

Blood-Boosting and Gastro-protective influences of Diets Supplemented with *Ganoderma lucidum* on Indomethacin Gastric Ulcer Induced Rats

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Abstract

Original Research Article

Ganoderma lucidum (Gl), collected from Saki, Oyo State, South Western Nigeria, was employed as dietary supplements in assessments of blood-boosting and gastro-protective actions on male Wistar rats (*Rattus norvegicus*). The antiulcer, biochemical and haematological influences were carried out in triplicates using standard methods. Data were expressed as Mean \pm SD and interpreted using ANOVA and test of significance was carried out using DMRT ($P \leq 0.05$). Gastric nitric oxide, malondialdehyde, sulfhydryl, hydrogen peroxide and H^+/K^+ -ATPase activities were significantly different when compared with ulcer untreated control (CU). When the same parameters were used in comparison with the normal control (CN), values obtained for haematological and plasma biochemical variables were not significantly different across all *G. lucidum* treatment groups. histopathological analysis of the stomach tissues revealed that there were marked ulcer inhibitions. *G. lucidum* treatment groups demonstrated blood enhancing and gastro-protective behavior through predominant antioxidant activities. The implications of these findings were discussed.

Keywords: *Ganoderma lucidum*, blood enhancer, Gastro-protection, and antioxidant.

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INTRODUCTION

Micro and macro fungi (moulds, yeasts, myxomycetes, morels, auricularias, stinkhorns, mushrooms, polypore, puffballs, etc) have been widely used by man from ancient time in conventional and non-conventional medicine, industries and agriculture [1-5]. Several fungi have great economic value to mankind [1-5]. For example, several fungi are utilized as food or food products, while others such as yeasts are used in brewery and bakery industries [6-8]; and some are used in medicine and pharmaceutical industries. Fungi have been implicated as good sources of antioxidant, anti-diabetic, hypo-cholesterolemic, antiviral, antitumor, anticancer, immune-modulatory, anti-allergic, nephron-protective, and antimicrobial agents [9-13]. Additionally, fungi are used in bioremediation, biodegradation, bio-control, chemical and biological purposes [6-8].

Ganoderma lucidum belongs to the Kingdom fungi, Order Polyporales of the Family Polyporaceae

[14]. There are 80 species in this family, many of which are found in tropical and sub-tropical regions of the world [14, 15]. *Ganoderma* spp are economically important because of their genetic diversity, bioremediation potential, and therapeutic properties [14]. *G. lucidum* has been widely used in places like China, Japan, Europe, America, Africa and Korea for thousands of years [16, 17]. This polypore has been used as immune-modulatory, antioxidant, antimicrobial, and antitumor agents [18, 19]. *G. lucidum* contains many phytochemicals like flavonoids, polyphenols, polysaccharides, steroids, and vitamins [20].

Blood is a body fluid in mammals that provides the cells with required nutrient materials. It also carries metabolic by-products away from the cells. Blood serves as a pathological reflective surface of toxicants and other consequences of animal exposure [21].

The gastrointestinal tract is generally subjected to dangerous swallowed substances, yet the involvement of the gastric membrane boundary system ensures the

digestive system's homeostasis [22]. Peptic ulcer has become a severe gastrointestinal condition and therefore is regarded as a leading cause of morbidity and mortality [23]. This happens due to inflammation in the mucosal membrane caused by pathogenic microorganisms. The aim of this study, therefore, was to investigate the blood-boosting and gastro-protective properties of *Ganoderma lucidum* from Saki, Southwest Nigeria in male Wistar rats.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh fruiting bodies of wild *G. lucidum* were collected from Saki (8,6726° N; 3,3943° E) in Oyo State, Southwest Nigeria, during the rainy season (June-August 2017). The identity of the fungus was confirmed based on the standard descriptions of Ostry *et al.* [24].



Fig-1: A photograph of *Ganoderma lucidum* as seen in Saki town. 99% identical to JQ520179.1 *Ganoderma lucidum* strain IUM 4100

Animal Model

Thirty-five (35) healthy male Wistar rats (100–110 g) used in this study were procured from the Animal House, College of Medicine, University of Ibadan, Nigeria.

