Bozok Journal of Science
Volume 3, No 1, Page 20-33 (2025)
Research Article DOI: 10.70500/bjs.1565734

Antitumour Activity of *Ganoderma mbrekobenum* on Monosodium Glutamate Induced Uterine Tumour in Wistar Rats *(Rattus norvegicus)*

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Received: 18/02/2025 Accepted: 24/03/2025 Final Version: 22/05/2025

Abstract

Ganoderma species is widely reported to be anticancer and antitumour but there seems to be no report on indigenous species against uterine tumours. Monosodium glutamate (MSG) has been shown to induce uterine tumours in high concentrations. Antitumour effect of the water extract of a *Ganoderma* species on Wistar rats given MSG was conducted. Wistar rats were fed with monosodium glutamate (MSG) to induce uterine tumour in the animals and were treated with water extract of the *Ganoderma mbrekobenum*. Heamatological, biochemical, histopathological examinations and acute toxicity test (LD50) were done on the animals. The heart and liver in groups fed with MSG had significant increase in weight but the groups treated with *G. mbrekobenum* reduced in weight. The MSG-treated group showed indications of anaemia and toxicity in the animals. Renal functions and lipid profiles of the rats were fairly altered while the hepatic functions were significantly ($p< 0.05$) altered. Also, the amount of estrogen in the rats was elevated. Animals exposed to *G. mbrekobenum* showed signs of recovery from the effects of MSG in the rats. The acute toxicity test showed no fatalities among the animals and the extract of the *G. mbrekobenum* was notably lowered the levels of estrogen in the rats. The histological experiment revealed the presence of growths/cysts in the uterine tissues of rats fed with MSG alone, whereas the rats treated with *G. mbrekobenum* (800mg/kg/body weight) exhibited no histopathological alterations. These findings indicate that the *G. mbrekobenum* could prevent and control uterine tumour in Wistar rats.

Keywords: Tumour, Monosodium glutamate, *Ganoderma* species, Toxicity and Heamatology

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1. INTRODUCTION

Ganoderma species are basidomycetes fungi pertaining to the Polyporaceae family and is among the most well-known traditional Chinese medicinal herbs (Sikandar et al., 2013). Lucidum in Latin means shiny or brilliant and it describes the fruiting body of the mushroom which has sculptures and varnishes. Crude extracts of *Ganoderma lucidum*, *G. colossum*, *G. resinaceum* and *G. boninense* from Nigeria showed activity against *Bacillus subtilis* and *Pseudomonas syringae* using thin layer chromatography agar overlay method. Antibacterial compounds were also isolated from *Ganoderma colossum* in the wild in Lagos, Nigeria (Ofodile et al., 2012). *Ganoderma* species was reportedly utilized in Nigeria to treat neoplasia and asthma (Oyetayo, 2011).

Uterine fibroids are benign (non-cancerous) growths known as leiomyomas or myomas that originate from the uterine muscular tissue. The size, shape, and location of fibroids can vary significantly. They could reside inside the uterus, on its exterior or inside its walls, or they might be connected to it via a structure resembling a stem. Uterine fibroids (leiomyoma's) signify most common tumour in women. These lesions disrupt the uterus's normal functions, leading to severe uterine haemorrhage, anaemia, poor embryo embedding, and ongoing pregnancy loss, preterm labour, obstruction of labour, pelvic distress as well as urine incontinence, which could conceal or resemble malignant tumours (Anne et al., 2012).

Uterine fibroids are characterized by their reliance on the ovarian hormones, progesterone and estrogen (Kumar and Sharma, 2014). Fibroid growth requires ovarian activity, and most fibroids diminish after menopause. Fibroid growth is significantly impacted by the abrupt fluctuations in estrogen and progesterone levels associated with early pregnancy and the postpartum phase. Age, genetic background, and ethnic origin are associated risks for uterine leiomyoma. African American women are more likely than Caucasian women to have uterine fibroids obesity, poor eating habits, inactivity, specific chemicals (such as monosodium glutamate), and some prescription medications that can raise levels of cholesterol, estrogen, and total protein (Nwaje et al., 2015; Airaodion et al., 2019) predispose women to uterine tumour. According to reports, fibroid and cramping during menstruation are most commonly caused by high levels of estrogen (Kumar and Sharma, 2014).

Monosodium Glutamate (MSG) is a glutamate salt that is produced by synthesizing L-glutamic acids and is utilized to enhance food flavor (Nwaje et al., 2015). Significant amounts of free glutamate are present in a variety of processed and prepared meals, including traditional spice sauce and some dishes from eateries (as MSG) both from natural sources and from added monosodium glutamate (Nwaje et al., 2015). MSG inhibits growth hormone release, which results in stunted growth and irreversible obesity and excessive weight gain (Airaodion et al., 2019). Obesity resulting from excessive cholesterol-related fat build-up in adipose tissue, which causes endocrinological disorders and cardiovascular ailments (Anne et al., 2012).

Ganoderma mbrekobenum is a medicinal mushroom species from the genus *Ganoderma.* Initially discovered in Ghana, it has since been found in countries such as India and Nigeria (Sikandar et al., 2013). *Ganoderma mbrekobenum* is a lignin and cellulose-degrading wood-decaying fungus contributing to lignin and cellulose degradation in forest ecosystems. It usually colonizes hardwood trees and is an important part of the nutrient cycle. This similar red-brown glossy appearance of the fruiting body is in accordance with many of the species belonging to the genus *Ganoderma* (Adotey et al., 2023).

Recent studies have noted *G. mbrekobenum* potential health benefits as antimalarial activity. Compounds with antimalarial activity have been isolated from this mushroom indicating potential for development of natural antimalarial (Adotey et al., 2023), and antimicrobial drugs. The anticancer extracts from *G. mbrekobenum* have significantly suppressed the growth of human liver, breast, and brain cancer cells, highlighting its potential as a source of anticancer agents. The medicinal effects are linked to bioactive compounds like triterpenoids and polysaccharides found in the mushroom(Adotey et al., 2023).

