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Improvement of Glucose Homeostasis and Hepatoprotective Effects of *Ganoderma applanatum* Polysaccharides Extract in MACAPOS 2 Obese Rats

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Abstract

The hyperglycaemia and hepatic steatosis are metabolic complications associated with obesity. This study was designed to investigate the glucose homeostasis improvement and hepatoprotective effects of *Ganoderma applanatum* polysaccharides (GAPs) extract in MACAPOS 2 obese rats. Obesity was induced in 6-8 week-old *Wistar* rats with a local high-fat diet for 16 weeks. The GAPs extract was orally administered to obese rats for two months at different doses (50, 100, and 150 mg/kg body weight). At the end of the study, the effects of GAPs extract were evaluated on blood glucose through oral glucose tolerance, insulin tolerance, and fasting blood glucose tests. Then, the hepatoprotective properties through lipid peroxidation (malondialdehyde and hydroperoxide), antioxidant activities (SOD, CAT, GPx), cytolysis function (ASAT, ALAT), and liver histological structure. The results showed that GAPs extract improved (reduces) glucose levels after the oral glucose tolerance test and significantly reduced blood glucose. Moreover, the administration of GAPs extract improved the liver architecture and significantly reduced hepatic malondialdehyde and hydroperoxide. ALAT and ASAT activities were significantly reduced whereas hepatic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were significantly increased in GAPs-treated groups. The GAPs extract administered at different doses showed beneficial effects on glucose homeostasis, and hepatoprotective potential in MACAPOS 2 obese rats.

Keywords: *Ganoderma applanatum*; Glucose homeostasis; Hepatoprotective; MACAPOS 2 Obese rats; Polysaccharide

Introduction

Obesity has become a serious health problem across the world. Its prevalence has rapidly increased worldwide over the last decades, probably due to combined genetic predisposition and profound lifestyle changes including sedentary habits and high-calorie dietary intake [1,2]. In 2016, over 39% of adults were overweight worldwide, and one-third were obese. About 51% of the global population is estimated to be obese by 2030 [3]. High consumption of energy-dense diets, such as high-fat diets with reduction of physical activities, is believed to be the leading cause of obesity in susceptible individuals [4-6].

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Obesity is associated with various phenotypic and metabolic complications including increased body weight, low-grade and systemic inflammation, insulin resistance, hyperinsulinemia, hyperleptinemia, hyperglycaemia, hyperlipidaemia, and hepatic steatosis [7-9]. Therefore, obesity represents a major medical and socio-economic burden, which leads to chronic metabolic development and psychological disorders like diabetes mellitus, hypertension, coronary artery disease, liver failure, and cancer [10,11]. Thus, the reduction of obesity is important to prevent metabolic disorders. Currently, lifestyle intervention and pharmacotherapy represent the common choices for long-term weight reduction, but their effectiveness is usually largely compromised due to poor compliance. In addition, pharmacotherapy is seriously challenged by the inevitable side effects [12]. Many efforts have therefore been devoted to developing more tolerated anti-obesity food bioactive [13].

Polysaccharides, important biological macromolecules, are widely found in nature and are considered to be the major bioactive components in edible and medicinal mushrooms [14]. They have recently attracted attention due to their no or less toxicity, their availability from widespread sources, and their large spectrum of activities. These polysaccharides are well known for their health benefits, such as anti-tumor, anti-inflammatory, anti-oxidant, and anti-dyslipidemia effects [15-17]. They can reduce body weight, leaky gut, and low-grade inflammation in various tissues, by physicochemical properties, such as water retention, and/or by prebiotic activity, such as altering the gut commensal microbiota and production of microbiota-derived metabolites [18,19]. Polysaccharides can also lower blood sugar and cholesterol levels by inhibiting the activity of digestive enzymes [20]. Therefore, the use of functional foods that are rich in polysaccharides provides health benefits and therapeutic strategies against obesity.

G. applanatum is a medicinal farming crop that grows within dead and living trees. Studies proved this genus to have potent biological properties such as antioxidative, antiviral, antitumor and immunomodulating activities [21]. These effects are attributed to a wide variety of bioactive components, such as triterpenes, polysaccharides, lectins, sterols, alkaloids, polypeptides, amino acids, phenols, and coumarins [22,23]. In addition, many *Ganoderma* species are commonly sold in Chinese herbal medicine markets for the prevention and treatment of various chronic diseases. Therefore, this study aimed to investigate the glucose homeostasis improvement and hepatoprotective effects of water-soluble polysaccharides crude extract of *G. applanatum* in MACAPOS 2 obese rats.

Methodology

Plant Material

The plant material consisted of fresh fruiting bodies of *G. applanatum*. It was collected in October 2016 in Santa (Northwest region of Cameroon) and identified at the Department of Biological Sciences, University of Bamenda by Pr. Rosemary Tonjock Kinge [24,25]. Then, *G. applanatum* was transported to the Laboratory of Phytoprotection and Valorization of Genetic Resources of the Biotechnology Centre of Nkolbisson, University of Yaoundé I. It was shade-dried for one month, then cut into small pieces, and powdered with a grinder. Thereafter the powered sample was stored at 20°C.