Experimental

The experimental animals were housed under pathogen-free conditions, in solid bottom polypropylene cages at 26.0–28.0 °C in the Animal Room of the Physiology Department with 70–80 percent relative humidity, 12hrs ambient light and dark cycles respectively for 2 weeks of acclimatization before and during the study. For the control tests, the animals had free access to their normal animal diets, and formulated diet supplemented by 20% w/w and 40% w/w of *G. lucidum* for the main experiment, and water *ad libitum*. The animals were randomly divided into 5 groups, with each group containing seven rats. Each experimental group was replicated thrice for 7 days, while the other group was for 14 days. Blood samples were obtained from the retro-orbital sinus on days 7 and 14 of the treatments for full haematological analysis. The

treatment groups were Control normal (CN), - control group not ulcer-induced; Control Untreated (CU) -ulcerated group (no ulcer-induced care was given); Control (Cm) - ulcerated group treated with 20 mg/kg of cimetidine and treatment groups fed with formulated feed supplemented with 20% and 40% of *G. lucidum*. The animals were fasted overnight before oral administration of indomethacin (40mg/kg) after 7th and 14 day of treatment, and sacrificed after 4 hours. Thereafter, their stomachs were excised, gently rinsed in cold phosphate buffer, blotted, and weighed while a portion was cut and formalin-fixed for histological evaluation. During the experimental studies, all the research animals were provided humane care in compliance with the ethics and procedure outlined in the National Academy of Science (NAS) Handbook for the Treatment and Use of Laboratory Animals; approved by the Institutional Animal Ethical Research Committee. Animals had unlimited access to water *ad libitum* during the period of the study.

Determination of Haematological Variables

The Dacie and Lewis [25] approach was used in blood analysis in haematological study.

Experimental Gastric Ulceration Induction

Method of Inas *et al.* [26] was used for indomethacin-induced ulceration

Macroscopic Analysis of Ulcer Scores

The gastric ulceration scoring technique was assessed by using the method of Elegbe and Bamigbose [27].

Histopathological Analysis

Morphologically, stained sections were analyzed and the microsomes were taken using the methods of Avwioro [28]

Biochemical Analysis of the Gastric Tissue

1. Determination of Lipid peroxidation: Lipid peroxidation was calculated by the method of Varshney and Kale [29].
2. Determination of gastric tissue Nitric oxide (NO): Nitrite concentration in the supernatant was calculated as an indication of NO activity that was detected by the Griess reaction [30]. Nitrite was determined using the method of Ignarro *et al.* [31].
3. Evaluation of Sulfhydryl Content: The amount of sulfhydryl in the tissue was measured using the method of Inas *et al.* [26].
4. Measurement of Mucosal-hydrogen peroxide (H₂O₂): Tissue levels of hydrogen peroxide (H₂O₂) were quantified using the method of Elegbe and Bamigbose [27].
5. Measurement of total concentration of mucosa proteins and gastric total protein was carried out using the method of Ignarro *et al.* [31].

6. Measurement of Hydrogen/Potassium anti-pump activities was carried out using the method of Ronner and Vasella, [32].
7. Mucin content determination: Mucin content was determined by the method of Winzle [33].
8. Statistical evaluation: Results were presented as Mean \pm SE, evaluated using ANOVA (two-way) and significance at $p \leq 0.05$.

RESULTS AND DISCUSSION

As shown in Table 1, percentage (%) ulcer inhibition increased in all the pretreated groups (Cm, 20*Gl*, and 40*Gl*) as compared with CU on the 7th and 14th day. The increase observed in groups supplemented with *G. lucidum* might be due to the presence of bioactive compounds such as flavonoids and polysaccharides that are known to possess antiulcer activities. This result was in agreement with Buswell *et al.* [34] who reported that the polysaccharides found in mushrooms are the source of their anti-inflammatory, and antiulcer properties.

As observed in Figure 2, on day 7, no significant increase was seen with the treatment groups formulated with 20% w/w and 40% w/w of *G. lucidum*, while on day 14, a significant increase (^{kkk} $p < 0.001$) was observed with 20*Gl* when compared with CU. However, when comparing treatment between 7th and 14th day of exposure, a significant difference was observed with 20*Gl* only. The increase in total protein observed with the *G. lucidum* supplement could be linked to gastro-protection which could be due to the presence of bioactive compounds present in *G. lucidum*. The results clearly showed that *Gl* had anti-inflammatory activity which could help in the regeneration and repair of cells [34].