Recent studies on medicinal mushrooms for HIV/AIDS patients in Africa have greatly increased the utility of mushrooms and were found to contribute to their recovery. Presently, surgery is the cheapest major treatment for uterine tumour which often grows again. According to Kumar and Sharma. (2014) analogues of gonadotropin-releasing hormone (GnRH) shrink fibroids and lessen related uterine hemorrhage by suppressing ovarian activity and lowering circulating levels of progesterone and estrogen. The use of GnRH agonists may remove the need for surgery in certain cases. When used before surgery, it reduces blood loss during surgery and decreases the size of the tumour making operation less complicated (Kumar and Sharma, 2014).

Moreover, Yoruba herbal healers in Nigeria call all *Ganoderma* species "olu iju." (Samuel et al., 2013) meaning mushroom for the treatment of fibroid but no scientific proof of their claims exits in literature. Hence, the crucial need to investigate the potentials of *Ganoderma* species isolated from the wild in Nigeria to control uterine tumour and provide alternative treatment for uterine tumour. This paper reports the antitumour effect of *Ganoderma mbrekobenum* in the uterus of Wistar rats fed with monosodium glutamate.

2. MATERIALS AND METHODS

2.1. Fungal Material

The *Ganoderma mbrekobenum* was isolated from dead trunk of a *Mangifera indica*, within Yaba College of Technology environment. The fungus was authenticated by the Mycologist as *Ganoderma*. The species of *Ganoderma* was identified by its ornamented, double walled basidiospore. Molecularly identified as *G. mbrekobenum* accession number PQ578264 and a voucher specimen (YCT Gano 201402) and placed in the Mushroom Research and Training Laboratory, Yaba College of Technology (Ofodile at al., 2022).

2.2. Experimental Animals and Monosodium Glutamate

Thirty-five adults female Wistar rats weighing 180-200g were used for this study (Ofodile et al., 2020). The animals were obtained and housed in the Animal Facility Centre of the College of Medicine, University of Lagos, (CMUL) Lagos and were allowed to acclimatize for 14 days.

The animals were divided among seven study groups at random of 5 rats each in plastic cages at room temperature (Ogbonnia et al., 2011). The animals were maintained under standard conditions (27± 2^{o}C and 12h light and dark cycle) and were fed with commercial rodent pellet diet (Pfizer) and water *ad-labitum.* Ethical clearance approval YCTESC 2016004SC was given by the Institutional Review Board of Yaba College of Technology in accordance with international standard on the care and use of experimental animals. Synthetic monosodium glutamate (MSG) was purchased from a vendor distribution shop in Yaba, Lagos. A stock solution (200 g/dm^3) of MSG was made.

2.3. Extraction of Mushroom Samples

The isolates were oven (55^{o}C) dried to a constant weight of approximately 5-7% moisture and crushed in an electric grinder to form coarse powder at the pharmacognosy, Department of the College of Medicine University of Lagos. Coarse powdered mushroom material (1000g) was soaked in 7L of distilled water overnight to prepare an aqueous extract. The solution was filtered using muslin cloth and then Whatman filter paper. The filtrate was dried with Büchi rotatory evaporator R-100 at a controlled temperature. The extract was stored at 4^{o}C in an airtight container until use (Ofodile et al., 2020).

2.4. Administration of MSG and Water Extract of *Ganoderma mbrekobenum*

Thirty-five female rats weighing 180-200g obtained from the Laboratory Animal Centre (CMUL) were allowed to acclamatize for 14 days and maintained under standard conditions (27±2^{o}C and 12h dark and light cycle. The animals were fed with commercial rodent pellet diet (Pfizer) and water *ad-labitum.* The rats were randomly placed into 7 groups of five rats each.

Rats in Group 1 served as the control group and allowed feed and water without any form of treatment. Rats in group 2 and 3 received oral daily doses of 600 and 800 mg/kg body weight of monosodium glutamate (MSG) respectively for a total of 60 days. Rats in group 4 and 5 received oral daily doses 600 and 800 mg/kg/wt of MSG for 30 days and 600 and 800 mg/kg/wt water extract of *G. mbrekobenum* for the next 30 days respectively. Rats in groups 6 and 7 received oral daily doses 600 and 800 mg/kg/wt. of MSG as well as 600 and 800 mg/kg/wt water extract of *Ganoderma* spp from the first day till 60 days respectively.

The animals were sacrificed on the 60th day by cervical dislocation after an overnight fast. Blood samples were collected in sterilised plain and anticoagulant bottles for analyses. The liver, kidney, heart and uterus were excised, blotted dried with filter paper and weighed.

2.5. Biochemical Assays

Serum levels of Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by method of Reitman and Frankel. (1957). Creatinine was determined using the alkaline picrate reagent (Cook, 1971). Albumin concentration was determined using the method of Doumas et al. (1971) while method of Evelyn and Malloy (1938) was employed to determine the serum bilirubin concentration of the samples, Kaplan (1965) method was used to determine the urea concentration, while King and King (1954) approach was used to determine the alkaline phosphatase activity. The total cholesterol, HDL cholesterol, and triglycerides were done using Tietz (1999) method.