Animals

The male albino 6-8 week-old *Wistar* rats (80-100 g) were raised in a pathogen-free environment under normal environmental conditions in the animal house of the Laboratory of Human Metabolism and Non-Communicable Diseases of the Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaoundé, Cameroon. These animals were kept in polypropylene cages with a metal mesh cover to acclimatize at an ambient temperature of about 25°C, 12 hours of light/dark cycle, and adequate ventilation. Then, food and tap water were given *ad libitum*. The animal handling and experiments were performed according to the European Union directives on ethical evaluation of animal experiments [26], adopted by the Cameroon Institutional National Ethics Committee, Ministry of Scientific Research and Innovation (N°: FWA-IRD 0001954).

Polysaccharides extract preparation

The water-soluble polysaccharides of *G. applanatum* were extracted as described in our previous study [24]. Briefly, 250 g of previously obtained powder was boiled with 5 L of distilled water at 100°C for 3 h. The mixture was filtered, and the supernatant was collected and precipitated at 4°C for 24 h with 95°C ethanol (1:3 (v/v)). The polysaccharides were collected by centrifugation (4000 g; 10 min), then dissolved in distilled water, and lyophilized. Water-soluble polysaccharides powder of *G. applanatum* obtained (yield: 2.14%) was stored at 4°C until use to prevent any enzymatic reaction. The percentage of polysaccharides yield was calculated according to the following formula [25].

$$\text{Polysaccharides yield (\%)} = (W_1/W_2) \times 100$$

Where W_1 was the weight of polysaccharides powder (g) and W_2 was the powder sample weight (g).

Diet composition and obesity induction

All ingredients used to prepare the standard and high-fat diet were obtained in Yaoundé market and processed according to the method described by Kamgang et al. [27].

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Table 1: Diet composition per 1000 g [27].

Groups	Ingredients in g										
	Maize	Wheat	Stepped cassava	Sucrose	Soya bean	Fish flour	Cabbage palm	Palm oil	Bones flour	Vitamins complex	Energy (kcal/kg)
ND	250	400	-	-	150	100	80	-	10	10	3400
HFD	80	110	220	50	280	30	-	200	20	10	4730

ND: Normal diet; HFD: High-fat diet

This high-fat diet was previously used in our laboratory to induce obesity by feeding the rat with it for a period of 16 weeks [24]. In fact, after two weeks of acclimatization period in the animal house, rats were randomly subjected to a normal diet and high-fat diet (Table 1). The food and tap water intake were measured every two days and the animal's weight was taken weekly for 16 weeks. At the end of this period, the Lee index allowed the selection of obese rats and was calculated by dividing the cube root of body weight (g) by the nose-to-anus length (cm) [28].

Research design and dose selection

The extrapolated doses from the traditional healer were used in this study. The experimental design and allocation of the rats were as follows:

- Group 1 or normal control (NC) group: five rats, were fed with normal diet, and received distilled water
- Group 2 or obese control (ObC) group: five obese rats, were feed with HFD, and received distilled water
- Group 3 or orlistat control (OrC) treated group: five obese rats, were feed with HFD, and received orally the reference drug orlistat (F. Hoffmann-La Roche SA) at 20 mg/kg body weight
- Group 4 or *G. applanatum* polysaccharides (GAPs50) extract treated group: five obese rats, were feed with HFD, and received 50 mg/kg of body weight (b/w)
- Group 5 or *G. applanatum* polysaccharides (GAPs100) extract treated group: five obese rats, were feed with HFD, and received 100 mg/kg of body weight (b/w)
- Group 6 or *G. applanatum* polysaccharides (GAPs150) extract treated group: five obese rats, were feed with HFD and received 150 mg/kg of body weight (b/w)

Evaluation of the effects of GAPs extract on glucose homeostasis

Oral glucose tolerance test

At the end of two months of treatment, the oral glucose tolerance test has been performed (day 56). In fact, after 12 hours an overnight fasting, rats were administered orally glucose at the concentration of 2.5 g/kg body weight, and blood glucose level was determined with a strip-operated blood glucose sensor (CERA-CHEK ™ 1 Code) [29-32]. The blood was collected from the tip of the tail vein at 0 (just before the administration of glucose), 30, 60, and 120 min, respectively. According to the manufacturer's instructions, rats with fasting blood glucose level of 140 mg/dL and above were considered hyperglycaemic meanwhile rats with fasting blood glucose of below 70 mg/dL were categorized as hypoglycaemic.

Insulin tolerance test

At the end of two months of treatment, the insulin tolerance test has been performed (day 58). After 12 hours an overnight fasting, rats were injected (sub-cutaneous injection) an insulin (ACTRAPID, Human insulin ADNr, Novo Nordisk Laboratory) at the concentration of 0.8 UI/kg body weight. Then, the blood glucose level was determined with a strip-operated blood glucose sensor (CERA-CHEK ™ 1 Code) and CERA-CHEK advantage test strips, using blood collected from the tip of the tail vein at 0 (just before the injection of insulin), 15, 30, and 60 min, respectively.

