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Effects of *Garnoderma lucidum* on Acetaminophen-Induced Liver Injury in Wistar Rats

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Abstract

Introduction: *Ganoderma lucidum* is considered to be a medicinal mushroom, widely used to prevent or treat different types of diseases including cancer, cardiovascular disease and hepatic dysfunction. This study aimed to evaluate the effect of *Ganoderma lucidum* on acetaminophen-induced liver injury in wistar rats.

Methods: Forty (40) male wistar rats were used for this study. Hepatoxicity was induced by oral administration of acetaminophenn (3000 mg/kg of body weight) for the last 21 consecutive days of the dietary of regimen *Ganoderma lucidum*. These rats were divided into eight cages each containing Five rats. Control Group 1 fed on feed and water only throughout the study, Group 2 received acetaminophen only, Group 3 received Acetaminophen + Standard drug (silymarin), Group 4 received Acetaminophen +100 mg/kg body weight of *Ganoderma lucium* extract, Group 5 received Acetaminophen + 200 mg/kg body weight of *Ganoderma lucidum* extract, Group 6 received Acetaminophen + 300 mg/kg body weight of *Ganoderma lucidum* extract , Group 7 received 100 mg/kg of *Ganoderma lucidum* extract, Group 8 received Acetaminophen+Standard Drug (silymarin) + 300 mg/kg body weight of *Ganderma lucidum* extract. Blood samples was collected via cardiac puncture within 24 hours of Sacrifice. The extent of the liver injury was determined by assessing the plasma levels of Tumor Necrosis Factor Alpha (TNF-α), Alpha Feto protein, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total bilirubin (TB), Conjugated bilirubin (CB), Unconjugated Bilirubin (UB), Albumin, Gamma Glutamyl Transaminase (GGT) and total protein (TP) using spectrophotometric method and ELISA as appropriate.

Results: Oral administration of Acetaminophen significantly increased the plasma levels of the parameters accessed, suggesting severe liver damage in the rats. However, the treatment of *Ganoderma lucidum* decreased these hepatotoxic indices at a significant level of $P < 0.01$ for TNF-α, AFP, ALT, AST, UB, TB, GGT and ALP, while Albumin and Conjugated Bilirubin were significantly decreased at a level of ($P<0.05$) in the *Ganoderma lucidum* + Acetaminophen-administered group compared to those of the control group.

Conclusion: Thus, the results of the present investigation demonstrates that the *Ganoderma lucidum* provides significant hepatoprotective activity against acetaminophen-induced liver injury in wistar rats.

Keywords: *Ganoderma lucidum;* Liver; Wistar Rats; Acetaminophen; Hepatoxicity

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1. Introduction

Ganoderma lucidum also called lingzhi or reishi mushroom is a polypore fungus that is red-varnished, kidney-shaped cap and peripherally inserted stem gives it a distinct fan-like appearance. When fresh, the *Ganoderma lucidum* is soft, cork-like, and flat. It lacks gills on its underside, and instead releases its spores via fine pores. Depending on the age, the pores on its underside may be white or brown [1]. *Ganoderma lucidum* and its close relative*, Ganoderma tsugae,* grow in the northern Eastern Hemlock forests. These two species of bracket fungus have a worldwide distribution in both tropical and temperate geographical regions, growing as a parasite or saprotroph on a wide variety of trees. Similar species of Ganoderma have been found growing in the Amazon [2]. *Ganoderma lucidum* is used for cancer, aging, boosting the immune system to prevent or treat infections, and for many other reasons, but there is no good scientific evidence to support these uses [3].

The family Ganodermetaceae Taxonomy describes polyphorebasidomycetous fungi having a doubled wall basidospore in all 219 species within the family have been assigned to the genius *Ganoderma lucidum* [4] owing to its irregular distribution in the wild and increasing demand of *Ganoderma lucidum* as a medicinal herb attempts were made to cultivate the mushroom [5] Different member of *Ganoderma lucidum* needs different conditions for growth and cultivation [6] and the different types are favoured in different geographical regions in South China black *Ganoderma lucidum* is popular and red *Ganoderma lucidum* is preferred in Japan, *Ganoderma lucidum* they thrive under hot and humid conditions and many wild varieties are found in the subtropical regions of the orient since the early 1970's *Ganoderma lucidum* has become a major source of mushroom artificial cultivation of *Ganoderma lucidum* had been achieved using substrates such as grain, sawdust, woodlogs [7].