2.6. Assessment Of Serum Total Protein Content

Serum total protein was determined by the Biuret method described by Gornall et al. (1949). Distilled water (0.1 mL) was added into test tubes containing serum sample solution (0.5 mL) to bring the volume to 1.5 mL of serum each test tube. Distilled water (1.5 mL) was given to the blank. Following the mixing of the combination, 0.2 mL of 5% sodium deoxycholate (DOC) in 0.01 N KOH was added. For the experiment, 2 mg/mL of standard bovine serum albumin (BSA) was employed. Biuret reagent (1.50 g $CuSO_4$. $5H_2O$, 6.0 g sodium potassium tartrate and 300 mL of 10% NaOH per liter) was added including the blank. The absorbance of the tubes was measured at 540 nm in a spectrophotometer against the blank (tube 1) after they were combined in a vortex mixer and incubated at 37 °C for 15 minutes.

2.7. The Estradiol (Estrogen) Content Measurement

Enzyme Immunoassay (EIA) was used to measure estradiol as previously mentioned by Meyer et al. (1997). The serum sample (4 mL) was brought to pH 3.5 using acetic acid, then extracted using 12 mL of pH 3.5 diethyl ether. The sample was evaporated and extracted again using the same diethyl ether. The residue was dissolved in 12 mL of assay buffer (40 mL of PBS, 0.1% BSA, pH 7.2) and, upon evaporation, combined to yield 3.2 mL in PBS (pH 7.5), the sample was dissolved in 12 mL of 100% methanol. Each serum's (4 mL) estradiol content was examined, the precise antigen-antibody response and the retention period (11.4 min) were detected. Between 0.15 pg (80% displacement of the labeled antigen) and 7.2 pg (20% displacement of the marked antigen of estradiol per 4 mL), there was a working interval. The EIA curve for calibration was created using 40% methanol.

2.8. Histopathology Experiment

The histopathology experiment followed the protocol outlined by Nweke and Akpuaka. (2019) Uterine tissues from the animals underwent fixation in 10% formal saline and subsequent processing involved sequential immersion in ascending alcohol grades. Following this, the tissues were cleared using xylene and subsequently embedded in paraffin wax. Tissue sections, 3-5 µm thick, were obtained using a rotary microtome. These sections underwent deparaffinization and hydration before being stained with haematoxylin and eosin dye.

For microscopic examination at a magnification of x150, the stained sections were mounted using a neutral dibutylphthalate xylene medium. The evaluation of uterine pathological changes was confirmed by histologist and pathologist in Faculty of Medicine, University of Lagos.

2.8.1 Acute Toxicity (LD50) Test

Acute toxicity was determined using the method of Ogbonnia et al. (2011). Dried powdered *Ganoderma* species (500g) was soaked in 5L of water and allowed to stand in the fume cupboard overnight and filtered with muslin cloth and then with a Whatman (No 1) filters paper. It was then freeze dried to give water extract of appreciable quantity. Thirty-five Swiss albino mice (20-25g) of both sexes, obtained from the Laboratory Animal Center of the College of Medicine, University of Lagos, maintained under standard condition (27±2ºC and 12h dark and light cycle) were fed commercial rodent pellet and water *ad libitum.* They were permitted to acclimate for 14 days. The rats were randomly distributed into 7 groups containing five animals per group. Rats in Groups 1 served as control group and received 0.4 mL of water orally while Group 2-6 received oral daily doses of 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 mg/g body weight extract of *Ganoderma mbrekobenum*. The animals were observed (24hs post treatment) for behavioural changes.

2.9. Statistical Analysis

The gathered data were expressed as mean ± Standard Deviation (SD), and statistical analysis was conducted using Analysis of Variance (ANOVA) with Turkey post hoc tests at a significance level of 5%. The statistical analysis for all parameters was conducted utilizing IBM SPSS 26.0 software.

3. RESULTS AND DISCUSSION

Body weight of the rats in the groups administered with MSG alone increased significantly ($p<0.05$) but the rats treated with the extracts of *Ganoderma mbrekobenum* gained weight non-significantly ($p >0.05$) compared with control. The heart, had a significant gain in relative weight in animals in groups 2 and 3 compared to the control Figure 2. The uterus gained weight significantly in group 6 animals compared to the control. The kidney had a non-significant weight gain in animals administered with MSG alone (group 3) compared to the control which was normalised in the groups treated with *Ganoderma* after administration and together with MSG (5, 6 and 7). The liver showed significant weight gain in the rats administered with MSG (600 mg/kg/bw) alone but non-significant reduction was observed in the groups treated with *Ganoderma* spp compared with the control Figure 2. Animals fed with MSG became bigger after some days of administration of monosodium glutamate alone.

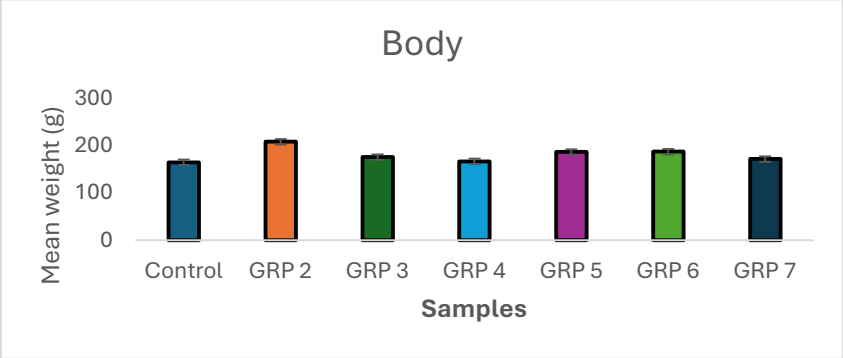

Figure 1: Effect Of Oral Administration of Monosodium Glutamate (MSG) And *G. mbrekobenum* On the Mean Weight of Wistar Rats (G, ± SD) (N=7), 60 Days Treatment.
Key: Group 1: Control, Group 2: MSG (600mg/Kg), Group 3: MSG (800mg/Kg), Group 4: MSG + *Ganoderma* Spp (600mg/Kg), Group 5: MSG+*Ganoderma* Spp (800mg/Kg), Group 6: *Ganoderma* Spp+MSG (600mg/Kg), Group 7: *Ganoderma* Spp+MSG (800mg/Kg).