Fasting blood glucose

At day 60, the fasting blood glucose of each of the rats was determined with a strip-operated blood glucose sensor (CERA-CHEK ™ 1 Code) and CERA-CHEK advantage test strips, using blood collected from the tip of the tail vein after an overnight fasting period of 12 hours.

Collection of serum and liver homogenates preparation

After two months of treatment, the 12 h overnight-fasted rats were sacrificed by decapitation under ether anaesthesia. The blood was collected in the dry tube, allowed to stand for at least 30 min at room temperature, and then centrifuged (2500 g, 15 min). The supernatant (serum) was collected.

After dissection under aseptic conditions, the liver was collected and weighed. It was immediately washed with an ice-cold saline solution (NaCl 0.9%) and weighed, and 200 mg were homogenized in 1 mL of a Tris-HCl (0.2 M, pH 7.4) buffer solution. The solutions were centrifuged (2500 g, 25 min) and the supernatants (homogenates) were collected.

The serum and homogenates were stored at -20°C in eppendorf tubes for biochemical analysis.

Evaluation of the hepatoprotective effects of GAPs extract

Calculation of the hepatosomatic index

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The hepatosomatic index (HI) was calculated according to the following formula [33].

HI (%) = (liver weight/rat body weight) x 100

Assessment of liver cytolysis function

This was achieved through the evaluation of the serum activity of aspartate amino transaminase (ASAT) and alanine amino transaminase (ALAT). ASAT and ALAT activities were evaluated using commercial detection kits (Biolabo, France-Abbott Laboratories, Germany) based on the manufacturer's instructions.

Evaluation of the ability to protect the liver against lipid peroxidation

The ability of GAPs extract to protect the liver against lipid peroxidation was evaluated through malondialdehyde and hydroperoxide levels in the homogenates. Malondialdehyde level was measured according to the method described by Yagi [32] and hydroperoxide level was measured via the oxidation of (Fe^{2+}) into (Fe^{3+}) using the FOX reagent.

Hepatic antioxidant activities evaluation

The antioxidant enzyme (SOD, CAT and GPx) activities were evaluated. The superoxide dismutase (SOD) activity was measured according to the method described by Misra and Fridovich [29], by measuring the ability of SOD to inhibit the photoreduction of nitro blue tetrazolium (NBT). Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%. Riboflavin (2 mM final concentration) was added to start the reaction and absorbance was recorded at 480 nm between 20-80 s. The catalase (CAT) activity was measured according to the method described by Sinha [31]. The rate of H_2O_2 decomposition was followed by measuring the decrease in absorbance at 570 nm. The glutathione peroxidase (GPx) activity was measured by Ellman [30] method based on the development of a yellow color when 2,2-dithio-5,5'-nitrodibenzoique acid (DTNB) is added to compounds containing sulfhydryl groups.

Evaluation of the effects of GAPs extract on the histological structure of the liver

This was done through the hematoxylin and eosin staining coloration. The fresh liver tissue samples were fixed in formol solution (10%). The tissues were dehydrated in ascending concentrations of ethanol (70-100%), then cleared with xylene. All tissues were then embedded in the paraffin wax and sliced into 4 µm thick sections. All samples were then stained with hematoxylin and eosin, then examined by a light microscope (Olympus Optical, Tokyo, Japan).

Statistical analysis

The results were expressed as mean ± standard error of the mean. The statistical analysis was performed using Graph Pad Prism 7.0.0. Tukey's test was used to compare the means of different groups. The difference was significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Results

Effects of *G. applanatum* polysaccharides extract on body weight

As shown in Table 2, there was a significant ($p < 0.001$) difference between normal control and obese groups concerning the body weight gain. After two months of treatment, the body weight gain in the highest dose GAPs150 (6.31%) was lower than that of ObC (7.72%) rats.

Table 2: Effects of GAPs extract on body weight gain after two months of treatment.

Groups	Body weight		
	Initial body weight (g)	Final body weight (g)	Weight gain (%)
NC	255 ± 2.35	305.4 ± 3.8	19.76 ± 0.76
ObC	380.6 ± 2.54***	410 ± 3.89***	7.72 ± 1.11***
OrC	366.2 ± 1.83***	378.2 ± 4.6^{***c}	3.30 ± 1.69***
GAPs50	377.6 ± 2.98***	408.2 ± 5.6$^{***\delta}$	8.09 ± 0.96$^{***\alpha}$
GAPs100	371.8 ± 1.74***	410.2 ± 4.8$^{***\delta}$	10.32 ± 0.98$^{**\alpha}$
GAPs150	370.6 ± 3.09***	394 ± 5.2***	6.31 ± 0.99***

n = 5; NC: normal control; ObC: obese control, OrC: orlistat control; GAPs 50: obese rats treated with GAPs extract 50 mg/kg; GAPs 100: obese rats treated with GAPs extract 100 mg/kg, GAPs 150: obese rats treated with GAPs extract 150 mg/kg; GAPs: G. applanatum polysaccharides; significant difference: * p < 0.05, ** p < 0.01, *** p < 0.001 compared to NC; ^{a} p < 0.05, ^{b} p < 0.01, ^{c} p < 0.001 compared to ObC, and $^{\alpha}$ p < 0.05, $^{\beta}$ p < 0.01, $^{\delta}$ p < 0.001 compared to OrC.

