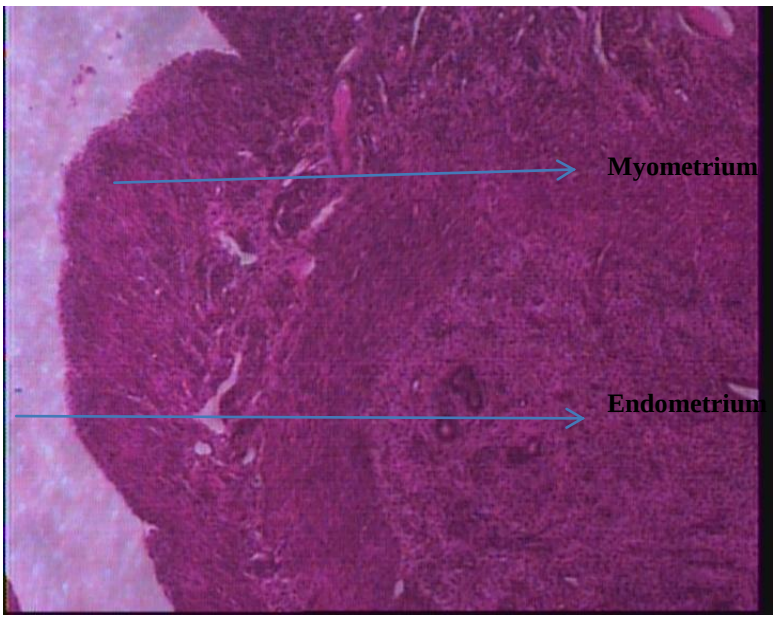

Figure 4. Photomicrographs of hematoxylin and eosin stained uterum cross-section of *P. amarus* treated rats after withdrawal of MSG (post administration) showing a normal histological picture of a uterine tissue. Magnification x 400.

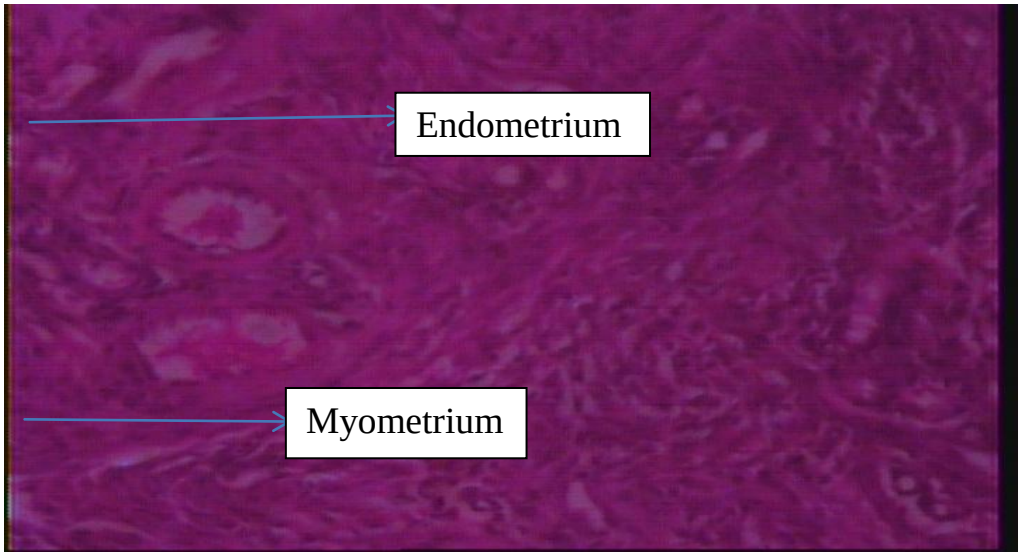

Figure 5. Photomicrographs of hematoxylin and eosin stained uterum cross-section of the MSG administered rat following self-recovery processshowing a normal histological picture of a uterine tissue. Magnification x 400.

RESULT

Table 1 shows the effect of treatment on serum lipid profile. The mean total cholesterol of the rats placed on MSG was 114.41±9.95 mg/dL and this was significantly higher than 87.03±5.89 mg/dL observed in the control subjects. Co- administration of *P. Amarus* and MSG prevented alterations in serum cholesterol level as the observed cholesterol level in group 3 rats (94.03±3.01 mg/dL) was not significantly different from the normal control group (group 1). The observed serum cholesterol level following withdrawal of MSG without treatment (99.74±1.38 mg/dL) was not significantly different from both the control group and the observed level when MSG administered rats were treated with *P.* amarus (group 4). Result of serum triglyceride was similar to that of the cholesterol. However, it was also observed that the serum triglyceride level after the withdrawal of MSG both in the rats that were treated with *P. amarus* (151.63±15.31mg/dL) and that not treated (130.51±9.78 mg/dL) though lower than that of the test control (174.18±16.19 mg/dL) group (group 2), it was however higher than that of the normal control group (102.42±2.11 mg/dL). The LDL-C level of the test control rats (54.98±7.39 mg/dL) was raised above the normal control value (20.63±4.79 mg/dL) and was not different from the value observed after MSG was withdrawn (with or without treatment). The LDL-C level observed when MSG was co administered with *P. amarus* (27.79±3.80 mg/dL) was also not different from that of the normal control group. Table 1 also indicates that rats administered with MSG (test control) showed a lowered HDL-C level compared with the normal control group (group 1). Co-administration of MSG and *P. amarus* however raised the HDL-C level to the pre-treatment level (40.44±3.08 mg/dL. The observed HDL-C value when *P. amarus* was administered after the withdrawal of MSG (35.45±5.59 mg/dL) was not different from that of the rats that were allowed to recovered without treatment (39.50±2.98 mg/dL) and was also not different from the normal control group.