As seen in Figure 3, no significant difference was observed with both 20*Gl* and 40*Gl* on day 7 and 14; however, a reduction in MDA level was observed with only 20*Gl*. In figures 5 and 6, a significant increase (^l $p < 0.05$) in sulfhydryl content was only observed with 40*Gl* on day 7 when compared with ulcer untreated control. (CU). Both treatments (20*Gl* and 40*Gl*) significantly increased in hydrogen peroxide activity only on day 14. Reduction in MDA and increase in sulfhydryl and hydrogen peroxide activities are indications of the antioxidant property of *G. lucidum*. This could serve as exogenous antioxidants to supplement the endogenous antioxidant enzyme found in the body. This antioxidant could be gastro-protective in function.

On day 7, only supplemented group of 20*Gl* significantly increased in nitric oxide while for day 14, both 20*Gl* and 40*Gl* significantly increased nitric oxide content. However, between days 7 and 14 of treatment, no significant difference was observed with 20*Gl* treatment, while a significant increase (^{zzz} $p < 0.001$) was observed with treatment 40*Gl* on day 14 as compared

with day 7 (Figure 4). In this study, the increase in values of NO may confer gastro-protective function on *Gl* by acting as a vasodilator which aided gastric blood flow. It may also be responsible for prevention of secretion of acids and stimulation of mucus production. This may help in giving protection to the gastrointestinal tract. This result supports the findings of Moura-Roucha *et al.* [35] who reported that NO is very useful in gastric ulcer healing.

On days 7 and 14 respectively, significant increase with 20*Gl* and a significant decrease with 40*Gl* in H^+/P^+ ATPase were observed when compared with CU (figure 7). The significant reduction in H^+/P^+ ATPase observed with 40*Gl* treatment may indicate proton pump inhibition; thus, providing a gastro-protective effect on the rat. This is in agreement with Xu *et al.* [19] who also reported *G. lucidum's* antiulcer potential.

On the 7th and 14th day of treatment, significant increases were observed in mucin content with 20*Gl* and 40*Gl* (Figure 8). An increase in gastric mucin content observed in this study showed that both 20*Gl* and 40*Gl* conferred stomach defense on both day 7 and day 14, making it to be gastro-protective. This result is compatible with that of Sabiu *et al.* [36], who reported that drugs that increase intracellular mucin secretion will speed up ulcer cure.

As shown in Table 2, there were no significant differences in the effects of 20% w/w and 40% w/w *G. lucidum* (*Gl*) on white blood cells, lymphocytes, neutrophils, monocytes and eosinophils as compared to CN for both days of treatment. From the results, the increase in white blood cells on day 7 and decrease observed on day 14 of the treatment could be linked with the immunomodulatory properties of the feeds. This is in agreement with the findings of Al-Obaidi [18], who reported on the immune-regulatory properties of *G. lucidum*.

From the result in Table 3, no significant differences in packed cell volume, erythrocyte sedimentary rate, reticulocyte, haemoglobin, red blood cells and platelet count were observed with 20*Gl* and 40*Gl* on the 7th and 14th day of treatment when compared with CN. An increase in platelet count caused by the effect of both concentrations of *G. lucidum* supplemented diets on Wistar rats on both days of treatment showed a boosting effect that aided blood clotting time during any injury. An increase in packed cell volume, haemoglobin, and red blood cells observed with both concentrations of *G. lucidum* supplemented diets on Wistar rats indicated an improved oxygen transport and erythropoiesis; thus having a blood-boosting effect.

Table 4 shows that on the 7th and 14th day of treatment, there were no significant increases in albumin, globulin, blood urea nitrogen, and creatinine counts with 20GI and 40GI. Based on the values of creatinine and blood urea nitrogen, which were not significantly different from the effects of *G. lucidum* supplemented diets as compared with CN, one can conclude that the feed was good for consumption.

From the above studies, one can conclude that *Ganoderma lucidum* has therapeutic biological activities with regards to blood boosting, antioxidant, and antiulcer properties. Hence, it could serve as a source of drug products which can be readily available, safe, and cheap with little or no side effects.

Table-1: Effects of *G. lucidum* supplemented diets on ulcer score, ulcer index, and ulcer percentage inhibition in Indomethacin- Induced Gastric Ulcerated rats.