Effects of GAPs extract on glucose homeostasis

Glucose tolerance test

The results of the oral glucose tolerance test are presented in Figure 1. The analysis of the area under the curve (AUC) shows that blood glucose was higher in the obese control (ObC) group compared to the normal control (NC) group ($p < 0.001$). The administration of GAPs extract (GAPs 50, 100, and 150) limited the increase of blood glucose, as shown by the AUC compared to the ObC group. The same observation was noted with the orlistat control (OrC) group when compared to the ObC group.

Effect on insulin sensitivity

During the insulin sensitivity assessment, the blood glucose levels progressively increased in obese control (ObC) rats compared to normal control (NC) rats as illustrated by AUC (Figure 2). The administration of GAPs (GAPs 50, 100, and 150) decreased the blood glucose level, as shown by the AUC compared to the ObC group. In the orlistat control (OrC) rats, a significant ($p < 0.05$) decrease of blood glucose was also observed.

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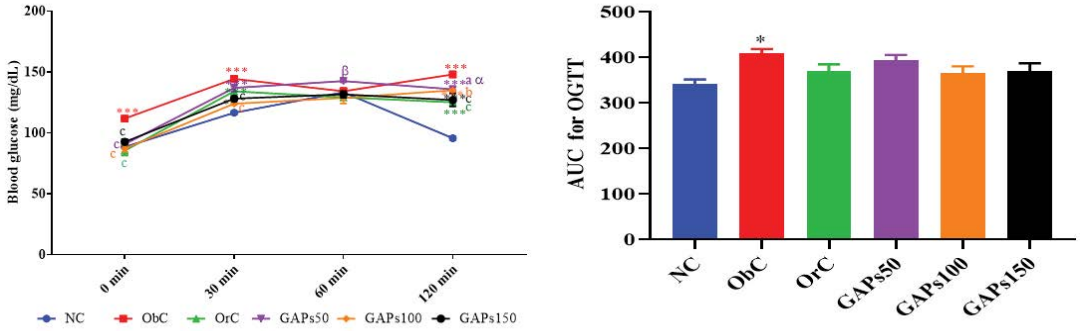
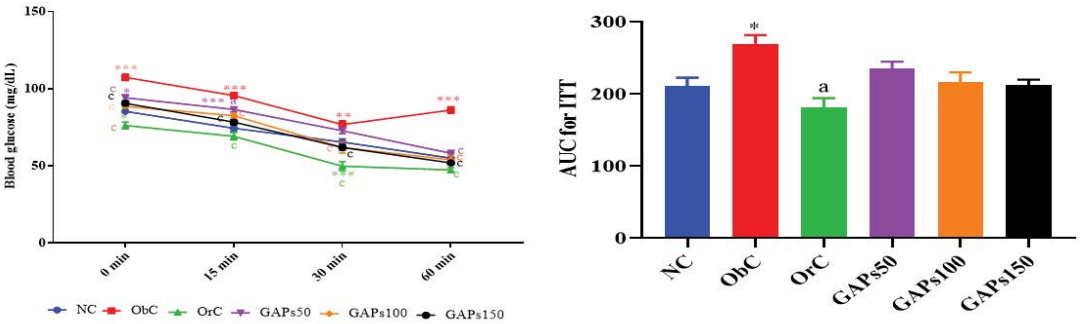


*n = 5; AUC: area under the curve; OGTT: oral glucose tolerance test; NC: normal control; ObC: obese control, OrC: orlistat control; GAPs 50: obese rats treated with GAPs extract 50 mg/kg; GAPs 100: obese rats treated with GAPs extract 100 mg/kg, GAPs 150: obese rats treated with GAPs extract 150 mg/kg; GAPs: G. applanatum polysaccharides; significant difference: * p < 0.05, ** p < 0.01, *** p < 0.001 compared to NC; [a] p < 0.05, [b] p < 0.01, [c] p < 0.001 compared to ObC, and [α] p < 0.05, [β] p < 0.01, [δ] p < 0.001 compared to OrC.*

Figure 1: Blood glucose levels and the calculated area under the curve (AUC) after the oral glucose tolerance test of GAPs in all groups of rats.



*n = 5; AUC: area under the curve; OGTT: oral glucose tolerance test; NC: normal control; ObC: obese control, OrC: orlistat control; GAPs 50: obese rats treated with GAPs extract 50 mg/kg; GAPs 100: obese rats treated with GAPs extract 100 mg/kg, GAPs 150: obese rats treated with GAPs extract 150 mg/kg; GAPs: G. applanatum polysaccharides; significant difference: * p < 0.05, ** p < 0.01, *** p < 0.001 compared to NC; [a] p < 0.05, [b] p < 0.01, [c] p < 0.001 compared to ObC, and [α] p < 0.05, [β] p < 0.01, [δ] p < 0.001 compared to OrC.*

Figure 2: Blood glucose levels and the calculated area under the curve (AUC) after the insulin tolerance test of GAPs in all groups of rats.