Table 2 shows an increase in both atherogenic and coronary risk indices in rats placed on MSG (test control) compared with the normal control (group 1).

Both parameters were however significantly reduced when MSG and *P. amarus* were co-administered and the observed value of 1.45±0.03 (AI) and 2.33±0.02 (CRI) were not different from the normal control values of 0.90±0.01 (AI) and 1.90 ±0.02 (CRI). When MSG was withdrawn after 28 days of administration, the AI of 1.77±0.03 observed in rats treated with *P. amarus* was not different from 1.60±0.02 observed in the rats that were allowed to recover without treatment on their own. The observed AI values were however significantly higher than that of the normal control value of 0.90±0.01 and that of the test group 1 (1.45±0.03). This observation was similar to that of the coronary risk index alteration.

Table 3 is the result of treatment on fasting blood glucose (FBS) and total protein level. Administration of MSG raised the fasting blood glucose level from the normal control value of 82.00±0.93 mg/dL to 112.00±3.86 mg/dL. The FBS level of rats co-administered with MSG and *P. amarus* (86.50±0.81 mg/dL) was however not significantly different from that of the normal control value. The FBS observed when MSG was withdrawn after 28 days of administration in rats that treated with *P. amarus* (97.67±1.65 mg/dL) was not different from the value observed when the rats were allowed to recover without treatment (90.00±1.29 mg/dL). The values observed were however higher than that observed in the rats that were co administered with MSG and *P. amarus.* Table 3 also shows that no significant alterations occurred in the serum total protein ofin all the treatment groups when compared with the control and with each other.

Result of the effect of treatment on total body and organ weight ratio is presented in Table 4. Although no significant variation was observed in the initial bodyweight of all the rats, the final body weight observed showed a significant increase in that of the rats administered with MSG (196.67±8.82 g) when compared with the normal control group (165.83±2.71 g). The final body weight of rat co-administered with MSG and *P. amarus* (165.00±4.28 g) was however not different from that of the normal control group. Although the final body weight after the withdrawal of MSG were not different between the treated and the untreated groups, the observed value of 194.17±6.38 g in the treated group was also not different from that of the test control group (group 2). This result is similar to the observed variation in the uterus and the kidney weight. No significant variation was however observed in the liver weight when compared among the groups. Result of the organ to body weight ratio indicates that no difference occurred in the values observed for all the organs studied.

Figure 1-5 is the result of the histopathology of the uterus after treatment. The figures indicate that there were no significant alterations in the architecture of the uterus when the treated groups were compared with the normal control. Both the endometrium and myometrium were normal in all the groups.

DISCUSSION

One of the major adverse effects of MSG that has generated a lot of interest is its role in the development of uterine fibroid (Faerstein *et al*. 2001, American Diabetic Association, 2004, Sadlonova *et al*. 2008).Ongoing studies are targeted at understanding the relationship between fibroid development and cardiovascular disease risk (Olugbenga et al., 2014). The present study in addition to investigating the antilypidemic action of *P. amarus* during MSG in take also tends to give provides more insight on the association between MSG intake and the risk of cardiovascular diseases.

This will help in understanding the relationship between smooth muscle cell proliferation and cardiovascular disease development (one of the world's leading causes of death). One of the major risk factors for the development of cardiovascular disease is dyslipidemia. Dyslipidemia usually involve elevated plasma levels of triglycerides, total, LDL and VLDL cholesterol and a low level of HDL cholesterol (Franz et al., 2002, Shen, 2007, Ajani *et al*., 2014,Faponle *et al*., 2015a).