Treatment	Mean ulcer score (mean \pm SEM)		Ulcer index (mm ²)		Percentage inhibition (%)	
	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
CN	0.0 \pm 0	0.0 \pm 0	0.0	0.0	0	0
CU	6.0 \pm 2.3	6.0 \pm 2.3	0.18	0.18	0	0
Cm	3.5 \pm 1.2	1.83 \pm 0.4	0.11	0.06	41.67	69.44
20GI	1.67 \pm 0.24	1.17 \pm 0.34	0.05	0.04	72.17	80.5
40GI	2.83 \pm 0.2	1.0 \pm 0.44	0.09	0.03	52.78	83.33

Values are expressed as Mean \pm SE.(n = 3). All values were not significantly different from control normal (CN) at p<0.05. 20GI=20%w/w of *Ganoderma*

lucidum in feed, 40GI = 40% w/w of *Ganoderma lucidum* in the feed, induced. Ulcer untreated control (CU). (Cm)- Control treated with 20 mg/kg of cimetidine.

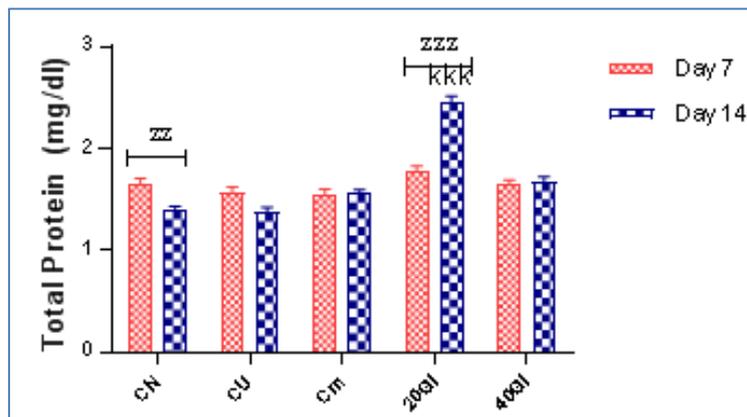


Fig-2: Effects of *G. lucidum* supplemented diets on total protein on indomethacin-induced gastric ulcerated rats in 7 and 14th day of exposure

Effect of *Ganoderma lucidum* supplemented diet on the Histological changes in the gastric mucosa of Indomethacin Induced gastric ulcerated rats.

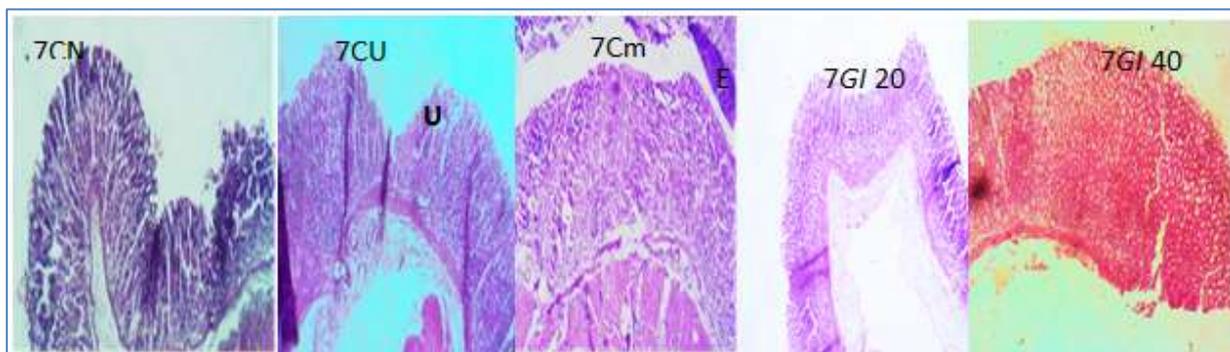


Plate-1: Photomicrograph of stomach section (Day 7 stained by Haematoxylin and Eosin stain MAG.X 100) showing 7CN: There is no observable lesion in the gastric mucosa, 7CU: There is marked ulceration (U), necrosis of chief cells and inflammation in the gastric mucosa, 7Cm: There is erosion (E) of the gastric mucosa, 7GI20: There are necrosis and loss of mucous neck cells in the gastric mucosa., 7GI40: There is no observable lesion