Effect on fasting blood glucose

The results of the administration of GAPs on treated groups after two months of treatment are presented in Figure 3. The fasting blood glucose significantly ($p < 0.001$) increased in the ObC group compared to the NC group. However, GAPs extract treatment at 50 mg/kg, 100 mg/kg, and 150 mg/kg significantly ($p < 0.001$) lowered fasting blood glucose.

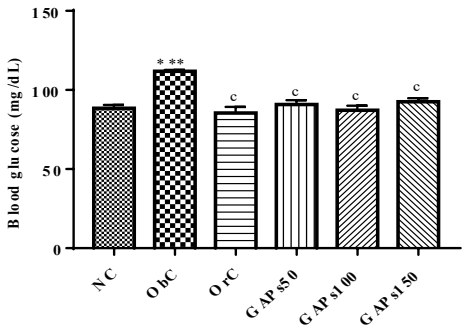
Hepatoprotective effects of GAPs extract

Effect on liver somatic index

Table 3 shows the results of the effects of GAPs on the hepatosomatic index (HI) after two months of treatment. There was no significant difference between ObC, and tests groups (GAPs 50, 100, 150, and OrC). However, a decrease of HI was observed after the administration of GAPs extract at 50, 100, and 150 mg/kg of body weight. This decrease was significant ($p < 0.05$) in the group receiving GAPs extract at 150 mg/kg of body weight.

Ability to protect against lipid peroxidation

Figure 4 presents the results of the ability of GAPs extract to protect the liver against lipid peroxidation through evaluation of MDA, and hydroperoxide levels after two months of treatment. The level of MDA and hydroperoxide was significantly ($p < 0.001$) high in the ObC compared to NC rats. The administration of GAPs extract at 50, 100, and



*n = 5; NC: normal control; ObC: obese control, OrC: orlistat control; GAPs 50: obese rats treated with GAPs extract 50 mg/kg; GAPs 100: obese rats treated with GAPs extract 100 mg/kg, GAPs 150: obese rats treated with GAPs extract 150 mg/kg; GAPs: G. applanatum polysaccharides; significant difference: * p < 0.05, ** p < 0.01, *** p < 0.001 compared to NC; [a] p < 0.05, [b] p < 0.01, [c] p < 0.001 compared to ObC, and [α] p < 0.05, [β] p < 0.01, [δ] p < 0.001 compared to OrC.*

Figure 3: Effect of GAPs on fasting blood glucose of rats.

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Table 3: Effects of GAPs extract on hepatosomatic index after two months of treatment.

Groups	Hepatosomatic index (%)
NC	3.37 ± 0.09
ObC	2.52 ± 0.07***
OrC	2.59 ± 0.09***
GAPs50	2.40 ± 0.06***
GAPs100	2.37 ± 0.05***
GAPs150	2.26 ± 0.04***α

*n = 5; NC: normal control; ObC: obese control, OrC: orlistat control; GAPs 50: obese rats treated with GAPs extract 50 mg/kg; GAPs 100: obese rats treated with GAPs extract 100 mg/kg, GAPs 150: obese rats treated with GAPs extract 150 mg/kg; GAPs: G. applanatum polysaccharides; significant difference: * p < 0.05, ** p < 0.01, *** p < 0.001 compared to NC; a p < 0.05, b p < 0.01, c p < 0.001 compared to ObC, and α p < 0.05, β p < 0.01, δ p < 0.001 compared to OrC.*

150 mg/kg of body weight significantly ($p < 0.001$) decreased MDA and hydroperoxide levels. The reference drug (orlistat) also reduced the levels of MDA and hydroperoxide.

Effect on hepatic antioxidant activities

The results of the effect of GAPs extract on hepatic antioxidant activities through evaluation of the enzymatic activity of SOD, CAT, and GPx after two months of treatment are presented in Figure 5. SOD, CAT activities, and GSH levels were significantly ($p < 0.001$) high in the ObC compared to NC rats. The administration of GAPs extract at 50, 100, and 150 mg/kg of body weight significantly ($p < 0.001$) lead to a decrease in SOD, CAT and GPx activities. The same results were obtained in rats treated with orlistat.

