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## Effect of a Long Term Consumption of a Diet Supplemented with Leaves of *Gongronema latifolium* Benth. on Some Biochemical and Histological Parameters in Male Albino Rats

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**Abstract:** This study investigated the effect of long term consumption of *Gongronema latifolium* on some biochemical and histological parameters in male rats. Biochemical parameters evaluated included serum protein, haemoglobin, cholesterol, lipid peroxidation, white blood cells, glutathione-s-transferase, superoxide dismutase, alanine transaminase, aspartate transaminase and alkaline phosphatase. The histological changes of the liver, intestines and testes were examined. The long term feeding of a *Gongronema latifolium* -supplemented diet caused significant increases ( $p < 0.05$ ) in cholesterol, haemoglobin and white blood cells. The diet exhibited antioxidant properties by reducing lipid peroxidation and increasing superoxide dismutase activity. There was a significant increase ( $p < 0.05$ ) in glutathione-s-transferase and decrease in the liver enzymes namely Alanine transaminase, aspartate transaminase and alkaline phosphatase suggesting a role of *Gongronema latifolium* in detoxification and hepatoprotection. However, *Gongronema latifolium* -supplemented diet did not cause any significant changes in weight and serum protein. The histology of the hepatic plate appeared irregular while the portal tracts showed a lifted basement membrane and few inflammatory cells. The intestinal mucosa showed an elongated and broadened villi. There were no histological changes on the testes.

**Key words:** *Gongronema latifolium*, haemoglobin, white blood cells, lipid peroxidation, glutathione-s-transferase, superoxide dismutase

### INTRODUCTION

*Gongronema latifolium* Benth. is a tropical rain forest plant widely used for culinary and medicinal purposes in Nigeria (Ugochukwu *et al.*, 2003). It is widely dispersed in the African forest and family farms as wild, semi-wild and cultivated (Okafor, 1980). It climbs by twining around a support and can also run along the ground producing adventitious roots. *Gongronema latifolium* belongs to the family Asclepiadaceae and is a perennial plant that produces milky latex when pricked. It produces leaves that are simple, entire and pale green with mature petioles. The leaves measure about 4-8 cm long ovate with cordate base and acute apex. *Gongronema latifolium* leaves contain nutrients such as amino acids, carbohydrate, minerals and phytochemicals (Osagie and Eka, 1998; Morebise *et al.*, 2002). Some of the constituents especially the phytochemicals are responsible for the bitter and astringent taste of the leaves of the plant. *Gongronema latifolium* is widely used in folklore medicine in the management of diabetes, stomach pains

and worm infestation (Akah and Okafor, 1992; Burkill, 1994). It is used to stimulate appetite and rapid contraction of womb during postpartum (Okafor, 1980). It is believed to improve growth in children and also used in treatment of cough (Osagie and Eka, 1998; Burkill, 1994).

Some of these medicinal effects of *G. latifolium* are due to its hypoglycaemic, antioxidant, antiinflammatory, antimicrobial, hepatoprotective, hypotensive and hypolipidemic properties (Ugochukwu and Babady, 2002; Ugochukwu *et al.*, 2003; Morebise *et al.*, 2002; Nwanjo and Alumanah, 2005; Nwinyi *et al.*, 2008). Its culinary uses include as spice and vegetable in soups, stews and salads (Osagie and Eka, 1998). The antioxidant and liver enzymes namely glutathione-s-transferase, superoxide dismutase, alanine transaminase, aspartate transaminase and alkaline phosphatase and the phytochemicals were studied to confirm the antioxidant and hepatoprotective effects of *G. latifolium*. Generally there is abundant information on some biological and medicinal effects of *G. latifolium* without a concomitant work on its effects on

animal histological characteristics. This study is aimed at contributing information on other biochemical and histological changes associated *G. latifolium* consumption.

**MATERIALS AND METHODS**

**Collection and processing of leaves of *G. latifolium*:** Fresh leaves of *G. latifolium* were harvested from a family garden in Nsukka, South-Eastern Nigeria. The leaves were sorted, picked, air-dried and milled to a coarse powdered form. This is the *G. latifolium* powder. The proximate composition of *G. latifolium* powder was determined according to the procedure of AOAC (1990).

**Formulation of *Gongronema latifolium* -supplemented diets:** *Gongronema latifolium* -supplemented diet (GLSD) was formulated by incorporating 5% of *G. latifolium* powder with other feedstuffs including maize, corn flour, fish meal, groundnut meal, bone meal and vitamin premix. The result of the proximate composition of the *G. latifolium* powder was considered in the formulation of an isocaloric and isonitrogenous *Gongronema latifolium* -supplemented diet (GLSD). A control diet (CD) was also formulated alongside GLSD consisting of all the feedstuffs without the inclusion of *G. latifolium* powder.

**Grouping and feeding of animals:** Twenty male albino rats aged six weeks old and with an average weight of 45 g were used for the experiment. The rats were randomly distributed into two groups namely Control Group (CG) and *G. latifolium* group (GLG) consisting of ten animals each. The control group were fed the Control Diet (CD) while the *G. latifolium* group (GLG) were fed *G. latifolium* -supplemented diet (GLSD) and given water *ad libitum* for six months. The average weights of animals in the two groups were recorded monthly.

**Phytochemical screening of leaves of *Gongronema latifolium*:** A preliminary phytochemical screening for the presence of tannins, phytosterols, glycosides, flavonoids, anthocyanidins, alkaloids and saponins were carried out on the leaves of *G. latifolium* (Odebiyi and Sofowora, 1978).