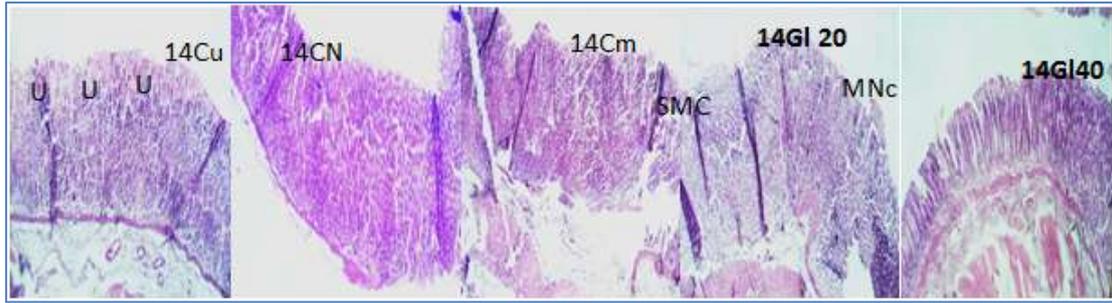


Plate-2: Photomicrograph of stomach section (Day 14 stained by Haematoxylin and Eosin stain MAG. X 100) showing; 14Cu: There is ulceration of the mucosa with hemorrhagic exudate (U), 14CN: There is no observable lesion, 14Cm: There is a loss of surface mucous cells (SMC), 14GI20: There is necrosis and sloughing of mucous neck cells (MNC), 14GI40: There is no observable lesion

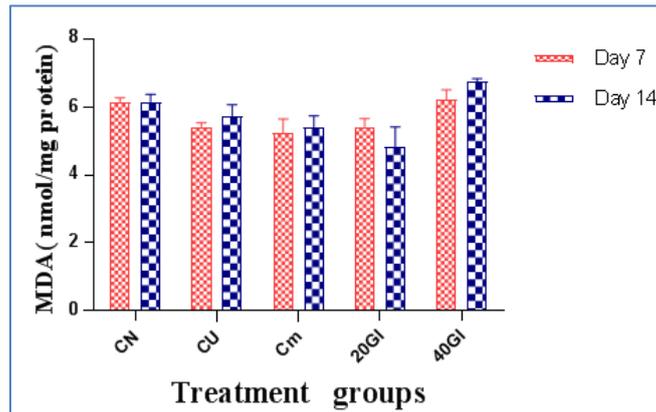


Fig-3: Effects of *G. lucidum* supplemented diets on Malondialdehyde (MDA) Level on indomethacin-induced gastric ulcerated rats for 7 and 14 day exposure periods

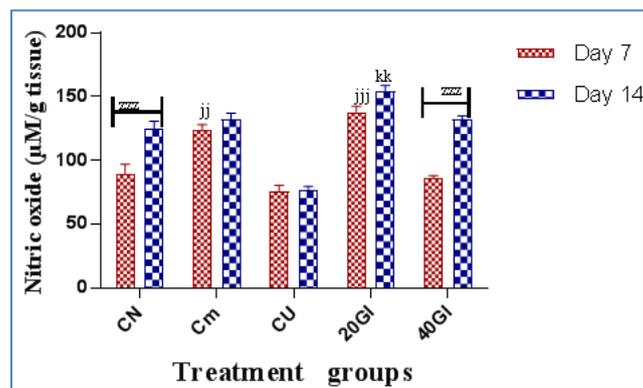


Fig-4: Effects of *Ganoderma lucidum* supplemented diets on Nitric Oxide (NO) Content on indomethacin-induced gastric ulcerated rats for 7 and 14 day exposure periods

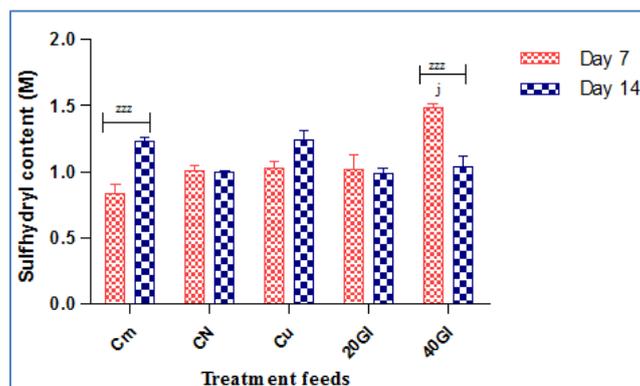


Fig-5: Effects of *G. lucidum* supplemented diets on Sulfhydryl Content on indomethacin-induced gastric ulcerated rats for 7 and 14 day exposure periods