Ganoderma lucidum has anti-oxidative effects when supplemented. It also has a therapeutic effect on insulin resistance, reduces the risk of prostate cancer, and can help treat a variety of conditions associated with metabolic syndrome. *Ganoderma lucidum* is well known for its anti-cancer effects. It is able to activate natural killer cells, increasing their activity and the body's ability to fight tumors [8]. Supplementing *Ganoderma lucidum* reduces the chances of metastasis, which is when cancer spreads to another part of the body. *Ganoderma lucidum* has a variety of mechanisms, but they are focused on moderating the immune system [9]. The lingzi mushroom is able to reduce immune system activity when the system is overstimulated, and boosts the immune system when it is weakened. In general, *Ganoderma lucidum* increases the amount of active immune system cells. *Ganoderma lucidum* is usually well-tolerated with few significant side effects. *Ganoderma lucidum* also contains a substance that may act like a blood thinner, potentially triggering bloody stools, nosebleeds, and easy bruising [10]. It should not be used if you are taking anticoagulants like warfarin or are scheduled to have surgery as it may increase the risk of bleeding. *Ganoderma lucidum* may also cause your blood pressure to drop and should be avoided if you are taking antihypertensive medications. Doing so may lead to hypotension (low blood pressure), triggering dizziness, fatigue, nausea, and blurry vision. Due to the lack of safety research, *Ganoderma lucidum* should be avoided in children, pregnant women, and breastfeeding mothers [11]. The popular edible mushroom *Ganoderma lucidum* has been widely used for the general promotion of health and longevity in Asian countries. The dried powder of *Ganoderma lucidum* was popular as a cancer chemotherapy agent in ancient China [12]. This study aimed to evaluate the effect of *Ganoderma lucidum* on acetaminophen-induced liver injury in wistar rats.

2. Materials and methods

2.1. Study area

The study was carried out on wistar rats at the animal house of Babcock University, Ilishan Remo, Ogun State.

2.2. Study design

This is a cross-sectional study, which was used to establish the magnitude of liver damage caused by acetaminophen that will be reflected directly by the levels of cytosolic and mitochondria liver enzymes in wistar rats.

2.3. Collection of plants

Formulated extracts of *Ganoderma lucidum* was purchased from the Pharmacy, Lagos Nigeria. The process of extraction of this formulated extract was correctly documented.

2.4. Experimental design

Forty (40) animals were used for the study to be divided into eight groups of five rats per group, the details of their treatment is highlighted below, they received this treatment for a period of three weeks during which they were placed on standard rats pellets diets

- Group 1: Normal control, administered drinking water only throughout the experiments
- Group 2: Acetaminophen (3000 mg) only
- Group 3: Acetaminophen (3000 mg) + standard drug (N- Acetyl cysteine)
- Group 4: Acetaminophen (3000 mg) + *Ganoderma lucidum* (100 mg/kg body weight)
- Group 5: Acetaminophen (3000 mg) + *Ganoderma lucidum* (200 mg/kg body weight)
- Group 6: Acetaminophen (3000 mg) + *Ganoderma lucidum* (300 mg/kg body weight)
- Group 7: *Ganoderma lucidum* (100 mg/kg body weight)
- Group 8: Acetaminophen (3000 mg) + Standard drug + *Ganoderma lucidum* (300 mg/kg body weight)

2.5. Animal sacrifice

Rats were made to fast overnight following the last administration and undergo cervical dislocation before being sacrificed. Blood was collected by cardiac puncture into lithium heparin tubes for biochemical analysis. The serum was separated after centrifugation at 4200rpm at room temperature for 5 minutes. The livers were carefully excised, cleared of adhering tissues and weighed. The weight of the animals was recorded in grams and expressed as g/kg body weight. A small portion of the above-mentioned organs/ tissue was fixed in 10% formalin and subsequently prepared for histology.

2.6. Procedures of data collection

Liver Enzymes (AST, ALT, ALP, GGT, Albumin, Bilirubin, Total Protein) were assayed using Fortress reagent kits and Tumor Necrosis Factor Alpha and Alpha Feto Protein were assayed using ELISA (Enzyme Linked Immunosorbent Assay) reagent Kits as well as spectrophotometry.

2.7. Statistical analysis

The Data obtained from the biochemical analysis was analyzed with Statistical Package for Social sciences (SPSS) version 20. The data was expressed in mean ± standard error of mean (SEM). The difference among the means was analyzed by one-way student-t-test and p-values < 0.05 will be considered statistically significant.

