# EFFECT OF THE AQUEOUS EXTRACT OF Ganoderma lucidum ON THE HAEMATOLOGY, OESTRADIOL, CHOLESTEROL AND PROTEIN LEVELS OF WISTAR RATS FED WITH MONOSODIUM GLUTAMATE

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Monosodium glutamate (MSG) at high concentration has been reported to alter the physiological and biochemical states of animals and humans. Ganoderma lucidum (G. lucidum) is a polypore mushroom reported to possess many medicinal attributes such as anticholesterolemia and the control of hormonal disorders. The present study investigated the effect of water extract of G. lucidum in the changes of haematology, oestradiol, cholesterol and protein levels of Wistar rats induced by MSG. Haematological analysis was determined from plasma, while oestrogen, serum total protein and cholesterol levels were determined from the serum of the rats. Results showed that MSG significantly raised the level of oestrogen ( $62.5 \pm 0.28$  pg/mL) in the rats which was significantly reduced in the rats fed with MSG for 30 days before treating them with the extracts of G. lucidum ( $30.85 \pm 12.94$ pg/mL-44.15 ± 0.92 pg/mL) and in rats fed concurrently with MSG and G. lucidum. The cholesterol level was significantly reduced in the rats treated with MSG and G. lucidum (200 mg/kg) concurrently compared to rats fed with MSG alone. The white blood cell (WBC) and red blood cell (RBC) levels were within normal in rats fed with both MSG and G. lucidum as in the control group while the rats fed with MSG only had low WBC, neutrophil (NEU) and RBC. This could imply that G. lucidum ameliorates the effect of MSG on serum oestrogen. serum cholesterol, WBCs, NEU, platelets and lymphocytes.

*Keywords: Ganoderma lucidum*, haematological analysis, hormonal test, Monosodium glutamate, Wistar rats

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#### INTRODUCTION

The fungal genus *Ganoderma* contains about 200 species and is a member of the Ganodermataceace, is a mushroom family characterised by unique double-walled basidiospores (Ofodile *et al.* 2005; Zhou *et al.* 2015). *Ganoderma lucidum (G. lucidum)* is known as *lingchi* in Chinese, *reishi* in Japanese, *yeonghi* in Korea. The taxonomy of *Ganoderma* species is globally known to be chaotic and the taxonomic relationships among taxa are still under investigation (Zhou *et al.* 2015). It is a bracket fungus and the most laccate species of *Ganoderma*. The genus has a worldwide distribution in both tropical and temperate regions, growing as a parasite or saprophyte on wide varieties of hard wood trees (Smith and Sivasthamparan 2003); Hennicke *et al.* 2016; Loyd *et al.* 2018).

Reishi has been used as a medicinal herb to treat many diseases in Asia such as hepatitis, hypertension, hypercholesterolemia and gastric cancer (Martínez-Montemayor *et al.* 2019). Reishi is also reported to contain immune enhancing substance and to improve human health (Tang *et al.* 2006; Cao *et al.* 2012; Loyd *et al.* 2018). Recent researches report that *G. lucidum* contains about 400 bioactive substances that are mostly polysaccharides and triterpenes (Basnet *et al.* 2017). These compounds have anti-inflammatory, radical oxygen scavenging, antitumour, immune-enhancing and antimicrobial activities (Sanodiya *et al.* 2009; Jin *et al.* 2012)