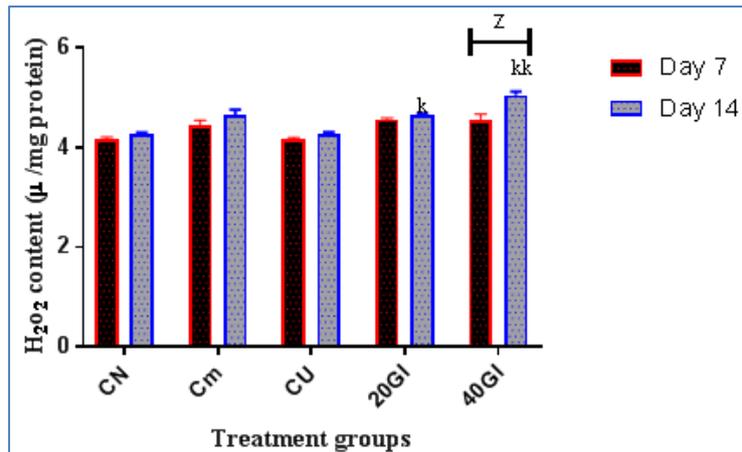


Fig-6: Effects of *G.lucidum* supplemented diets on hydrogen peroxide content on indomethacin-induced gastric ulcerated rats for 7 and 14 day exposure periods

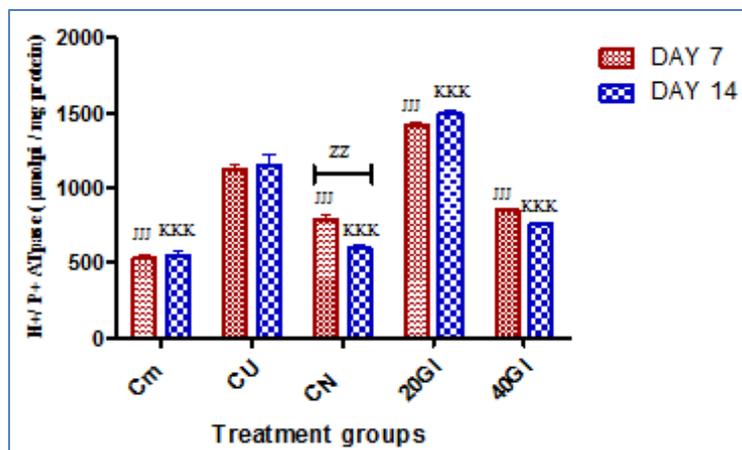


Fig-7: Effects of *G. lucidum* supplemented diets on Hydrogen Potassium pump activities in rats for 7 and 14 day exposure periods

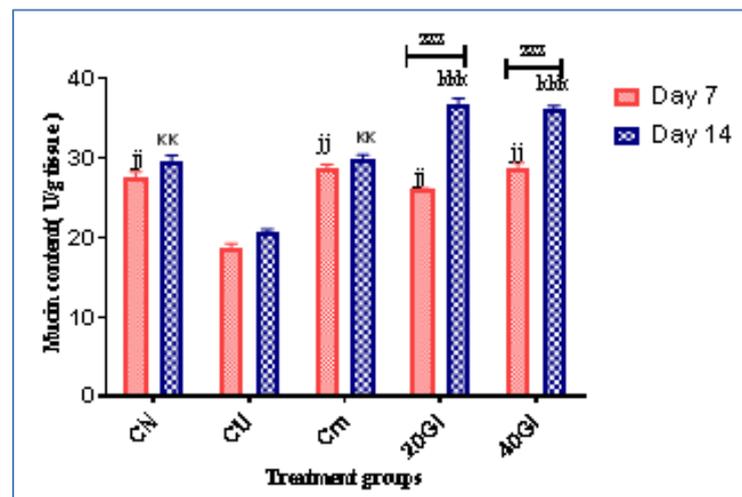


Fig-8: Effects of *G. lucidum* supplemented diets on Mucin content in rats for 7 and 14 day exposure periods

Keys of significance; Following one –way Anova, ⁱ $p < 0.05$, ⁱⁱ $p < 0.01$, ⁱⁱⁱ $p < 0.001$ at 7 days and ^k $p < 0.05$, ^{kk} $p < 0.01$, ^{kkk} $p < 0.001$ at 14days compared with the corresponding ulcer untreated control (CU). Using two-way Anova, ^z $p < 0.05$, ^{zz} $p < 0.01$, ^{zzz} $p < 0.001$,

between 7 and 14 days exposure periods. 20Gl = 20% w/w of *Ganoderma lucidum* in feed, 40Gl = 40% w/w of *Ganoderma lucidum* in feed.(Cm)- control treated with 20 mg/kg of cimetidine.(CN),-Control normal.

