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Full Length Research Paper

Immunomodulatory and toxicological properties of some selected *Ganoderma* mushrooms species

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The toxicological and immunodulatory properties of some selected *Ganoderma* species was investigated using animal bioassay. The result of the haematological analysis showed that there was no significant difference in the total white blood cell (WBC) count value between the control animal and those fed with *Ganoderma* extract, while there was increase in the packed cell volume (PCV) of the rat fed with the extract. The differential WBC count showed that lymphocytes and eosinophil increase in the animals fed with *Ganoderma* extract, while the neutrophil reduced. However, the monocytes remained constant in the treated and control group of animals. Histopathological analysis revealed haemorrhage, reduction and detachment of the glomerular of the kidney while the intestinal section showed degeneration and erosion of the crypts of villi and cellular infiltration. There was necrosis in the heart, expansion and waxy contraction. The *Ganoderma* extract contain immunodulatory constituents that are toxic.

Key words: Toxicological, immunodulatory, *Ganoderma*, histopathology.

INTRODUCTION

Mushrooms have been valued throughout the world as both food and medicine for thousands of years. They represent a major and yet largely untapped source of potent pharmaceutical products. Apart from their nutritional potentials, mushrooms are also sources of physiologically beneficial bioactive substances that promote good health (Sagakami et al., 1991; Wasser and Weis, 1999). They produce a wide range of secondary metabolites with high therapeutic value (Demain, 1999). *Ganoderma* is a traditional Chinese medicine prescribed for the treatment of chronic hepatopathy, hypertension, bronchitis, arthritis, neurasthenia and neoplasin in China and other countries of the Orient (Arisawa et al., 1986; Dudhgaonkar et al., 2009; Majagi and Patil, 2009; Chen et al., 2009). Microbial metabolites that have the ability to enhance immunogenic reactions and produce antibodies are indications of bioactivity (Behal, 2000; Nduka, 2007). *Ganoderma* is a medicinal mushroom that has antidiabetic, antioxidant, immunomodulatory, antitumor and antimetastatic activities (Kimura et al., 2002). These mushrooms attract international due to wide variety of their biological activities as immunimodulatory, cardiovascular, respiratory and antinoceptive (active against pain) effects (Ha et al., 2000; Chang and Mshigeni, 2001). Our body temperature and wealth of nutrients provide an ideal home for these micro-organisms to thrive.

The human immune system has the essential function of protecting the body against the damaging effects of microbial agents that are pathogenic. The system comprises innate (non-specific) and acquired (specific) immunity. Natural killer (NK) cells complement system, macrophages, antigen presenting cells (APCs) and neutrophils make up the innate immune system, and mount an immediate non-specific response to foreign microbial agents. Apart from these natural mechanisms, there are additional factors that stimulate and suppress host immunity. Immunostimulants enhance the overall immunity of the host, and present a non-specific immune

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Abbreviations: WBC, White blood cell; **PCV,** packed cell volume.

Table 1. Heamatological parameters of the experimental animals.

	PCV (%)	WBC/mm^3 (×10^3)	LPC (%)	NTP (%)	MNC (%)	ESP (%)
CON	48.67 ± 6.5^a	4.93±1.02^b	36.67 ± 3.06^a	59.33 ± 8.96^a	0.33 ± 0.58^a	0.00 ± 0.00^a
GTE	54.33 ±7.64^a	4.29 ± 1.29^b	55.33 ± 11.72^a	44.33 ± 11.93^a	0.33 ± 0.58^a	0.33 ± 0.58^a

Values are mean ± SD of three replicates.

response against the microbial pathogens. They also work to heighten humoral and cellular immune responses by either enhancing cytokine secretion or by directly stimulating B- or T-lymphocytes (Benny and Vanitha, 2004). However, little information is available on the toxicity of local *Ganoderma recinacium* (Ganodermataceae). Toxicology is the study of the adverse effects of chemical substances on living organisms (Schrager, 2008). It is the study of symptoms, mechanisms, treatments and detection of poisoning, especially the poisoning in humans. G. *resinaceum* may constitute a potentially useful resource for new and safe drugs for the treatment of various ailments. Thus, toxicity studies will play an important role in identification and isolation of new compounds from crude extracts.