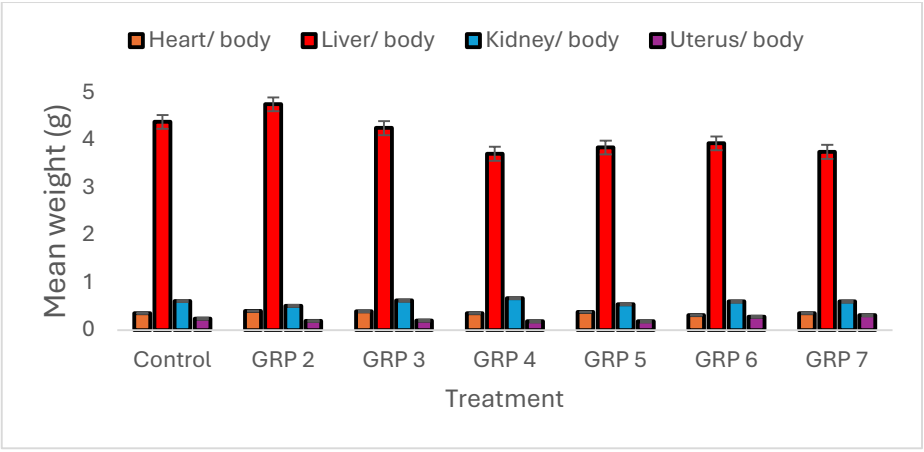

Figure 2: Effect Of Oral Administration of Monosodium Glutamate (MSG) And *Ganoderma* Spp on The Mean Relative Weights of The Heart, Liver, Kidney and Uterus of Wistar Rats G, ± SD) (N=7), 60 Days Treatment
Key: Group 1: Control, Group 2: MSG (600mg/Kg), Group 3: MSG (800mg/Kg), Group 4: MSG + *Ganoderma* Spp (600mg/Kg), Group 5: MSG+*Ganoderma* Spp (800mg/Kg), Group 6: *Ganoderma* Spp+MSG (600mg/Kg), Group 7: *Ganoderma* Spp+MSG (800mg/Kg).

Effect of the administration of MSG and the water extract of Ganoderma species on the haematology of the rats is shown on Tables 1 and 2.

The administration of MSG caused a significant decrease in the White Blood cells (WBC), Red Blood (RBC) Haemoglobin (HGB), Blood platelets (PLT) and packed cell volume (HTC), in the animals given only MSG alone compared to the control $p < 0.05$. The administration of a higher dose of the extract of *G. mbrekobenum* at any time during the treatment produced a non-significant increase in the above parameters (Group 5 and 7) compared to control. Except for RBC and HGB where the increase was significant showing traces of recovery in groups 4, 5, 6 and 7 (Table 1). There were no obvious changes in the levels of Mean cell volume (MCV), Mean corpuscular haemoglobin (MCH) and mean capsular haemoglobin concentration (MCHC) in all the animals treated with MSG and *Ganoderma* spp (Table 2).

Table 1. Effect of Oral Administration of MSG and Extract of *G. mbrekobenum* on the WBC, RBC, HGB And HCT Rats Of Rats N=7, 60 Days Treatment
Key: WBC: White blood cell, RBC: Red blood cell, HGB: Haemoglobin PCV (hct): Packed cell volume, *G*. spp: *Ganoderma* species, MSG: monosodium glutamate*=p<0.05 when compared with group Group 1: Control, Group 2: MSG (600mg/kg), Group 3: MSG (800mg/kg), Group 4: MSG + *G*. spp (600mg/kg), Group 5: MSG+*G*.spp (800mg/kg), Group 6: *G*.spp+MSG (600mg/kg), Group 7: *G*.spp+MSG (800mg/kg).

	MEAN EFFECT						
Parameter	Control	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6	GRP 7
$WBC_{(10^3/\mu L)}$	7.7±0.37	5.2±0.70*	5.2±0.09*	5.7±0.17*	6.7±0.25*	9.0±0.18*	6.5±0.20*
$RBC_{(10^3/\mu L)}$	6.46±0.19	4.46±0.23*	5.85±0.19*	6.08±0.22	6.71±0.38	7.62±0.27*	7.01±0.31*
HGB(g/dL)	12.5±0.43	8.0±0.28*	10.4±0.22*	11.3±0.31*	12.8±0.37*	11.4±0.24*	12.4±0.16*
HCT (%)	38.8±0.97	24.9±1.12*	32.8±1.00*	35.5±1.15*	38.9±1.05	43.0±1.01*	38.9±1.17

Table 2: Effect of Oral Administration of MSG and Extract of *Ganoderma* Spp PLT, MCV, MCH and MCHC of Rats, N=7, 60 Days Treatment
Key: Platelet, PLT, MCV: Mean cell volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean capsucular haemoglobin concentration, G. spp: G. mbrekobenum, MSG: monosodium glutamate*=p<0.05 when compared with group Group 1: Control, Group 2: MSG (600mg/kg), Group 3: MSG (800mg/kg), Group 4: MSG + G. spp (600mg/kg), Group 5: MSG+G.spp (800mg/kg), Group 6: G.spp+MSG (600mg/kg), Group 7: G.spp+MSG (800mg/kg). n=7

	MEAN EFFECT						
Parameter	Control	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6	GRP 7
PLT (103/μL	726±12.92	516±17.02*	447±11.02*	480±18.10*	542±15.02*	810±16.11*	628±11.02
MCV(FL)	60.1±1.47	55.9±0.97*	56.1±1.02*	58.4±1.50	58.1±1.27	56.5±1.91	55.6±1.13
MCH(PG)	19.3±0.57	17.9±0.37*	17.7±0.41*	18.5±0.39	19.0±0.32	18.9±0.61	17.9±0.52
MCHC (%)	32.2±0.28	32.1±0.19	31.7±0.29	31.8±0.33	32.9±0.28	31.5±0.35	32.3±0.77

Table 3 illustrates the impact of the oral administration of MSG and *G. mbrekobenum* extract on the renal function indices of rats. There was non-significant decrease in total protein and DB ($p<0.05$) in animals administered with MSG (Group 2 &3) while the urea decreased significantly ($p<0.05$) in the same group compared with the control group. The level of TB was significantly ($p<0.05$) increased in rats administered with MSG (800 mg/kg/body weight) which was decreased non-significantly in the treated groups compared with the control (Table 3).