Result from this study indicates that MSG administration is correlated with increase total cholesterol, tryglyceride and LDL-C and a decrease in HDL-C. This thus suggests that MSG administration may induce dyslipidemia and therefore may predispose to cardiovascular diseases. This assertion is supported by our result on athrogenic and coronary risk indices where we have observed that MSG administration caused a significant increase in atherogenic and coronary risk indices. This result agrees with previous studies as reported by Ebesunun *et al*., (2007), Basse*yet al*., (2012), Ajani*et al*, (2014). LDL particles are atherogenic because they can easily penetrate and form stronger attachments to the arterial wall, and they are more susceptible to oxidation. Because less cholesterol is carried in the core of small LDL particles, subjects with predominantly small LDL particles have higher numbers of particles at comparable LDL cholesterol levels (Robertson, 2004). Oxidized LDL is pro-atherogenic because once the particles become oxidized they acquire new properties that are recognized by the immune system as "foreign." Thus, oxidized LDL produces several abnormal biological responses, such as attracting leukocytes to the intima of the vessel, improving the ability of the leukocytes to ingest lipids and differentiate into foam cells, and stimulating the proliferation of leukocytes, endothelial cells, and smooth muscle cells (Chen *et al*., 2003) all of which are steps in the formation of atherosclerotic plaque.

A high plasma triglyceride level has also been reported as both an independent and synergistic risk factor for cardiovascular diseases (Shen, 2007, McBride, 2007) and is often associated with hypertension abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus (Franz *et al*., 2002, McBride, 2007). Our study with MSG as reported in this study also indicates that it significantly raised serum triglyceride level. A study by Bassey *et al*., (2012) reported that MSG may increase hepatic lipid catabolism via upregulation of oxidative genes. MSG was reported in his study to specifically activate genes involved in the bile acid pathway including key regulatory enzyme, cholesterol - 7 –α hydroxylase (CYP7A1).

Report from the present study also suggests that co- administration of MSG with *P. amarus* offers protection against predisposition to dyslipidemia and cardiovascular diseases.Furthermore, we also observed based on the data from our study that following withdrawal of MSG, further metabolic alterations were halted in the lipid profile of the rats.

This tends to suggest that when the offensive agents were withdrawn, the metabolic system of the subjects revert back to normal. This observation was similar to that which was also seen when the rats were placed on *P. amarus* after the withdrawal of MSG. Based on this observation, we therefore opined that the plant, though mayprovide a protective action against dyslipidemia, it however may offerno beneficial advantage when administered after dyslipidemia condition has been induced. The atherogenic and coronary risk indices of the two groups of subjects also donot differ between each other.

Previous studies have demonstrated that lowering plasma total cholesterol (TC), low density lipoprotein cholesterol (LDLC) and increasing high density lipoprotein cholesterol (HDLC) are beneficial in preventing risk of cardiovascular disease (Bordia 1981, Agbedana and Akanji, 1988, Faponle et al., 2015a,b).High HDL exerts a protective effect by enhancing reverse cholesterol transport by scavenging excess cholesterol from peripheral tissues, which it esterifies with the aid of lecithin: cholesterol acyltransferase, and delivers to the liver and steroidogenic organs for subsequent synthesis of bile acids and lipoproteins, and eventual elimination from the body (Ademuyiwa *et al*., 2005, McBride , 2007) and inhibiting the oxidation of LDL as well as the atherogenic effects of oxidized LDL by virtue of its antioxidant (McBride , 2007) and anti-inflammatory property. The result of the final body weight indicates that MSG induced significant increase in the final body weight of the rats. Organ weights are widely accepted in the evaluation of test article-associated toxicities (Black, 2002, Bucci, 2002, Wooley, 2003).In this study, no significant difference was observed in the liver and kidney weight ratio in all the treated groups. However, we observed a significant increase in the uterine weight ratio of the MSG administered rats when compared with all other groups. Wooley (2003) noted that organ weight changes are often associated with treatment related effects. It can be the most sensitive indicator of an effect of drug toxicity, as significant differences in organ weight between treated and control animals may occur in the absence of any morphological changes. Another report by Gur and Warner, (1993) noted that in addition to experimental factor, other factors that may influence animal organ weights include strain of animal, age, sex and environmental conditions. In rodents and non-rodents, normal reproductive cycling and the effects of age has been reported to cause notable inter animal variation in uterine and ovarian weights (Rani *et al*., 2007). Interpretation of reproductive organ weights from animals with evidence of stress or exhibiting significant body weight changes must therefore take into account that organ weight changes might represent secondary effects of treatment on the reproductive cycle rather than a direct toxic effect of the test article (Bucci, 2002). Data from our study therefore suggest that MSG may not be directly toxic to the organs but rather it may alter the reproductive cycle. This assertion is supported by the histopathological study of the uterus, where no significant alterations were observed in the endometrium and myometrium of all the treated rats when compared with the control. This result also agrees with previous studies that reported that MSG induces increase in serum estrogen which has been shown to cause cellular hypertrophy and fibroid development (Obochi *et al*., 2009).

CONCLUSION

Based on the result of this study, MSG administration may predispose to hyperlipidemia thus increased the susceptibility to cardiovascular disease.

The result also suggest that MSG may alter the reproductive cycle and may thus induce uterine fibroid and that co administration of MSG and *P. amarus* we have reported in this study may offers a protective effect against this metabolic disorder but may not be able to reverse the metabolic dysfunction. The study also suggests that uterine fibroid development is positively correlated with cardiovascular disease risk.

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