The deleterious effects of monosodium glutamate (MSG) have been shown in animal and human clinical experiments (Eskes 1998; Belluardo *et al.* 1990; Nayanatara *et al.* 2008; Obochi *et al.* 2009; Eweka, Eweka and Om'Iniabohs 2010; Eweka, Igbigbi and Ucheya 2011; Meraiyebu *et al.* 2012) and according to Muhammad *et al.* (2014), the substance induces uterine tumour (fibroid) by increasing the level of oestrogen in rats. MSG is a salt of glutamate, synthesised from L-glutamic acids and used as a flavour enhancer in foods; binder and filler for nutritional supplements, in prescription drugs, intravenous fluids given in hospitals and in the chicken pox vaccines (Ikonomidou and Turski 1995). MSG causes reduction in the secretion of growth hormones, leading to stunted growth and irreversibility in obesity, excessive weight. Essentially due to accumulation of excess fats in adipose tissue which arises from high cholesterol levels leading to cardiovascular diseases and endocrinological disorder (Obochi *et al.* 2009). Various processed and prepared foods such as traditional seasonings sauce and certain restaurant foods contain significant levels of free glutamate, both from natural sources and from added MSG (Rodriguez *et al.* 1998; Airaodion *et al.* 2019).

Studies have shown that MSG administered to adult rats caused change in the levels of biochemical parameters such as lipids, alterations in the levels of thiobarbituric acid reactive substances (Ahluwalia *et al.* 1996; Choudhary *et al.* 1996; Anwar and Mohamed 2010) and significant alteration in cholesterol and total protein levels (Manivasagam and Subramaniam 2004; Airaodion *et al.* 2019). According to Singh and Pusha (2005), MSG also induced an oxidative stress, hyperlipidemia and hyperglycemia. However, it is still widely used in baby's foods and in many foods sold by vendors and for cooking in many homes in Nigeria.

This paper therefore reports the preliminary experiment to find out the effect of *G. lucidum* on Wistar rats fed with MSG and assesses the possibility of using the mushroom to ameliorate the impact of MSG on the haematology, oestrogen, serum total protein and cholesterol levels in female albino rats.

### METHODS

### **Collection and Extraction of Mushroom Sample**

Basidiocarps of *G. lucidum* were obtained from decaying wood of *Delonix regia*, family Fabaceae within Yaba College of Technology, Yaba Lagos, South West, Nigeria. The mushroom species was identified according to Ofodile (2006) and voucher specimen (YCT Gano 201401) was deposited in the Mushroom Laboratory, Yaba College of Technology. Air-dried and powdered basidiocarps of *G. lucidum* (160 g) was soaked overnight in 1.5 L of distilled water. The extract was then filtered using Whatman filter paper into weighed beakers and residue was discarded. The filtrate was dried with a Büchi rotatory evaporator. The dried extract (36.5%) yield was reconstituted in hot water (90°C) following the modified method used by Ofodile *et al.* (2005) and this was used for the animal experiment.

### **Test Animals**

Twenty-five adult female Wistar rats aged 6 weeks weighing 120 g–160 g were obtained from the animal house of National Institute of Medical Research (NIMR) and transferred to the animal house, Yaba College of Technology where the experiment was carried out. Ethical clearance with approval number YCTESC 2016001SC was given by the Institutional Review Board of Yaba College of Technology in accordance with the International Standard on the Care and Use of Experimental Animals. The animals were randomly assigned into five study groups, five rats per group. They were acclimatised for 14 days in plastic cages under standard conditions (27  $\pm$  2°C and 12 h light and dark cycle). The animals were fed commercial rat chow and water ad-libitum throughout the period of the experiment.

#### Administration of Extract and MSG

Synthetic MSG was obtained from a vendor distribution shop in Mushin, Lagos, Western Nigeria and granules of the MSG (100 g) were dissolved in 500 mL of distilled water (1 g/5 cm<sup>3</sup>).

All rats were treated daily for a period of 60 days. The rats in group 1 (control) received water ad-libitum and were fed through gastric intubation. Animals in groups 2 and 3 were treated concomitantly with 100 mg/kg body weight MSG each and extract of *G. lucidum* (100 mg/kg and 200 mg/kg), respectively. Animals in groups 4 and 5 were first administered 100 mg/kg MSG for 30 days; two of the animals were then sacrificed to check for the necessary parameters; the animals were regarded as the sixth group. The remaining rats were treated with 100 mg/kg and 200 mg/kg *G. lucidum*, respectively, for another 30 days. The rats were sacrificed through cervical dislocation and their blood was collected into ethylenediaminetetraacetic acid (EDTA) and plain bottles. The liver, kidney and uterus were removed from the body of the animals and weighed, serum from plain bottles were prepared by centrifugation (6000x g, 30 min) and used for determining the serum total oestradiol, total protein and total cholesterol while the blood in EDTA bottles were used for haematological analysis.