Creatinine and albumin were not altered significantly in the rats administered with MSG compared with the control which were maintained in the groups treated with the extract of *Ganoderms* spp. Uric acid was also increased non-significantly in rats administered with MSG and maintained throughout the treated groups. The administration of the extract of *G. mbrekobenum* either after 30 days of MSG or together with MSG maintained significant decrease in urea and non-significant decrease in TP and DB.

Table 3: Effect of Oral Administration of MSG and Extract of *Ganoderma mbrekobenum* on Renal Function Indices of Rats
Key: Total protein (TP), Total bilirubin (TB) Direct bilirubin (DB), Creatinine (creat.), Serum albumin (ALB)

Parameter	**MEAN EFFECT**						
Renal Function	Control	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6	GRP 7
TP g/dL	7±0.19	6.4±0.08	6.6±0.53	5.5±0.21	6.4±0.29	5.7±1.11	6.0±0.22
TB mg/dL	0.6±0.02	0.5±0.03	0.7±0.01	0.6±0.01	0.6±0.03	0.5±0.01*	0.6±0.01
DB mg/dL	0.4±0.03	0.3±0.01	0.4±0,.003	0.3±0.02	0.3±0.003	0.3±0.01	0.3±0.01
Urea mg/dL	31±1.02	24±1.15*	27±0.68*	27±1.08*	25±1.41*	27±1.00*	27±1.47*
Creat. mg/dL	0.9±0.04	0.8±0.02	1.0±0.04	0.8±0.03	0.9±0.02	1.0±0.02	0.9±0.02
Alb g/dL	3.8±0.11	3.9±0.09	3.5±0.19	3.9±0.08	3.8±0.04	3.0±0.17	3.6±0.10
Uric Acid mg/dL	2.7±0.09	3.1±0.04	3.4±0.10	3.6±0.16	3.7±0.1*	2.5±0.09	3.2±0.07

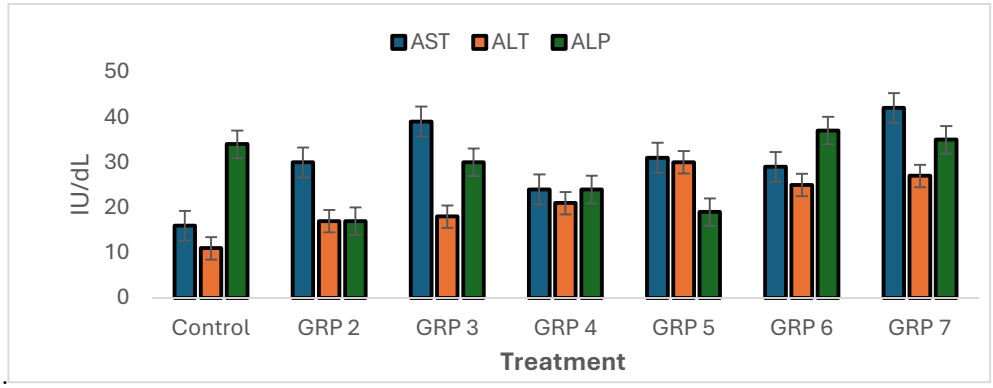

Figure 3: Effect of Oral Administration of MSG And Extract of *Ganoderma* Species on Hepatic Function of Rats
Key: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) Group 1: Control, Group 2: MSG (600mg/kg), Group 3: MSG (800mg/kg), Group 4: MSG + *G*. spp (600mg/kg), Group 5: MSG+*G*. spp (800mg/kg), Group 6: *G*.spp+MSG (600mg/kg), Group 7: *G*.spp+MSG (800mg/kg). n=7

The lipid profile of rats treated with MSG and the extract of *G. mbrekobenum* are shown in Figure 4. There was a significant increase in the total cholesterol level in the treated rats compared to the control. There was a significant decrease in HDL animals in animals administered with MSG alone ($p<0.05$) while no significant change was observed in the HDL cholesterol level in the rats treated with *G. mbrekobenum* compared to the control. A significant increase in LDL level was recorded in animals fed with MSG alone and all rats treated with *G. mbrekobenum*. There was a significant decrease in treated rats in triglyceride level compared to the control $p<0.05$.