MATERIALS AND METHODS

Collection of mushroom samples treatment

Fruiting bodies of *G. resinaceum* were collected from the forest Igunshin in Akure south local government area of Ondo State, Nigeria. In the laboratory, the basidiocarps were washed with freshwater and brushed with a soft brush before being dried in an oven at 45°C for 5 days. They were identified and authenticated in the Department of botany and microbiology, University of Ibadan, Nigeria.

Preparation of the mushroom extracts

The mushrooms were oven dried at 45°C for 5 days, ground and milled into powder using blender prior to extraction (Jonathan and Fasidi, 2003). The powder of mushroom was extracted by soaking 20 g in 200 ml of methanol with stirring for 72 h and then filtered. The extracting solvent was evaporated to dryness using rotary evaporator.

Experimental animals

A 8-10 week old albino rats weighing 38 to 45 g comprising of both sexes were used. The animals were purchased from the Department of animal production and health, Federal university of technology, Akure, Nigeria and housed in separate cages, kept in a clean environment and fed with food and water *ad libitum*. They were allowed to acclimatize to the laboratory condition for 10 days (Table 1).

Acute toxicity

The experimental animals were divided into two groups (control and tested groups). The animals in the control group were fed with standard diet (Feed Master©) grower mash. Exactly 1000 mg/ml per body kg of *G. resinaceum* extract dissolved in distilled water was administered orally to the test group. The animals in the control received sterile distilled water. The animals were observed for signs and symptoms such as hyperactivity, sluggishness, anorexia and diarrhea during the experiment. The experiment lasted for 21 days. The animals were sacrificed at the end of the experiment following international ethics on animal bioassay. The blood was collected in ethylene diamine tetraacetic acid (EDTA) bottles for hematological analyses and various organs (heart, liver, kidney and intestine) were removed, washed in normal saline, blot dry with filter paper and weighted in order to determine the weight of different organs and then fixed in 10% formalin.

Blood analyses

Hematological analyses were performed at the Federal university of technology health centre, Akure, Nigeria. Full blood cell counts were carried out to determine the packed cell volume (PCV), white blood cell (WBC), neutrophil and lymphocytes using standard techniques.

Histological studies

The heart, liver, kidney and intestine of the animal were removed and grossly examined. Histopathological analysis was also carried on them by staining with haematoxylin-eosin before examined under the light microscope. Histological processing and interpretation was done at the Histology department state specialist hospital, Akure, Nigeria.

Tissue processing

The tissue were fixed in normal saline for 24 to 48 h and then processed through a series of ethyl alcohol of ascending strength (70, 80 and 95%) for a period of 1 h and twice in absolute alcohol and twice in xylene (1 h each) in order to render the tissue elements transparent. The tissues were then infiltrated with molten paraplastic at 58°C. The transparent tissues, after clearing the entire element from it, were embedded in a solid mass paraplast. The blocks were removed. The solid mass was then trimmed free of excess paraplast, leaving some free margin around the embedded.

DISCUSSION

Histopathological study of medicinal plants is an aspect of pharmacognosy in Nigeria that has not been given much attention in order to determine the toxic or beneficiary effects of medicinal plants when used for a long period of time as food or medicine to various tissues and organs of the body. Gross examination of the organs of different groups of animals showed no colour changes

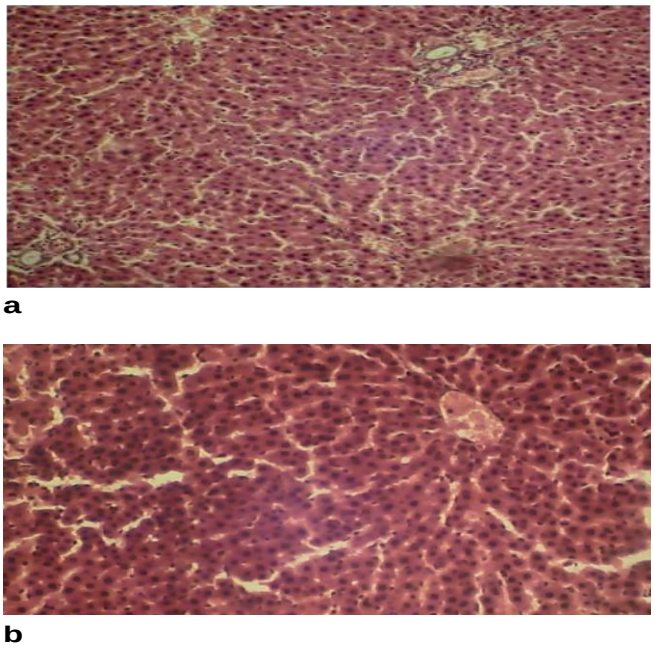

Figure 1. (a) Photomicrograph of liver section of the normal rats (control) H + E ×100; (b) photomicrograph of liver section of the treated rats showing thickening of the hepatocytes of the liver section of rats fed. H + E ×100.