### **Determination of Total Cholesterol**

Serum (0.1 mL) was pipetted into test tubes and absolute ethanol (10 mL) was added to each tube and mixed on a vortex mixer for 10 sec. The tubes were centrifuged for

5 min at full speed. Extracts (2.0 mL) were pipetted into new test tubes. The blank received 2.0 mL distilled water. Then, 2.0 mL of the colour reagent (diluted 40 mL iron stock solution to 500 mL with concentrated  $H_2SO_4$  and dispensed with automatic dispenser) was slowly added to all test tubes including the blank and mixed gently. The iron stock solution was prepared by dissolving 5.0 g FeCl<sub>2</sub>.6H<sub>2</sub>O in 200 mL concentrated  $H_2PO_4$ . The cholesterol working standard solution was prepared by adding 2.0 mL cholesterol stock solution (0.1 mg/mL cholesterol standard) to 98 mL absolute alcohol. The tubes were then covered with parafilm and allowed to stand at room temperature for 30 min. The absorbance read at 550 nm in 6400/6405 spectrophotometer against the reaction blank. The average mg/mL value of cholesterol was calculated. The concentration of the unknown was calculated using the ratio formula below (Obochi *et al.* 2009):

Concentration of cholesterol = Conc. of std = concentration of standard
standard

### Determination of Serum Total Protein

Serum total protein was done using the Biuret method according to Gornall *et al.* (1949). Serum sample solution (0.5 mL) was pipetted into test tubes and 1.0 mL in distilled water added to bring the volume to 1.5 mL in each tube. Tube 1 (the blank) received 1.5 mL distilled water. The suspension was mixed and 0.2 mL of 5% sodium deoxycholate (DOC) in 0.01 N potassium hydroxide (KOH) was included and mixed to make the suspension more soluble. The concentration of the standard bovine serum albumin (BSA) used in the assay was 2 mg/mL. Then, of 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 tubes were mixed in a vortex mixer and incubated at 37°C for 15 min and the absorbance was read at 540 nm against the blank (tube 1) in a spectrophotometer.

### Determination of Oestradiol (Oestrogen)

Oestradiol was determined using enzyme immunoassay (EIA) described by Meyer *et al.* (1997). Serum sample (4 mL) was put to pH 3.5 with acetic acid and extraction was done with 12 mL of diethyl ether (pH 3.5), evaporated and re-extracted with diethyl ether (pH 3.5). The residue was dissolved in 12 mL of assay buffer (40 mM PBS, 0.1% BSA, pH 7.2) and pooled to give 3.2 mL in PBS (pH 7.5) after evaporation; the sample was dissolved in 12 mL of 100% methanol. The content of oestrogen in each serum (4 mL) was analysed. The retention time (11.4 min) and the specific antigen-antibody reaction were identified. The experiment worked at the interval of 0.15 pg (80% displacement of labeled antigen) and 7.2 pg (20% displacement of labeled antigen of oestradiol per 4 mL). Methanol (40%) was used to prepare the EIA calibration curve.

## **Statistical Analysis**

Data collected were expressed as mean ± standard deviation (SD) and one-way analysis of variance (ANOVA) with Tukey post hoc were used for analysis at 5% level of significance.