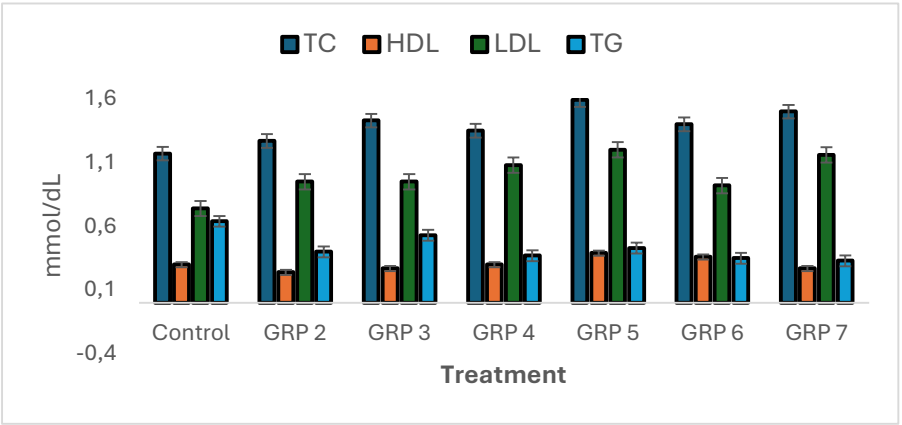

Figure 4: Effect of Oral Administration of MSG And Extract of *Ganoderma* Species on Lipid Profile of Rats**. Key:** High-density cholesterol (HDL), Low density cholesterol (LDL), Triglyceride (TG), Total cholesterol (TC) Group 1: Control, Group 2: MSG (600mg/kg), Group 3: MSG (800mg/kg), Group 4: MSG + *G*. spp (600mg/kg), Group 5: MSG+*G*.spp (800mg/kg), Group 6: *G*.spp+MSG (600mg/kg), Group 7: *G*.spp+MSG (800mg/kg). n=7

Figure 5 demonstrates impact of MSG and *G. mbrekobenum* extract on animal estrogen levels after administration. The level of estrogen was significantly increased ($p<0.05$) in the rats fed with MSG alone (MSG 600mg/kg/body weight and MSG 800mg/kg/ body weight). *G. mbrekobenum* significantly reduced the level of estrogen in the rats fed with MSG and *G. mbrekobenum* together and animals administered with *G. mbrekobenum* 30 days after MSG compared to the rats fed with MSG alone (197.53pg/ml) at $p<0.05$.

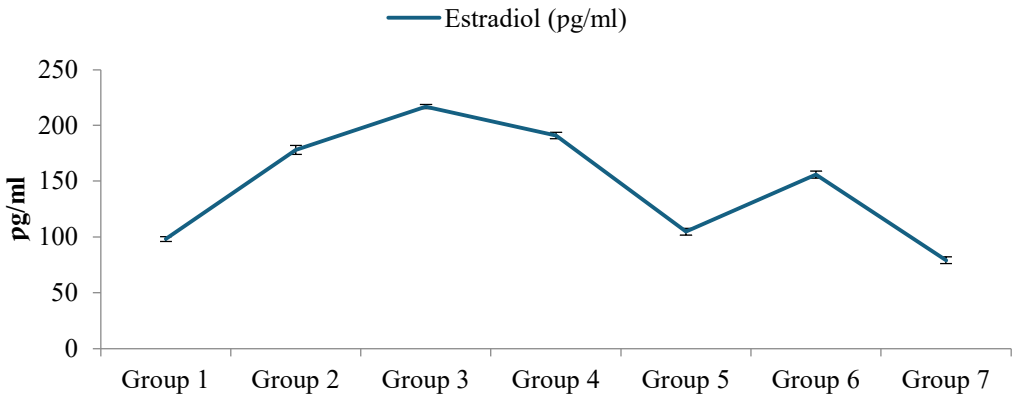

Figure 5: Shows the Effect of Administration of MSG and the Extract of *G. mbrekobenum* on the Estrogen Level of the Animals
Key: Group 1: Control, Group 2: MSG (600mg/kg), Group 3: MSG (800mg/kg), Group 4: MSG + G. spp (600mg/kg), Group 5: MSG+G.spp (800mg/kg), Group 6: G.spp+MSG (600mg/kg), Group 7: *G*.spp+MSG (800m**g**/kg).
Histopathology of the uterus of rats administered with monosodium glutamate and *G. mbrekobenum* is shown in Figures 6A-G.

The findings from the histological profiles supports the biochemical findings by the presence of the growth and cyst in the uterine tissues of the MSG treated groups, this supports the fact that MSG could result in growth in the uterus. The group of rats administered MSG and *G. mbrekobenum* together showed a better response to the damage that could have been induced by MSG when compared to the group given MSG for 30 days before treatment with *Ganoderma* species.

The female rats uterus (untreated) showed no signs of histological alterations (Figure 6A). Signs of cyst/growth were observed in animals administered with MSG alone at 600 mg/kg bwt which increased in size at 800mg/kg/bwt (Figure 6 B and C). Rats treated with *Ganoderma* species (600 mg/kg bwt) 30 days after feeding with MSG showed patches of growth in their uterus which disappeared in animals administered with 800mg/kg/bwt (Figure 6 D and E). Similar results were recorded in rats given MSG and *Ganoderma* the same time at both concentrations (Figure 6F and G) though the effect of MSG was less in these groups.

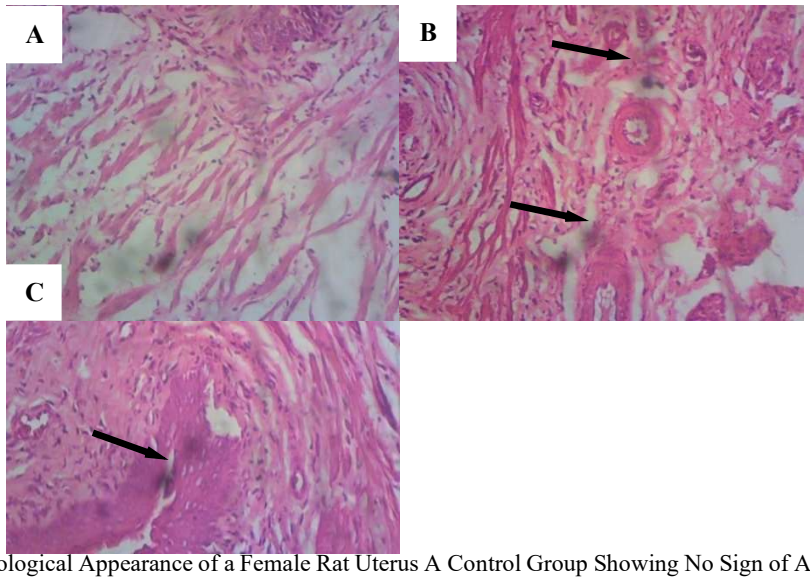

Figure 6: Histopathological Appearance of a Female Rat Uterus A Control Group Showing No Sign of Any Histological Change B Rat That Received 600mg/Kg of MSG Showing Cyst/Growth in The Tissue (Black Arrows) Compared to The Group C That Received 800mg/Kg MSG Showing Large Cyst/Growth in The Tissue (Black Arrows), H-E X150. MSG; Monosodium Glutamate, GI; Ganoderma Lucidum, H-E; Hematoxylene-Eosine.