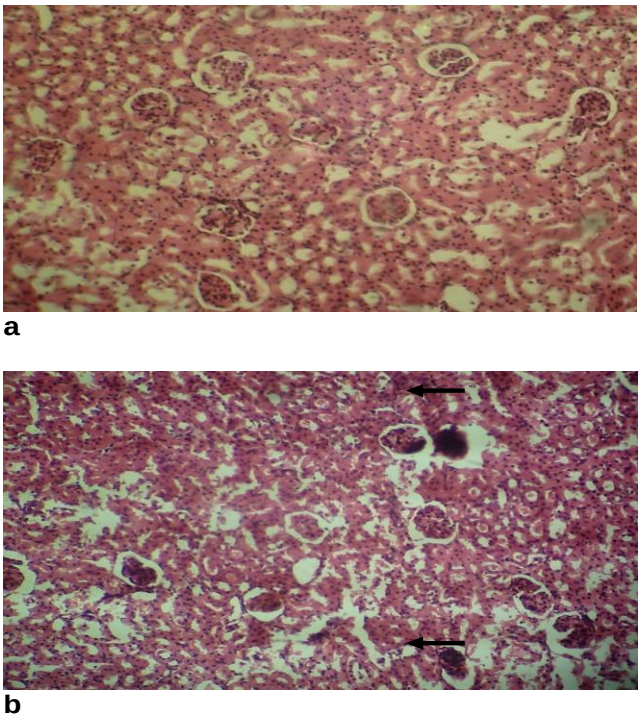

Figure 2. (a) Photomicrograph of kidney section of the control rats. H + E ×100; (b) photomicrograph of kidney section of the treated rats showing haemorrage and shrinking of glomerula (arrows) of kidney section of the rats treated with GRE. H + E ×100.

except that of liver which appeared dark-red and intestine which was pale when compared with the control. Autopsy at the end of the experiment period revealed changes in all the organs, though there were no changes in the body weight of the animals.

The hematological parameters of this study revealed an increase in the PCV compared with the control. The increase seen in the treated group might be as a result of the effect of the methanol extracts which have affected the hematopoiesis of the rats. It might also be as a result of exogenous uptake of mineral from the mushroom extracts. The hematopoietic system is very sensitive to toxic compounds and severs as an important index of the physiological and pathological status in both animal and man (Adeneye et al., 2006). Statistically, there is no significant difference in the PCV of control and treated groups. There is also no significant difference in the WBC observed in this experiment when compared with the control. The lymphocytes, neutrophil and monocytes levels also showed no significant difference when compared with the control.

The liver is the first organ that encounters all absorbed materials from the gastrointestinal tracts work and showed thickened hepatocytes with respect to the control (Figures 1a and b). The kidney is an excretory organ that removes metabolized and non metabolized toxic wastes from the body (Robbins et al., 1984), hence the organ is exposed to high concentrations of noxious materials that would cause lesions (Akinnawo et al., 2005).

There was haemorrhage, reduction and detachment of glomerula of the kidney section (Figures 2a and b). The intestinal section revealed degeneration and erosion of the crypt of the villi and the congestion of microvilli (Figures 3a and b). The smooth muscles of the heart (Figures 4a and b) showed necrosis, expansion and wavy contraction band of rat section. The heart, liver, kidney and intestine play significant role in various metabolic processes. The liver plays important role in xenobiotic function while digestion of food and absorption of water takes place in the intestine. The heart is the site where blood is circulated to other parts of the body for various processes and the kidney is the site for filtration and reabsorption. The antimicrobial and phytochemical activities of various *Ganoderma* sp were examined and their potencies were qualitatively and quantitatively evaluated by oral and histopatology studies. Sasidharan et al. (2008) reported that toxic effects of natural products on host cells must be considered as a substance that may exhibit an apparent biological activity by virtue of toxic effect on cells. Ogbonnia et al. (2008) reported that experimental screening is important to ascertain the safety and efficacy of natural products and to establish the components of these natural products. The orally administered dose of the extract interferes with the production of circulating red blood cells and WBC; and also produced side effects on the vital organs. A toxic