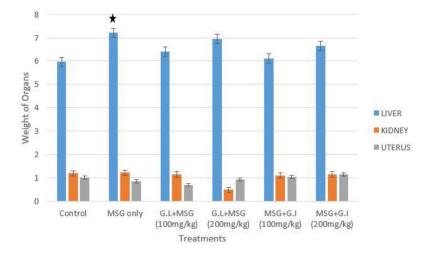
### RESULTS

The results of the haematological, oestrogen, serum protein and cholesterol assay conducted on the rats to access the effect of mushroom extract on the rats treated with MSG are presented in Table 1 and Figures 1–4.

Table 1 shows the results of the haematological analysis of the rats treated with aqueous extract of *G. lucidum* and MSG. The results showed that the level of white blood cells (WBCs) and red blood cells (RBCs) in the rats fed with only MSG decreased significantly (p < 0.05) compared with the control. Rats treated with MSG and *G. lucidum* [MSG + G. *lucidum* (200 mg/kg)] concurrently for 60 days recorded the same normal RBC counts as in the control. There were also significant increase in the RBC in rats that were treated with MSG and *G. lucidum* [MSG + G. *lucidum* (100 mg/kg)] concurrently for 60 days and those first fed with MSG for 30 days and treated subsequently with the doses of MSG + *G. lucidum* (100 mg/kg), MSG + *G. lucidum* (200 mg/kg) for another 30 days. *G. lucidum* also raised the white blood cells of the rat at the significance level of p < 0.05 in all doses and treatments.

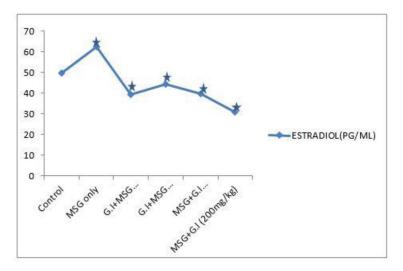
The haemoglobin (HGB), packed cell volume (HTC), mean capsular haemoglobin concentration (MCHC) and the neutrophil (NEU) levels were also lowered significantly (p < 0.05) with the treatment of MSG alone. Lymphocytes and platelets increased significantly (p < 0.05) in the group fed with MSG alone. Results also showed that the HGB, HTC, MCHC and NEU levels were raised significantly (p < 0.05) in rats fed with the different doses of the mushrooms and MSG compared to the rats fed with MSG alone. The difference in the level of MCHC between the animals treated with the mushrooms and the control were found to be insignificant. Treatment with *G. lucidum* in the different doses and treatments were observed to lower MCHC level almost to the volume of that of normal rats because the differences between them were insignificant. The blood platelets remained on the increase after treatment with the mushrooms at the significant levels (p < 0.05). Percentage lymphocytes were significantly reduced in rats treated with the mushrooms compared with the animals treated animals.

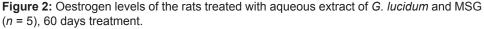
Figure 1 shows the weight of the liver, kidney and uterus of the animals before and after treatments. There was a significant increase in the weight of the liver of the rats treated with MSG compared with the control. Animals fed with MSG were also weak after some days of administration.



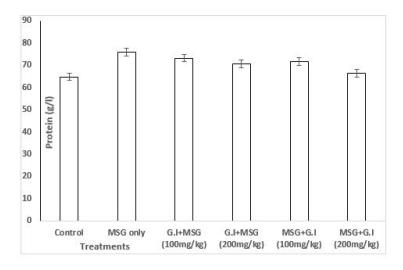
**Figure 1:** Mean weight ( $g \pm SD$ ) of organs of the liver, kidney and uterus treated with aqueous extract of *G. lucidum* and MSG, (n = 5), 60 days treatment.

Figure 2 shows that the mean value of oestrogen (pg/mL) in the group fed with MSG alone was significantly higher than that of the control. The animals in the groups treated with mushroom at different doses also showed significant (p < 0.05) reduction in oestrogen levels compared with those fed with MSG alone. The reduction in oestrogen in the groups treated with mushroom doses were not significant compared with the control.