Effect on serum transaminase activities

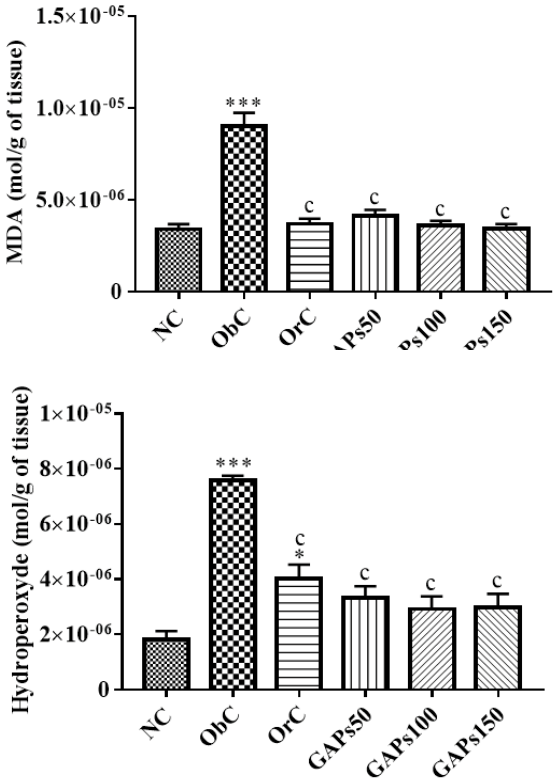
Figure 6 shows that the activities of serum transaminase (ALAT, and ASAT) significantly ($p < 0.001$) increased in obese control (ObC) compared to normal control (NC) groups. After two months of treatment, a significant ($p<0.001$) decreases of the above serum transaminase was noted in GAPs-treated groups (GAPs 50, 100, and 150), and the OrC group.

Effect on the histological structure of the liver

In Figure 7, the normal control (NC) rats revealed the normal structure of the liver with an intact central vein and normal hepatocytes with curved euchromatic nuclei radiating from the central vein. A dilated central vein was observed in the liver tissues of obese control (ObC) rats. An improvement in the hepatocyte structure and normal-sized central veins was observed in GAPs-treated groups (GAPs 50, 100, and 150), and the orlistat control (OrC) group.

Discussion

Mushrooms have been appreciated owing to their taste and favour for a long time. They possess numerous compounds with nutraceutical and therapeutic significance [34]. *Ganoderma* is a genus of polypore mushrooms that grow in decaying logs or tree stumps. The fruit body of *Ganoderma*, for its perceived benefits, has gained widely popular use as a dietary supplement in China, Japan, North America, and other regions of the world including Cameroon [35]. The most commonly characterized medicinal mushroom of the genus *Ganoderma* is *G. lucidum*, and it has been used in oriental medicine as a traditional remedy [36]. As per traditional Chinese medicine, *G. lucidum* supports numerous health benefits and many studies have demonstrated a vast array of pharmacological effects [37-39]. *G. lucidum* showed enormous potential in the treatment of modern deadly diseases such as antitumor [40,41]], antioxidant [42], immunomodulation [43], hepatoprotection [44], hypoglycaemic effect [45,46], reduction of blood cholesterol [47,48]. These biological activities of the genus *Ganoderma* have been considered due to the main compounds such as polysaccharides and terpenoids. In addition, other metabolites



*n = 5; MDA: malondialdehyde; NC: normal control; ObC: obese control, OrC: orlistat control; GAPs 50: obese rats treated with GAPs extract 50 mg/kg; GAPs 100: obese rats treated with GAPs extract 100 mg/kg, GAPs 150: obese rats treated with GAPs extract 150 mg/kg; GAPs: G. applanatum polysaccharides; significant difference: * p < 0.05, ** p <0.01, *** p <0.001 compared to NC; a p < 0.05, b p < 0.01, c p < 0.001 compared to ObC, and α p < 0.05, β p < 0.01, δ p < 0.001 compared to OrC.*

Figure 4: Effects of GAPs on MDA, and hydroperoxide of rats.