3. Result

A total of forty (40) male wistar rats were used for this study. They were stratified into eight(8) groups, Group 1 which is the control, Group 2 were given acetaminophen only, Group 3 were given acetaminophen and standard drug, Group 4 were given acetaminophen and 100 mg/kg body weight of *Ganoderma lucidum*, Group 5 were given Acetaminophen and 200 mg/kg body weight of *Ganoderma lucidum*, Group 6 were given acetaminophen and 300 mg/kg body weight of *Ganoderma lucidum*, Group 7 were given 100 mg/kg body weight of *Ganoderma lucidum*, Group 8 were given Acetaminophen, Standard drug, silymarin at a dose of 140 mg/kg of body weight and 300 mg/kg body weight of *Ganoderma lucidum*. The results from the study carried out on the effect of *Ganoderma lucidum* on acetaminophen-induced liver injury in wistar rats are presented in chats and figures below.

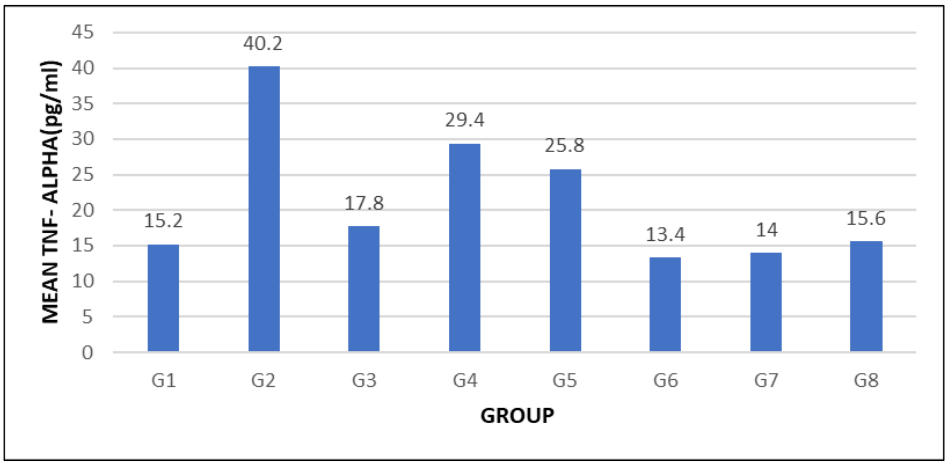

Figure 1 Comparison of Tumor Necrosis Factor levels Groups 1-8

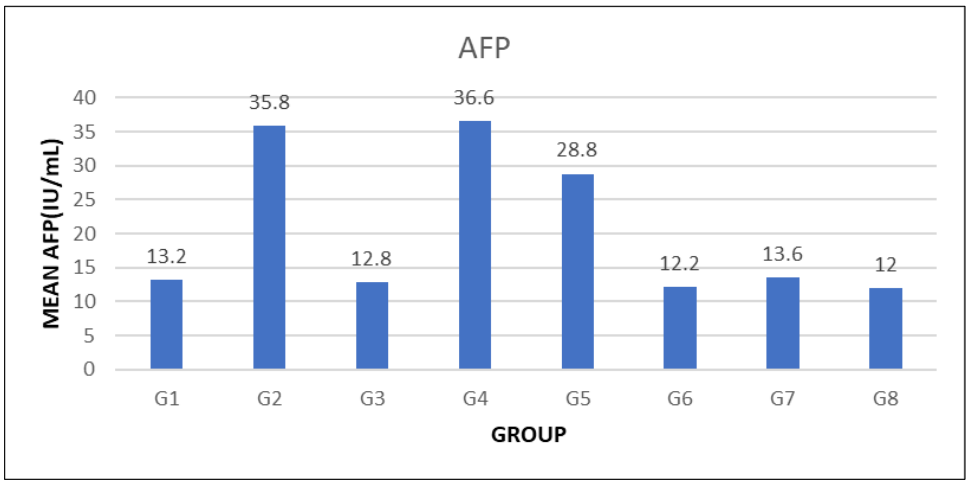

Figure 2 Comparison of Alpha Feto-Protein levels between Groups 1-8

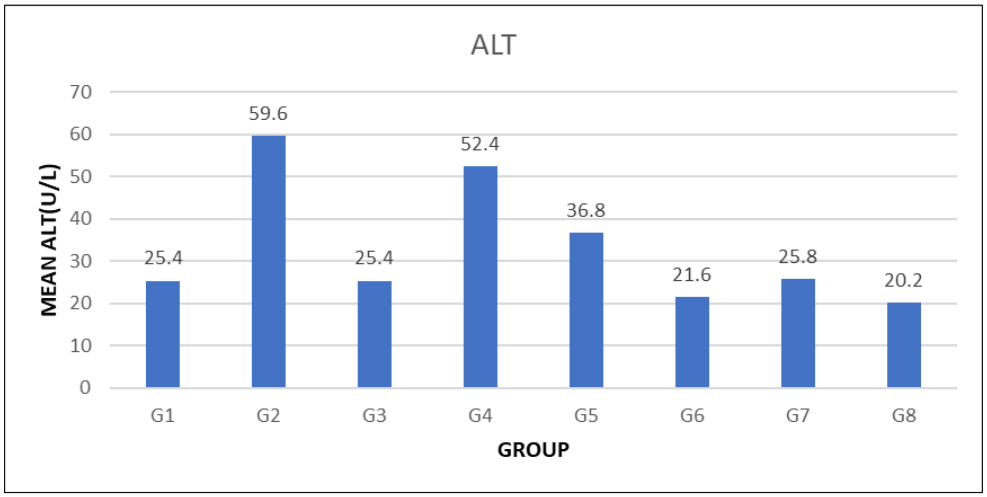

Figure 3 Comparison of Alanine Aminotransferase levels between Groups 1-8

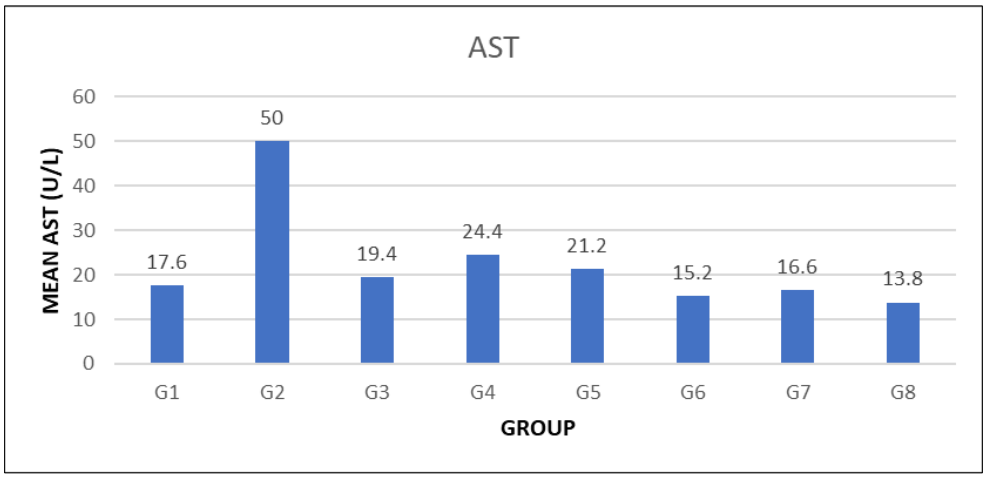

Figure 4 Comparison of Aspartate Aminotransferase levels between Groups 1-8