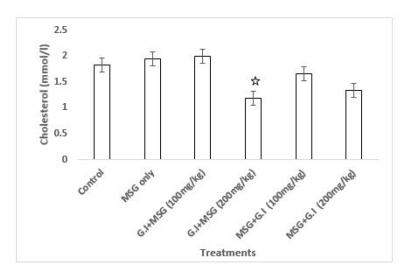


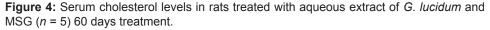


Figures 3 and 4 show that the mean value of protein and cholesterol, respectively, in rats fed with MSG alone were higher than others.



**Figure 3:** Serum protein levels in rats treated with aqueous extract of *G. lucidum* and MSG (n = 5) 60 days treatment.





The animals fed with MSG alone had the highest level of serum protein (76 g/L) which reduced slightly (73 g/L) in rats treated with 100 mg/kg *G. lucidum* and MSG, concurrently. The protein level reduced further in rats treated with the mushroom (200 mg/kg) and MSG concomitantly. Animals treated with MSG for 30 days before administrating

the extract of the mushroom (200 mg/kg) had the lowest protein (66 g/L) which was insignificant reduction compared to those treated with MSG (76 g/L).

There was no change in serum cholesterol level in the animals fed with MSG alone. The serum cholesterol significantly reduced in rats treated with *G. lucidum* and MSG concurrently even below the level in the control. Cholesterol level in rats first treated with MSG for 30 days before treating with the mushroom (100 mg/kg body weight) showed to some extent normal level of cholesterol as shown in Figure 4.

#### DISCUSSION

Haematological studies are important in investigating the degree of injury to blood and in determining stresses caused by nutritional, environmental, and pathological factors (Afolabi *et al.* 2010; Mashi *et al.* 2019).

Reduced RBCs, WBCs and NEU in rats fed with MSG only might have resulted from physiological reaction to the introduction of MSG (Obochi *et al.* 2009; Muhammad *et al.* 2014). According to Arunima *et al.* (2013), blood profile can change drastically under stress condition, infections and introduction of toxins. Decrease in RBCs may have occurred as a result of stress on the cells due to oxidative activities of MSG (Barnard *et al.* 2002). RBC is responsible for carriage of oxygen and carbon dioxide in the body. Hence, a reduced RBC count indicates a decrease in the level of oxygen that would be carried to the tissues and the level of carbon dioxide returned to the lungs (Ugwuene 2011; Isaac *et al.* 2013).

Low WBCs and RBCs in rats treated with doses of MSG only may be due to rapid destruction of the cells by the MSG and this could result in anaemia (Egbuonu *et al.* 2010). In this research the administration of MSG raised the level of oestrogen which could have caused the decrease in the HGB and RBCs. Arunima *et al.* (2013), also reported depletions of RBCs and HGB content by the administration of oestrogens which resulted in hyperlipaemia and haemodilution.

MSG in food provides a flavour that resembles naturally occurring free glutamate which is different from bitter, sweet, salt and sour tastes (Egbuonu *et al.* 2010). It stimulates taste and improves appetite but reports also showed that MSG is toxic to living cells (Egbuonu *et al.* 2010). Low HGB concentration in rats fed with MSG only showed weakness of the animals while those treated with doses of *G. lucidum* showed traces of recovery from low HGB as seen in Table 1.

Tawfik and Al-Badr (2012) reported that the kidney and liver of rats increased significantly in weight after feeding them with MSG at two different doses. They concluded that the increase could be from a rise in the activity of inflammatory agents that increased the size of the tissues of the liver and kidney. Result of the present work (Figure 1) also recorded significant increase in the weight of the liver in rats fed with MSG. The increase in weight of the kidney and uterus were not significant probably because the treatment doses of MSG used in this work was 6 times less than that used by Tawfik and Al-Badr (2012). In another experiment, MSG was observed to have toxic effects on the liver of adult Wister rats at high doses and the expansion may have affected the function of the liver (Eweka, Igbigbi and Ucheya 2011).