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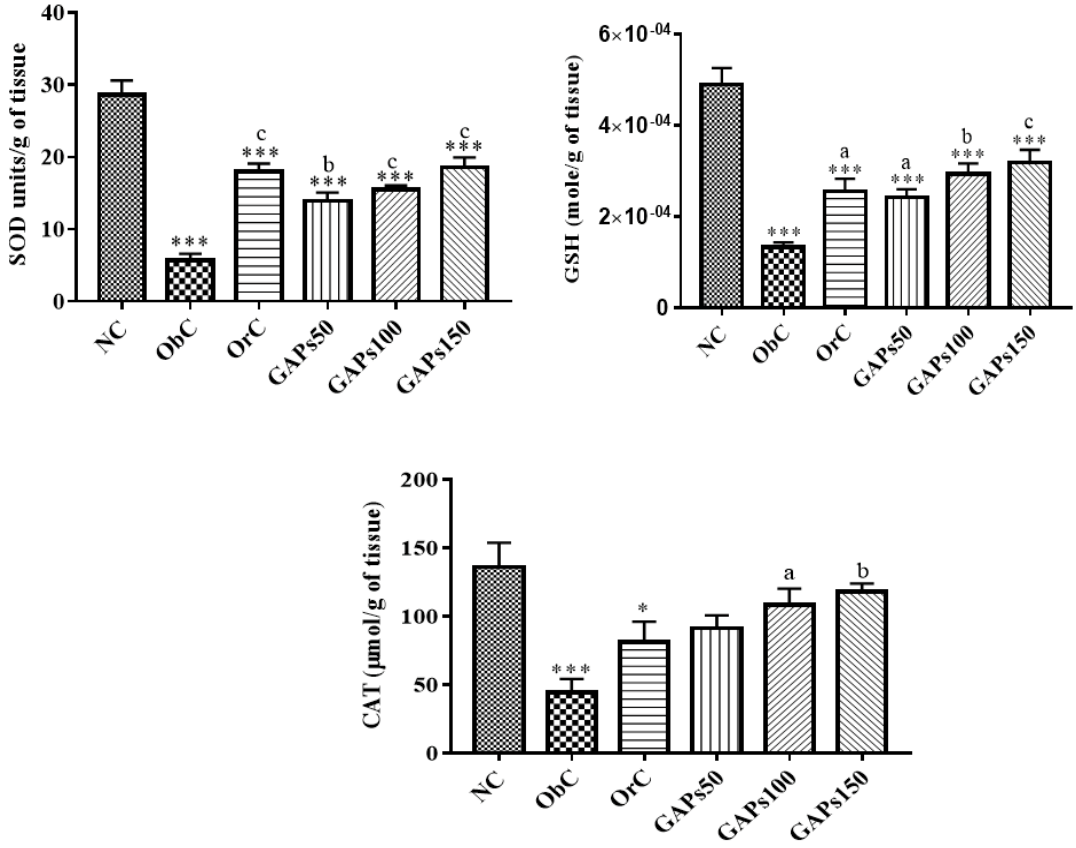
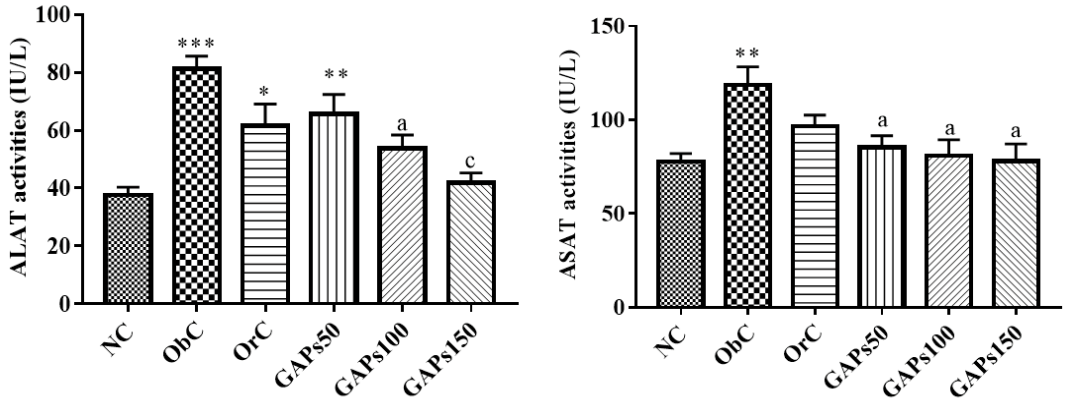


*n = 5; CAT: catalase; SOD: superoxide dismutase; GSH: glutathione, NC: normal control; ObC: obese control, OrC: orlistat control; GAPs 50: obese rats treated with GAPs extract 50 mg/kg; GAPs 100: obese rats treated with GAPs extract 100 mg/kg, GAPs 150: obese rats treated with GAPs extract 150 mg/kg; GAPs: G. applanatum polysaccharides; significant difference: * p < 0.05, ** p < 0.01, *** p < 0.001 compared to NC; [a] p < 0.05, [b] p < 0.01, [c] p < 0.001 compared to ObC, and [α] p < 0.05, [β] p < 0.01, [δ] p < 0.001 compared to OrC.*

Figure 5: Effects of GAPs on SOD, GSH, and CAT of rats.



*n = 5; ALAT: alanine transaminase; ASAT: aspartate transaminase; NC: normal control; ObC: obese control, OrC: orlistat control; GAPs 50: obese rats treated with GAPs extract 50 mg/kg; GAPs 100: obese rats treated with GAPs extract 100 mg/kg, GAPs 150: obese rats treated with GAPs extract 150 mg/kg; GAPs: G. applanatum polysaccharides; significant difference: * p < 0.05, ** p < 0.01, *** p < 0.001 compared to NC; [a] p < 0.05, [b] p < 0.01, [c] p < 0.001 compared to ObC, and [α] p < 0.05, [β] p < 0.01, [δ] p < 0.001 compared to OrC.*

Figure 6: Effect of GAPs on serum activities of ALAT, and ASAT of rats.