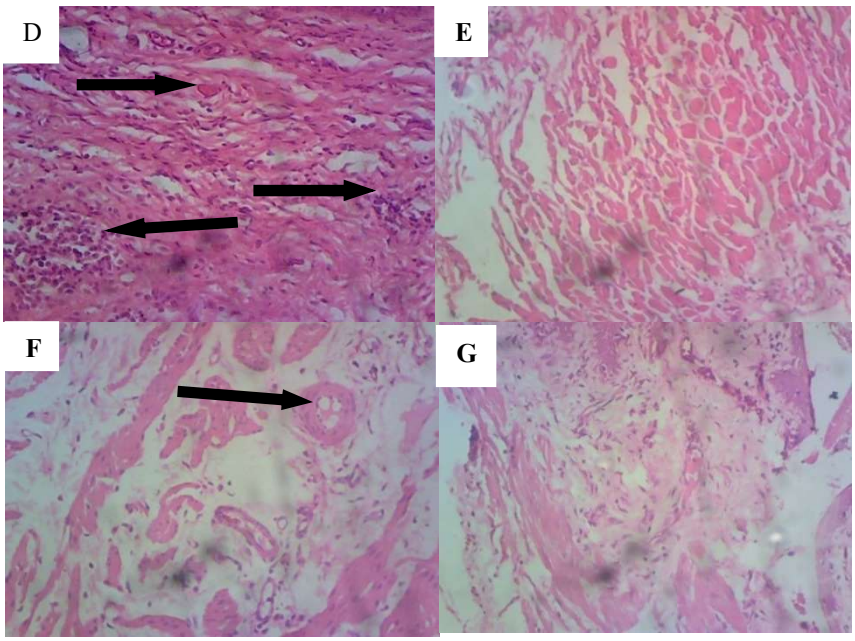

Figure 6: Histopathological Appearance Of A Female Rat Uterus **D** Rat That Received 600mg/Kg Of MSG+G.Spp Showing Small Patches Of Tissue Growth (Black Arrows) **E** Rat That Received 800mg/Kg Of MSG+G.I, **F** Showing No Sign Of Histopathological Changes **G** A Female Rat That Received 600mg/Kg Of G.Spp+MSG Showing Some Cyst Growth In The Tissue (Black Arrows) Compared To The Group Rat That Received 800mg/Kg Of G.I+MSG Showing No Histopathological Change To The Tissue, H-E X150. MSG; Monosodium Glutamate, GI; Ganoderma Lucidum, H-E; Hematoxylene-Eosine.

The acute toxicity study (Table 3) recorded 100% survival beyond 24 h for all the animals that received 20 g/kg body weight of the extract. The median acute toxicity (LD50) of the extract should therefore be over 20g/kg body weight. During the acute toxicity study of the extract, there were no observable alterations in the behaviour of the treated rats.

Ofodile et al. (2020) also reported weakness of the animals' days into the administration with MSG alone which was also observed in the present experiment. Previous studies, including those by Ofodile et al. (2020) reported a significant increase in the weights of both the kidney and liver of rats when fed with MSG alone. The observed augmentation was suggested to be linked to heightened inflammatory agents' activity, leading to an enlargement of the liver and kidney tissues. While a similar outcome was noted for the liver weight, the increase in kidney weight was not deemed statistically significant.

The weight gain in liver and Kidney could be due to the fact that MSG has an antherogenic effect on the body thus inducing proliferation of these organs directly involved with metabolism (liver and Kidney) and cardio-regulation (heart and kidney). The alteration, whether an increase or decrease, in the absolute or relative weight of an organ following the administration of a chemical or drug has been documented as an indicator of the toxic impact of that substance, Imafidon and Okunrobo (2010); explained that the enlargement may have affected the function of the liver. Non-significant changes in rats treated with *G. mbrekobenum* was also observed by Deepalakshmi and Mirunalini (2013).

Low levels of WBC, RBC, HGB and HTC recoded in the present work indicate the occurrence of microcytic aneamia like previous reports (Ghorbel et al., 2017). Heamatological analysis of administered extract help in assessing the toxicity of MSG and the extract of *G. mbrekobenum*. Heamatological analysis is vital in determining nutritional, environmental and pathological stresses and the degree of injury to the blood tissues. *G. mbrekobenum* increased the hemoglobin level in the animals which may be due to strong antioxidant effect of the mushroom preventing the destruction of RBC's from free radical formation (Hossen et al., 2018). This hematopoietic effect of *G. mbrekobenum* may be due to the antioxidant properties of its constituents (Hossen et al., 1918). The administration of the extract of *G. mbrekobenum* ameliorated the effect of the MSG and complied with previous work by Ofodile et al. (2020). Also, the effect of *G. mbrekobenum* could be said to be doze dependent. Hossen et al. (2018) also reported *Ganoderma lucidum* protected the animals against carbofuran-induced toxicity suggesting its antioxidant activities and presence of phenolic compounds which may be the case with the extract of *G. mbrekobenum* in the current report.