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ble 1: Heamatological parameters of the rats treated with aqueous extract of G. lucid
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Parameters	Control	MSG only	G. Iucidum (100 mg/kg) + MSG	G. Iucidum (200 mg/kg) + MSG	MSG + G. lucidum (100 mg/kg)	MSG + G. lucidum (200 mg/kg)
WBC (10 <sup>3</sup> /µL)	8.35 ± 0.071 ª	3.96 ± 0.014 <sup>b</sup>	8.605 ± 0.007°	8.506 ± 0.008 <sup>d</sup>	8.653 ± 0.004°	8.652 ± 0.003 <sup>c</sup>
RBC (10 <sup>6</sup> /µL)	7.853 ± 0.004ª	5.925 ± 0.05 <sup>b</sup>	7.282 ± 0.002∘	7.787 ± 0.005ª	7.404 ± 0.002 °	7.703 ± 0.004 <sup>d</sup>
HGB (g/dL)	15.54 ± 0.014ª	11.39 ± 0.014 <sup>b</sup>	13.76 ± 0.014∘	14.845 ± 0.007 <sup>d</sup>	14.315 ± 0.021◎	14.5 ± 0.000⁴
НСТ (%)	43.605 ± 0.007 <sup>ab</sup>	37.315 ± 0.020 <sup>d</sup>	42.11 ± 0.014°	44.555 ± 0.007ª	41.945 ± 0.064 <sup>bc</sup>	44.615 ± 0.021ª
PLT (10 <sup>3</sup> /µL)	387.1 ± 0.141ª	433.1 ± 0.141 <sup>b</sup>	592.55 ± 0.778⁰	486.5 ± 0.000 <sup>d</sup>	576.20 ± 0.283 <sup>e</sup>	557.4 ± 0.141 <sup>f</sup>
MCV (fl)	55.63 ± 0.042ª	62.4 ± 0.212ª	56.94 ± 0.057ª	58.05 ± 0.071ª	56.90± 0.000ª	56.755 ± 0.007ª
MCH (pg)	19.81 ± 0.014ª	18.965 ± 0.020ª	18.63 ± 0.042ª	19.21 ± 0.014ª	18.56 ± 0.014ª	$18.175 \pm 0.035^{a}$
MCHC (%)	35.71 ± 0.014ª	30.455 ± 0.007 <sup>b</sup>	34.255 ± 0.007ª	33.215 ± 0.021ª	33.865 ± 0.021ª	33.955 ± 0.007ª
Neu	2.753 ± 0.004 ª	0.699 ± 0.001 <sup>b</sup>	1.858 ± 0.011⁰	1.754 ± 0.006⁰	1.754 ± 0.006°	1.615 ± 0.021⁰
Neu (%)	33.42 ± 0.028ª	16.15 ± 0.071 <sup>b</sup>	29.445 ± 0.007ª	22.23 ± 0.042°	32.875 ± 0.035ª	29.74 ± 0.057 <sup>d</sup>
Lym (%)	57.355 ± 0.431 ª	77.185 ± 0.049 <sup>b</sup>	66.74 ± 0.057°	70.555 ± 0.007 <sup>d</sup>	54.65 ± 0.212 <sup>e</sup>	63.45 ± 0.141 <sup>f</sup>

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Vinodini *et al.* (2010) reported that MSG exposure to rats may probably exert adverse effect on renal functions. This study indicates that there was significant increase in NEU count which was significantly low in the group of rats that were fed with MSG only. The reason for the reduction could be a direct harmful impact of MSG on blood NEUs or injurious effect on blood production in the bone marrow, especially on the progenitor cells (Hall 2011; Ashaolu *et al.* 2011). The first line of defense against invading microorganism, toxic substances and foreign substances come from NEUs along with monocytes, which underscores the essential role NEUs in the body defense (Hall 2011).