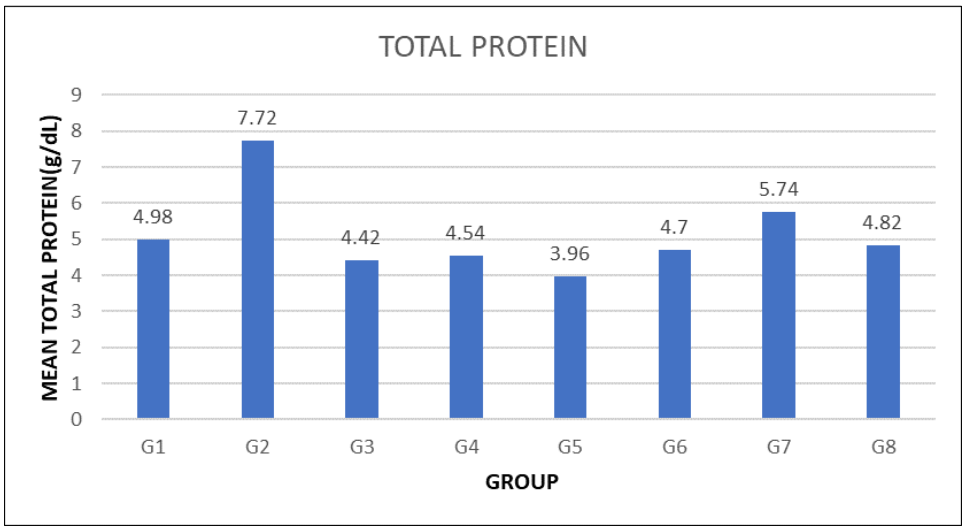

Figure 5 Comparison of Total Protein levels between groups 1-8

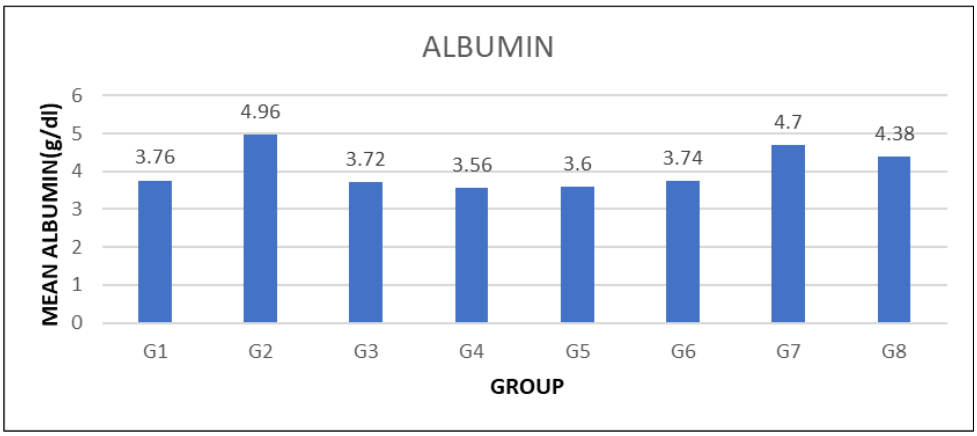

Figure 6 Comparison of Albumin levels between groups 1-8

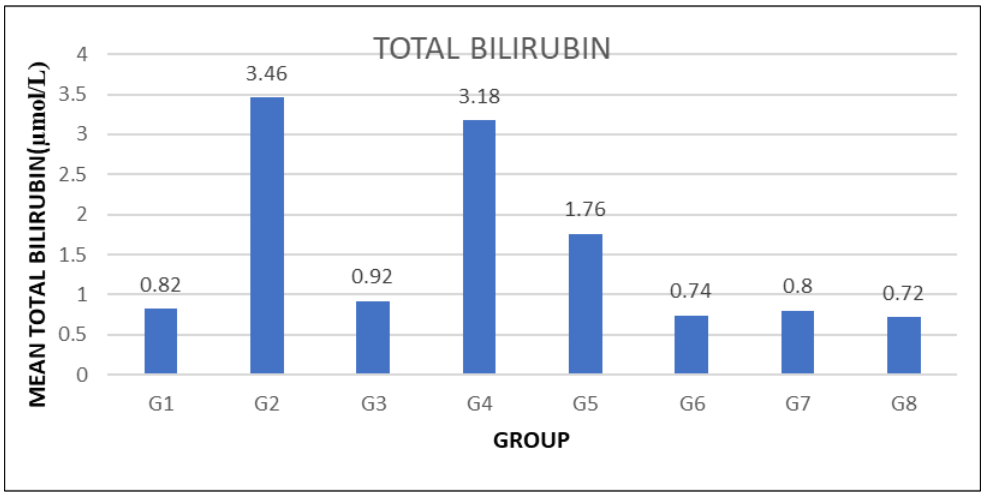

Figure 7 Comparison of Total Bilirubin levels between Groups 1-8

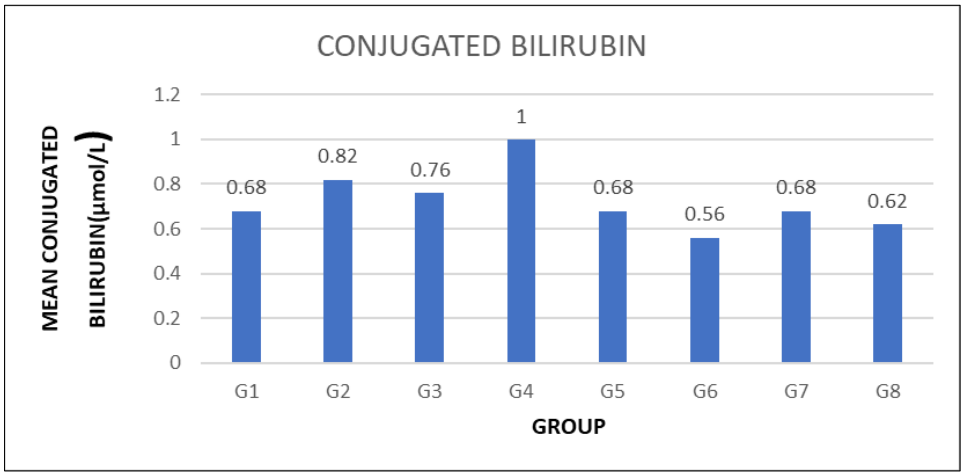

Figure 8 Comparison of Conjugated bilirubin levels between Groups 1-8

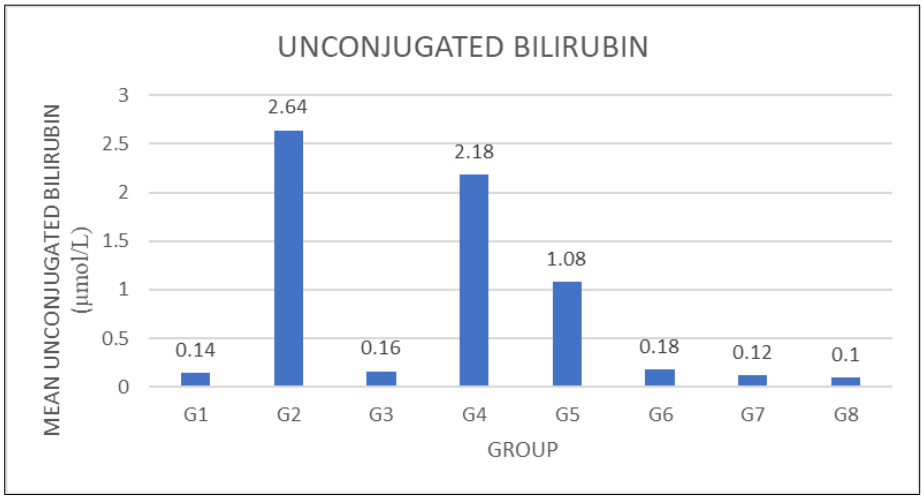

Figure 9 Comparison of Unconjugated Bilirubin levels between Groups 1-8

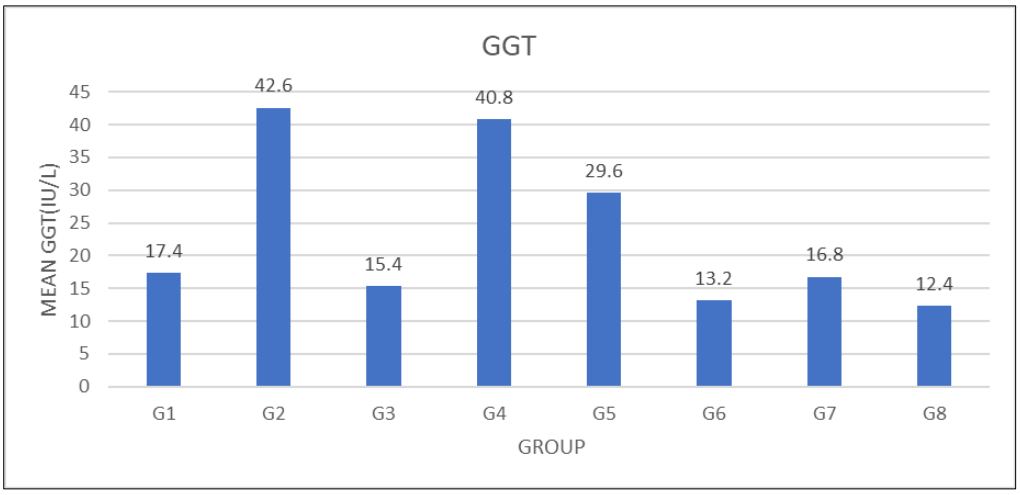

Figure 10 Comparison of Gamma Glutamyl Transaminase between Groups 1-8

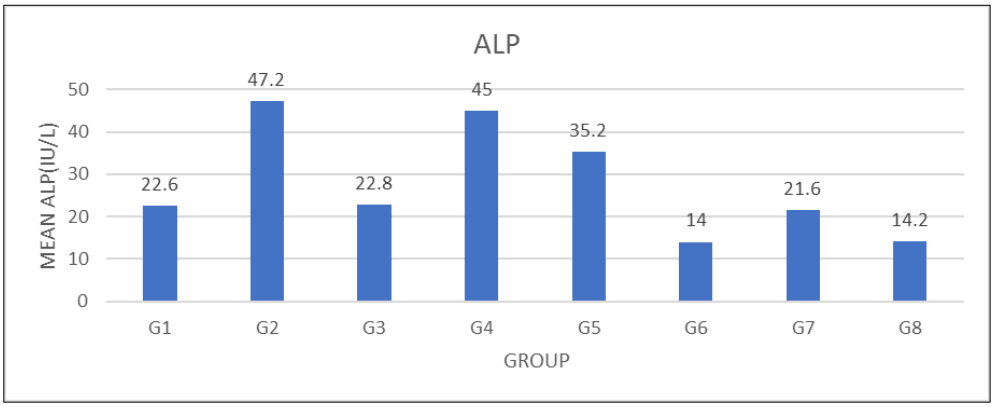

Figure 11 Comparison of Alkaline Phosphatase levels between groups 1-8

4. Discussion

In this study, the effect of *Ganoderma lucidum* was investigated by using acetaminophen for the induction of liver injury. Normally 95% of acetaminophen is metabolized in the liver by glucuronidation and sulfation. Only 5% of acetaminophen is metabolized by several of the P450 lysoenzymes system into the reactive intermediate N-acetyl-benzoquinone imine (NAPQI) [13]. The NAPQI is detoxified at the expenses of reduced glutathione (GSH). Thus, the overdose of acetaminophen depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction and development of hepatic necrosis which leads to the elevation of liver enzymes [14] but the intervention of *Ganoderma lucidum* brings about a complete reversal of this disorder.

The serum levels of liver enzymes, Tumor necrosis factor alpha and Alpha fetoprotein were determined to investigate the severity of liver damage caused by acetaminophen and also to establish the dosage concentration of *Ganoderma lucidum* extract that will exactly reverse liver damage in wistar rats. Tumor Necrosis Factor Alpha and Alpha Feto Protein can be important markers for foresight and determining the response to treatment of Liver injury. Acetaminophen overdose is the leading cause of drug-induced acute liver failure in many developed countries. Mitochondria oxidative stress is considered to be predominant cellular event in Acetaminophen-induced liver injury [15]. Accordingly, Silymarin, a known scavenger of reactive oxygen species (ROS), is an effective clinical antidote against Acetaminophen- induced acute liver damage; Correspondingly the intervention of *Ganoderma lucidum* is in accordance with this mechanism of action.

In this study, it was established that the mean values of TNF-αlevels in the animal subjects were significantly increased in Group 2 (Acetaminophen only) compared to the control group. Treatment with *Ganoderma lucidum* significantly

decreased the levels of TNF-α in the other groups such as *Ganoderma lucidum* + Acetaminophen group when compared to those of the group 2 which took Acetaminophen alone. There was a dose dependent effect of *Ganoderma lucidum* on TNF-alpha with 300 mg/kg group showing highest efficacy. This is in agreement with the study of Pellicoro *et al*., [16] who established unequivocally, that *Ganoderma lucidum* has a positive effect for tumor treatment. However, the research study of Wang *et al*., [17] argued that, it is the bioactive component of *Ganoderma lucidum* (Polysaccharides) that significantly reversed the elevated TNF-α that was caused by acetaminophen overdose in wistar rats and not necessarily the entire mushroom.

It was established that the mean values of AFP levels in the animal subjects were significantly increased in Group 2 compared to the control, significantly decreased in Group 3 but the AFP levels in Group 4 (acetaminophen + 100 mg) increased which means there was no effect on *Ganoderma lucidum* on AFP in Group 4; mean values of AFP levels Group 5, 6, 7 and 8 were significantly decreased. This study is in agreement with the study of Kwon *et al*., [18] who confirmed that *Ganoderma lucidum* is potent not only as an anti-tumor agent but hepatoprotective.

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) are the most specific indicators of hepatic injury and represent markers of hepatocellular necrosis. Oral administration of acetaminophen significantly increased the plasma levels of aminotransaminases (ALT and AST), while pretreatment of *G. lucidum* significantly decreased the levels of these enzymes in the *Ganoderma lucidum* + Acetaminophen rats to those of the control rats. Rises in these enzymes correspond to hepatocyte necrosis or abnormal membrane permeability, while depletion indicates healing of hepatic parenchyma [19]. Therefore, we speculate that the decreases of the elevated plasma levels of these enzymes might occur by the prevention of the leakages of intracellular enzymes in the *Ganoderma lucidum*+ Acetaminophen group. This is in agreement with the research study of Myer *et al*., [20] who submitted that *Ganoderma* lucidum substantially intervene in hepatocellular toxicities in Wistar rats. Kwon *et al*, [18] further established the hepatoprotective function of *Ganoderma lucidum* as shown by the cytosolic and mitochondria enzymes (ALT and AST respectively).