Imafidon and Okunrobo (2012) reported increased in protein and albumin levels in rats administered with palm oil, coconut oil and groundnut oil associated the effect with impairment in normal liver function, reported reduction in Alb and TB concentrations in the liver indicated liver damage which do not align with the result of this work. Concerning serum urea in rats administered with MSG and extract of *G. mbrekobenum*, significant reduction could indicate alteration in urea cycle. The proposition suggests that MSG increases the formation of reactive oxygen species (ROS) while concurrently diminishing antioxidant activities which has been linked as the biochemical basis for the altered cholesterol and total protein concentration. The observation of a low urea level in the current study suggests a compromised ability of the kidneys to effectively filter waste products from the blood and eliminate them through urine (Hossen et al., 2018). Urea and creatinine are used as biomarkers of renal function. Hence, deviations from normal biomarker concentrations suggest underlying pathological conditions, such as renal failure. However, in the case of these animals with decreased urea levels, this does not apply. Bilirubin, resulting from haemoglobin breakdown, has been demonstrated to possess antioxidant properties within human brain tissue. Also, high level of SUA was associated with slower rate of neurological functions in the present experiment.

The liver is vulnerable to damage from direct exposure to harmful substances due to its role in metabolizing and detoxifying compounds and xenobiotics an elevation of ALT and AST in blood parameters is indicative of liver and heart damage (Deepalakshmi and Mirunalini, 2013). It could also increase due to injuries of the heart, muscle, pancreas, kidney, or red blood cells Deepalakshmi and Mirunalini (2013).In this study, a significant change in the ALT and AST activities could be due to the toxic effect of MSG in

agreement resulting in proliferation, an increase in turnover or damage or in synthesis. The increase in ALT and AST may result in liver cell damage which is characterized by the transaminases action from damaged hepatocytes.

Phenolics and flavonoids in mushrooms contribute to inhibiting membrane lipid peroxidation and exhibit strong free radical scavenging activities, showcasing antioxidant properties for cellular protection and potential health benefits and the extract of *G. mbrekobenum* ameliorated HDL level (Hossen et al., 2018). The non-significant increase in total cholesterol level and decrease in HDL level coupled with significant increase in LDL suggest hypercholesteroemic effect in animals administered with MSG alone. The alterations could result in atherogenic and coronary risk indices, but the treatment with *G. mbrekobenum* capable of ameliorating the MSG alteration. A proposal suggests that MSG increases reactive oxygen species (ROS) production, diminishes antioxidant activities forming a biochemical basis for altered cholesterol and total protein levels.

Result is similar to a previous observation of an increase in the levels of estrogen found by Ofodile et al. (2020). No case has been reported of uterine tumour before adolescence but there are exceptional cases after menopause. This finding informs the concept that estrogen is the main feeder of uterine leiomyomas The size of the uterine tumour increased with increased concentration of MSG (Figure 5b and b). Estrogen was reported to stimulate the proliferation of fibroblasts isolated from uterine fibroids, thus indicating its influence to fibroid growth (Luo et al., 2014). Enzymearomatase activated by MSG catalyse the conversion of testosterone to estrogen, raising the production of estrogen. The administration of *G. mbrekobenum* as a preventive therapy (group 5 and 6) resulted in a significant reduction in the estrogen concentration when compared with the curative therapy (group 4 and 6). Estrogens exert their effects by binding to a nuclear receptor with high affinity known as the estrogen receptor (ER). *Ganoderma lucidum* was reported to indicate *in vitro* decrease. The MCF-7 breast cancer cells also exhibit the expression of estrogen receptor alpha (ER-alpha) (Kashimoto et al., 2010).

The median acute toxicity value (LD50) of the extract appears to be higher than 20.0 g/kg bwt, as all of the mice that received a dose of the extract equivalent to 20.0 g/kg body weight lived past the 24-hour observation period. Based on the findings reported by the extract can be categorized as non-toxic. This classification is supported by the fact that the oral LD50 (lethal dose for 50% of the population) exceeds 15 g/kg body weight, a significantly higher value than the WHO toxicity index of 2 g/kg (Ogbonnia et al., 2011). As a result, rats given the mushroom did not exhibit any acute toxicity, and the fatal value of the medium acute toxicity (LD50) was found to be greater than 2000 mg/kg, indicating that the mushroom was usually considered harmless. (GRAS).

Thus, the study suggests that monosodium glutamate should not be taken in high concentration to avoid damages to the liver, kidney or other organs of the body. Since monosodium glutamate can induce tumours in female, it is however important to avoid its intake and if taken at all it should not exceed 1.0g per day.

4. CONCLUSION

From the experiment, *G. mbrekobenum* can be used to reduce the effect of monosodium glutamate intake in the body to avoid acceleration of estrogen which is an indication of development of fibroid tumours. Hence, these findings support the fact that monosodium glutamate is detrimental to health in high concentration and can induce uterine tumour and that *Ganoderma* collected in Nigeria has the ability to prevent and control uterine tumour growth in rats. The LD50 value (20.0 g/kg body weight). The obtained results clearly indicate that the mushroom preparation can be safe for use.

ACKNOWLEDGMENT

We thank Mr B. C. Duncan, University of Lagos, Idi-Araba, Lagos, Nigeria for his technical assistance during this research work

AUTHOR'S CONTRIBUTIONS

Author 1: Conceptualized, supervised the research and wrote manuscript. **Author 2:** co-supervised research and edited manuscript, **Author 3**: edited manuscript, **Author 4:** Analysed the data. **Author 5:** edited manuscript, **Author 5:** Edited manuscript

CONFLICTS OF INTEREST

There is no Conflicts of interest.

RESEARCH AND PUBLICATION ETHICS

"This research was done in line with the guidelines of the Declaration of Nigeria, and authorized by the Institutional Ethical Group of Yaba College of Technology (Ethical code YCTESC 2016004SC and 16th February 2016).

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