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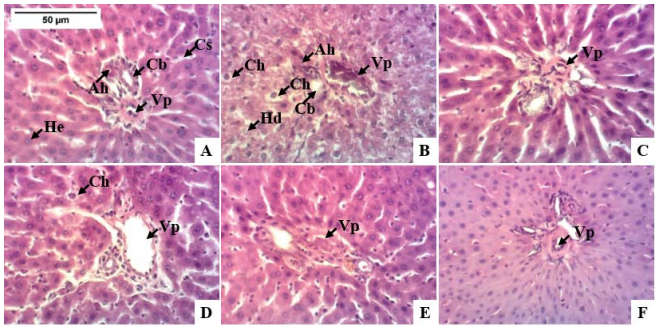

A: normal control; B (ObC): obese control; C (OrC): orlistat control; D (GAPs 50): obese rats treated with GAPs extract 50 mg/kg; E (GAPs100): obese rats treated with GAPs extract 100 mg/kg; F (GAPs150): obese rats treated with GAPs extract 150 mg/kg; GAPs: G. applanatum polysaccharides; Vp: portal vein; He: hepatocyte; Cs: sinusoid capillary; Ah: hepatic artery; Cb: biliary canaliculi; Hd: degenerated hepatocyte; Ch: hepatic cytolysis.

Figure 7: Effect of GAPs on liver histology of rats.

such as lectins, proteins, adenosine, and sterols have also been found to play an important role in these functions [49,50]. This study aimed to investigate the glucose homeostasis improvement and hepatoprotective effects of *G. applanatum* polysaccharides extract in MACAPOS 2 obese rats.

Feeding rodents an energy-dense food is a good strategy to induce obesity that resembles human obesity [51-53]. It is well known that obesity, dyslipidaemia, and glucose intolerance have indispensable relationships. The oral glucose tolerance test indicated that obese rats exhibited impaired glucose tolerance. This study demonstrated that oral administration of GAPs extract to obese rats decreased significantly blood glucose (Figure 1 and 2), increased insulin sensitivity (Figure 3), indicating an attenuation of dyslipidaemia and hepatic steatosis observed in MACAPOS 2 obese rat [24].

Oxidative stress, an imbalance between pro-oxidants and antioxidants, is involved in the pathogenesis of many diseases. Under normal physiological conditions, the elimination of reactive oxygen species (ROS) and the balance between the generation and removal of ROS are performed by intracellular antioxidants to resist oxidative damage. There is a close link between obesity and an increase in oxidative stress, and the production of pro-oxidant molecules [54]. Adipose tissue is capable of producing ROS. Indeed, NADPH oxidases (pro-oxidant enzymes) are present on adipocyte membranes and produce ROS which may be responsible for the oxidation of membrane lipids, especially polyunsaturated fatty acids characterized by a tissue increase in MDA, and hydroperoxyde. This increase in ROS production is followed by a decrease in anti-oxidant defences represented by SOD, CAT, and GPx [55]. Thus, the balance between generation and removal of ROS is broken, and excessive production of ROS will lead to oxidative stress and injuries of tissues [56,57]. MDA, and hydroperoxide, as lipid intermediates, are capable of disturbing the antioxidant defence and provide demonstrations of the pathogenic roles of oxidative stress [58]. In the present study, significantly increased activities of SOD, CAT, and GPx (Figure 5) as well as decreased contents of MDA, and hydroperoxide (Figure 4) in the liver tissue of obese rats treated with GAPs extract compared with the ObC group showed GAPs extract, as novel bioactive compounds could tend the antioxidative system in the liver to be normalized, thus improving liver function by accelerating regeneration and protection of hepatocytes.

It is well known that the activity of some enzymes in the serum is considered a biochemical, and sensitive indicator of hepatic injury [59]. It was reported that a high-fat diet could accelerate the formation of radicals *in vivo* and destroy the intrinsic antioxidant defence, thus reducing oxidative stress. Excess free radicals react with fatty acids, leading to the generation of lipid intermediates and the alteration of cellular membrane integrity [60]. The damaged permeability of the cell membrane results in that ALAT and ASAT could be leached out from hepatocytes into blood circulation [61]. In the present work, significantly increased serum activities of ALAT, and ASAT in ObC compared with NC groups (Figure 6) was observed. This indicated that hepatocytes were damaged, and their transport function as well as membrane permeability were altered, leading to the leakage of their contents out of the cells [62]. However, a significant decrease in the activities of ALAT and ASAT was observed after the administration of the GAPs extract (Figure 6). The results obtained suggest that GAPs extract could protect tissue membranes. GAPs extract, as a natural antioxidant supplementation, could recover the balance between generation and removal of ROS and reduce the level of oxidative stress [63]. Therefore, the elevated activities of ALAT and ASAT in serum could be reduced.

Furthermore, in comparison with the hepatic cellular architecture of rats from the normal control group, severe liver damages had occurred in the obese control group. These hepatic lesions were corrected in GAPs-treated groups. These findings were in line with the results of serum biochemical parameters (MDA, hydroperoxide, ALAT, and ASAT). However, other mechanisms of action associated with these hepatoprotective activities of GAPs extract need to be evaluated.

Conclusion

The results of this study showed the beneficial effects of *G. applanatum* polysaccharide extract on glucose homeostasis and liver protection against alterations due to the action of prooxidant agents. These beneficial effects result from the reduction of blood glucose, MDA, and hydroperoxide levels; an increase of ALAT, ASAT, SOD, CAT, GPx, and protection of the histological structure of the liver.

Competing Interests

The authors have declared that no competing interests exist.

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