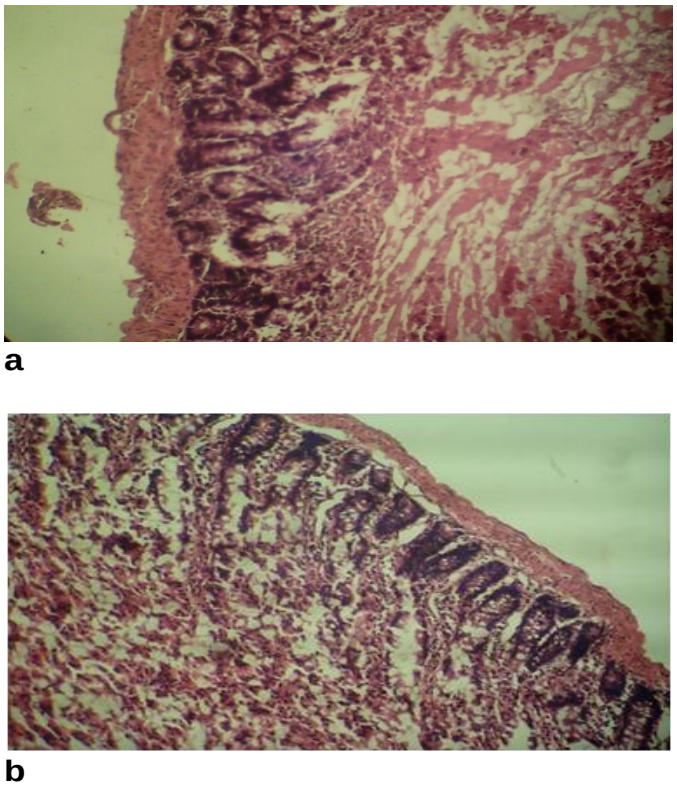

Figure 3. (a) Photomicrograph of intestinal section of control rats. H + E × 100; (b) photomicrograph of intestinal section of rats fed with GRE showing congestion of the microvilli. H + E ×100.

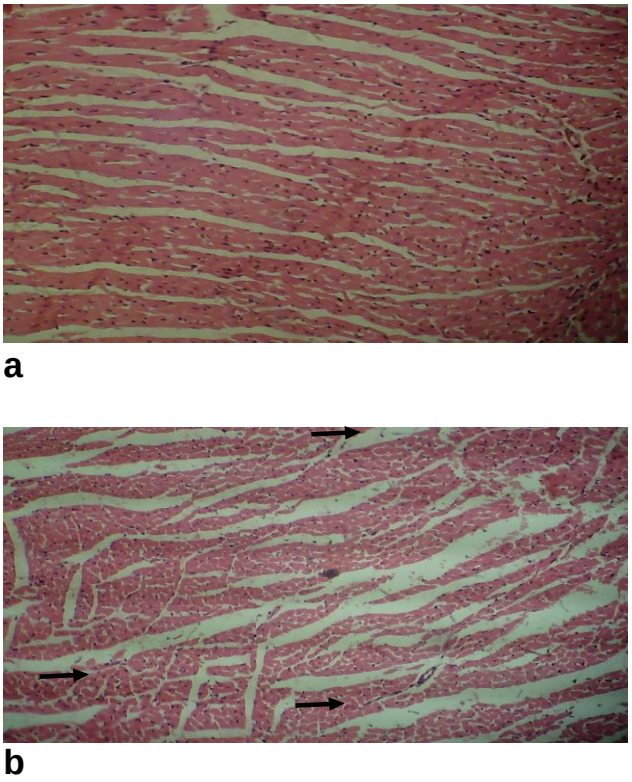

Figure 4. (a) Photomicrograph of showing smooth muscles of the heart for control rats. H + E × 100; (b) photomicrograph of smooth muscles of the heart for treated rats showing necrosis is setting in and a mild wavy contraction band unremarkable of any significant changes in the histology of the heart. H + E × 100.

substance at a lower dose might elicit pharmacological effects of interest in animal model for judging safety of *Ganoderma* sp. The majority of common dietary constituents do not have acute biological effect immediately after ingestion. However, when eaten daily over a life time, subtle long term effects may be observed. It could therefore, be concluded that indiscriminate large consumption of *Ganoderma* mushrooms for whatever reason over a long period as medicine is not be safe and could be hazardous to health.

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