Table-2: Effect of *G.lucidum* supplemented diets on white blood cells, lymphocyte, and neutrophils, monocyte, and eosinophils counts in Wistar rat

Groups	WHITE BLOOD CELL (10 ⁶ /μL)		LYMPHOCYTES (10 ³ /μL)		NEUTROPHILS (10 ³ /μL)		MONOCYTE (10 ³ /μL)		EOSINOPHILS (10 ³ /μL)	
	Day 7	Day14	Day 7	Day14	Day 7	Day14	Day 7	Day14	Day 7	Day14
CN	4183±44	2733±20	71.3±1.8	77.7±0.4	26±1.5	13.7±0.3	1.3±0.33	1.7±0.67	1.33±0.88	0.33±0.33
20Gl	4983±60	3733.3±30	66.3±1.33	71.3±0.2	30±0.5	16±0.6	1.7±0.33	1.3±0.33	2±0.58	1.33±0.05
40Gl	4517±16.7	3316.7±33	72.7±0.67	83.3±0.4	28.7±0.67	14.7±0.6	1.33±0.33	1.7±0.3	1.33±0.88	0.33±0.05

Values are expressed as Mean±SEM. (n = 3). All values were not significantly different from control normal (CN) at p<0.05.
20Gl=20%w/w of *Ganoderma lucidum* in feed, 40Gl = 40%w/w of *Ganoderma lucidum* in the feed.

Table-3: Effect of *G. lucidum* supplemented diets on packed cell volume, Erythrocyte sedimentary rate, and Reticulocyte, Haemoglobin, Red blood cell, and Platelets counts in Wistar rat

Groups	PCV (%)		ESR (mm/hr)		RECTIC (x 10 ⁹ /L)		HB (g/dl)		RBC (x10 ¹² /L)		PLATELETS (x 10 ⁹ /L)	
	Day 7	Day14	Day 7	Day14	Day 7	Day14	Day 7	Day14	Day 7	Day14	Day 7	Day14
CN	41±0.5	49.3±2.3	1.13±0.7	1.43±0.07	2.4±0.2	3.3±0.4	13.8±0.4	14.77±0.12	7.23±0.4	7.49±0.15	144333±15	94666±5
20Gl	40.33±0.67	52.67±2.19	1.17±0.2	1.13±0.07	2.7±0.1	2.97±0.03	14.6±0.7	16.13±0.06	7.4±0.4	8.38±0.2	127000±11	135333±6
40Gl	44.33±0.33	53.33±2.03	1.9±0.7	1.37±0.09	2.5±0.6	3.23±0.2	14.8±0.6	16.9±0.2	7.4±0.6	8.76±0.06	108666±15	134000±22S

Values are expressed as Mean±SEM. (n = 3). All values were not significantly different from control normal (CN) at p<0.05.
20Gl=20%w/w of *Ganoderma lucidum* in feed, 40Gl = 40%w/w of *Ganoderma lucidum* in the feed.

Table-4: Effects of *G. lucidum* supplemented diets on Albumin, Globulin, Blood urea nitrogen, and Creatinine in Wistar rat

.. GROUPS	ALBUMIN (g/dL)		GLOBULIN (g/dL)		BUN (g/dL)		CREATININE (g/dL)	
	Day 7	Day14	Day 7	Day14	Day 7	Day14	Day 7	Day14
CN	2.87±0.3	2.93±0.18	4.5±0.2	4.17±0.3	17.27±0.35	16.67±0.18	0.77±0.03	0.83±0.09
20Gl	3.13±0.0	3.5±0.15	4.67±0.09	4.23±0.17	17.9±0.3	17.43±0.22	0.97±0.17	1.1±0.06
40Gl	2.73±0.35	3.5±0.1	4.6±0.3	4.53±0.12	18±0.31	17.83±0.55	0.77±0.03	1.1±0.15

Values are expressed as Mean±SEM. (n = 3). All values were not significantly different from control normal (CN) at p<0.05.
20Gl=20%w/w of *Ganoderma lucidum* in feed, 40Gl = 40%w/w of *Ganoderma lucidum* in the feed.

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