In this study, it was established that the mean values of TP, Albumin, GGT and ALP levels in the animal subjects were significantly increased in Group 2 compared to the control, while pretreatment of *G. lucidum* significantly decreased the levels of these enzymes in the *Ganoderma lucidum* + Acetaminophen groups to those of the control group. There was a dose dependent effect of these parameters (TP, Albumin, GGT, ALP) on *G. lucidum* with the highest dose of 300 mg/kg of body weight manifesting relative highest level of potency. This is in agreement with the research study of Hodgman & Garrard, [21] who affirmed that this noble mushroom successfully reversed the elevated total serum protein and albumin in acetaminophen-induced liver injury in wistar rats.

Gamma glutamyl transferase (GGT) and Alkaline Phosphatase are also enzymes that are released from bile ducts and are raised in liver diseases [22]. Plasma protein provides information about the severity of the parenchyma liver disease or necrosis as well as synthetic capacity [23]. Alkaline Phosphatase as a marker that was reversed by *Ganoderma lucidum* is in agreement with the research study of Larson *et al*., [24] who accredited hepatoprotective activity to bioactive components of *Ganoderma lucidum*. Activity of the microsomal enzyme GGT reflects the extent of intracellular oxidative stress in drug and xenobiotics detoxification function [25]. Thus, the drug Acetaminophen at high doses elevated the enzyme GGT and the intervention of *Ganoderma lucidum* brought about a complete reversal. This is in agreement with the research study of Myer *et al*, [20] who discovered that hepatoprotective ability is conferred on this King of mushroom, *Ganoderma lucidum*.

Plasma bilirubin levels are related to the functions of hepatic cells [26]. The rise in the bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte [27]. Toxic levels of acetaminophen which affect the function of the liver can cause an increase in bilirubin. This is as a result of the liver losing its ability to remove and process bilirubin from the blood stream; thus, losing its conjugational ability hence the rise in unconjugated bilirubin.

In this study, it was established that the mean values of Serum Total Bilirubin and Unconjugated Bilirubin levels in the animal subjects were significantly increased in Group 2 (Acetaminophen only) compared to the control group; Treatment with *Ganoderma lucidum* significantly decreased the levels of total and unconjugated bilirubin in the other groups such as *Ganoderma lucidum*+ Acetaminophen groups when compared to those of the group 2 which took Acetaminophen alone. There was a dose dependent effect of *Ganoderma lucidum* on both total and unconjugated bilirubin with 300 mg/kg group showing highest efficacy. This finding is in agreement with the research study of Yuen *&* Lai, [9] who concluded that protection against liver injury was as a result of the administered extracts of *Ganoderma lucidum* in the wistar rats. Furthermore, Burke *et al*, [28] submitted that this noble mushroom successfully reversed the elevated bilirubin in acetaminophen-induced liver injury in wistar rats. Unconjugated bilirubin which is a waste product

of haemoglobin breakdown after 120 days is taken up by the kupffer cells of the liver, where it is converted by the enzyme uridine diphosphophoglucuronate glucuronosyltransferase (UDT) into soluble conjugated bilirubin. The unconjugated bilirubin which is insoluble, crossing the Brain barrier resulting to KENICTERUS; condition characterized as imbecile.

In this study, the parameters TNF-α, AFP, ALT, AST, Total Protein, Total Bilirubin, unconjugated Bilirubin, GGT, ALP significantly reduced the toxic level of acetaminophen as observed in group 2 (Acetaminophen only) at a significant level of $p < 0.01$. Therefore, this study agrees to the anti-inflammatory / anti- oxidative, anti- tumor effect of *Ganoderma lucidum* extract [29]. It further suggests that *Ganoderma lucidum* ameliorates the hepatocellular damages concomitantly with the improvement of structural integrity, as shown in so many other studies.

5. Conclusion

This present study showed that severity of liver damage caused by acetaminophen overdose was reflected by the increased levels of the cytosolic and mitochondria liver enzymes (ALT And AST), Tumor markers (AFP and TNF- alpha), Total serum Protein, Albumin, GGT, ALP and Bilirubin in wistar rats. This was effectively reversed by the potent *Ganoderma lucidum* especially at a concentration of 300 mg/kg body weight of *Ganoderma lucidum.*

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from Babcock University Health Research Ethics Committee (BUHREC),147/20.

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