The cholesterol level was slightly higher than the control in the rats treated with MSG only and was reduced to almost normal when treated with MSG + 200 mg/kg (body weight) *G. lucidum.* Walaa, Sanaa and Eldurssi (2012) also reported increase in cholesterol in rats fed with MSG only. According to Airaodion *et al.* (2019) MSG also increased total protein and cholesterol and induced fibroid in experimental rats. The protein level was however high in the rats fed with MSG only. This increase in protein may be related to the effect of MSG on the specific genes encoding for these protein as demonstrated in a study by Radwan (2005) who revealed that coumarin caused qualitative and quantitative changes in tissues (brain, liver and kidney) protein fractionation pattern of chicken.

Cholesterol is useful for the normal function of all animal cells and is a basic component of their cell membranes. However, too low cholesterol was reported to be detrimental to the body (Alagwu *et al.* 2011; Samarghandian *et al.* 2011). Alagwu *et al.* (2011) reported hypocholesteremic action in albino rats fed with honey where total cholesterol was significantly lower compared to controls. Hypocholesterolemia has also been reported to be associated with depression and suicide attempt in adults of the Mexican population. It was linked with increased risk of death due to injuries or suicide (Wu *et al.* 2016; Segoviano-Mendoza *et al.* 2018; Messaoud *et al.* 2017). Significantly low cholesterol observed in the present research could be a hypocholesteremic action. On the contrary, *G. lucidum's* hypocholesterolemic properties was recommended for the treatment of high serum cholesterol concentration and amelioration of hyperglycaemia (Wang *et al.* 2012; Meneses *et al.* 2016).

The oestrogen level was also significantly high in rats treated with MSG only, which may have led to increased proliferation of cells because the proliferation was determined by the availability of oestrogen (Obochi *et al.* 2009). The normal range of serum oestrogen levels in intact female rats reported by Shi-Juan *et al.* (1996) is 30 pg/mL–500 pg/mL depending on the stages of the estrus cycle. Synthetic chemicals such as polychlorinated biphenyls (PCBs) can introduce oestrogen-like hormones into the body thereby increasing the size of the fibroid (Fuschs-Young *et al.* 1996). High level of oestrogen has been reported to be the most common cause of fibroid and painful menstruation (Szekeres 1996; Barnard, Scialli and Bobela 2002). The result of the current experiment showed that the oestrogen level in the rats fed with MSG and *G. lucidum* reduced even below the control and this could be an indication that *G. lucidum* has the attribute of stopping cell proliferation. Oestrogens carry out their action by binding to a high affinity nuclear receptor, the oestrogen receptor (ER) (Jiang *et al.* 2006). *G. lucidum* was shown to inhibit the spread of breast cancer MCF-7 and MDA-MB-231 cells by the inflecting the oestrogen receptor (ER) and NF- $\kappa$ B signaling (Jiang *et al.* 2006).

According to Ofodile *et al.* (2005), Yihuai *et al.* (2003), Ofodile, Ogbe and Oladipupo (2011), *G. lucidum* possesses numerous pharmaceutically active compounds, polysaccharides, triterpernoids, adenosine and its derivatives, protein and used to treat various human diseases such as hepatitis, hypertension, hyperglycemia and cancer. Reishi dietary supplements (DS) are valued for their immunomodulating, anticancer, antiviral and antitumour actions (Wasser and Weis 1999; Kashimoto *et al.* 2010) and as an

adjunct therapy for cancer (Zhong *et al.* 2019). Commercially available whole *Ganoderma* mushroom GLE also showed selective inhibition of breast cancer cell (Martinez-Montemayor *et al.* 2019)

### CONCLUSION

MSG altered the heamatological, protein, cholesterol and oestrogen levels of the animals and weakened the rats but *G. lucidum* mitigated the impact of MSG on the weight of the liver, oestrogen levels and heamatological parameters in the rats.

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