**Collection of blood, organs and tissue samples:** At the end of the feeding period of six months, six animals each from the control group and *G. latifolium* group were anesthetized and decapitated. Blood samples were collected in labelled sample bottles with drops of Ethylenediaminetetraacetic acid (EDTA). Serum samples

were collected in sample bottles without EDTA and allowed to clot before being centrifuged at 5000 rpm for 10 min. The liver, intestines and testes were promptly excised soon after decapitation and stored in 10% formyl saline.

**Determination of biochemical parameters:** Total serum protein was determined by the Lowry *et al.* (1951). Total and free cholesterol was determined by the method of Searcy and Bergquist (1960). Lipid peroxidation was determined by the method of Wallin *et al.* (1993). Hemoglobin and White Blood Cells (WBC) were determined according to methods described by Dacie (1975) and Dacie and Lewis (1991). Glutathione-s-transferase (GST) was assayed according to the method described by Habig *et al.* (1974). Superoxide dismutase (SOD) was assayed by the method of Jewette and Rocklin (1993). Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were determined using Randox test kits, UK according to the method of Reitman and Frankel (1957).

**Histological examination of tissues and organs:** Processing and histological examination of the sections of liver, intestine and testes of the rats in the two experimental groups were done according to procedures described by Disbrey and Rack (1974) and Drury and Wellington (1967).

**Statistical analysis:** The results obtained were expressed as Mean±Standard Error of Mean (SEM) for triplicate determinations. A One-way Analysis of Variance (ANOVA) for a completely randomized design and Duncan’s multiple range tests were used to analyse experimental data. Values were considered significant at p<0.05.

**RESULTS AND DISCUSSION**

Table 1 represents the result of the phytochemical screening of the leaves of *G. latifolium* which show that

Table 1: Phytochemical constituents of leaves of *Gongronema latifolium*

Substances	Results
Phenolic substances	++++
Flavonoids	++
Anthocyanidins	+++
Phytosterols	++
Tannins	+
Saponins	+
Alkaloids	ND
Glycosides	++
Cyanogenic glycosides	++
Cardiac glycosides	ND

+: Presence, ND: Not detected

Table 2: Effect of *Gongronema latifolium*-supplemented diet (GLSD) on some biochemical parameters (n = 6)

Experimental groups(Diet)	Weight (%)	Protein (mg mL <sup>-1</sup> )	Cholesterol (mM L <sup>-1</sup> )	Hemoglobin (g dL <sup>-1</sup> )	WBC (10 <sup>3</sup> mm <sup>-3</sup> )	Lipid peroxidation index (nmol MDA mL <sup>-1</sup> )
Control diet (CD)	60.0±7.28	261±16.4	1.43±0.18	9.8±0.50	5.13±0.29	5.03±0.23
<i>Gongronema latifolium</i> -supplemented diet (GLSD)	59.5±7.98	289±13.66	0.95±0.10*	12.1±1.06*	9.17±0.33*	2.84±0.28

\*p<0.05

Table 3: Effect of *Gongronema latifolium* -supplemented diet (GLSD) on some enzyme levels (n = 6)

Experimental groups (Diet)	GST (IU L <sup>-1</sup> )	SOD (U mg <sup>-1</sup> protein)	AST (IU L <sup>-1</sup> )	ALT (IU L <sup>-1</sup> )	ALP (IU L <sup>-1</sup> )
Control diet (CD)	5.41±0.82	70.7±3.68	88.7±7.41	45.3±5.3	88.6±8.96
<i>Gongronema latifolium</i> -supplemented diet (GLSD)	7.28±1.21*	28.7±3.68*	61.0±9.61*	32.0±5.0*	67.3±5.56

\*p<0.05

it contained tannins, phytosterols, glycosides, flavonoids, anthocyanidins and saponins. Alkaloids and cardiac glycosides were not detected.

The long term effect of feeding of *G. latifolium*-supplemented diet (GLSD) on weight and some biochemical parameters are presented in Table 2. The GLSD did not cause any significant change in weight when compared to the control diet. The increase in serum protein by GLSD was not significant (p<0.05). The reduction in the cholesterol level from 1.43±0.18 to 0.95±0.10 Mm L<sup>-1</sup> by GLSD was statistically significant (p<0.05). The lipid peroxidation level was reduced from 5.03±0.23 and 2.84±0.28 mg mL<sup>-1</sup> in animals fed GLSD. This reduction in lipid peroxidation was significant (p<0.05). Hemoglobin and WBC levels were also significantly increased (p<0.05) in the animals fed with GLSD.

There were significant increases (p<0.05) in the activities of GST and SOD in the animals fed GLSD. The liver enzymes including AST, ALT and ALP were significantly reduced (p<0.05) by GLSD (Table 3).

The histological features of liver, intestine and testes of the animals fed Control Diet (CD) and *Gongronema latifolium*-supplemented diet (GLSD) are presented in Fig. 1a, b, 2a, b and 3a, b. The hepatocytes of the rats fed GLSD were clustered and had a distorted architecture. The hepatic plate appeared irregular and the portal tracts showed a lifted basement membrane and few inflammatory cells. There were histological changes in the intestinal mucosa of rats fed with GLSD. The mucosa showed elongated and broadened villi and dilated vessels of lamina propia. There were no visible histological changes in the testes of the animals fed with GLSD when compared to the control group.

This study showed that the consumption *Gongronema latifolium*-supplemented diet (GLSD) for a long term did not result in any significant changes in the weight of the animals. Consumption of food in the absence of disease normally leads to increase in weight. The unpalatability of GLSD due to the presence of bitter substances in the leaves of *G. latifolium* reduced the quantity of the feed consumed by the rats and hence their

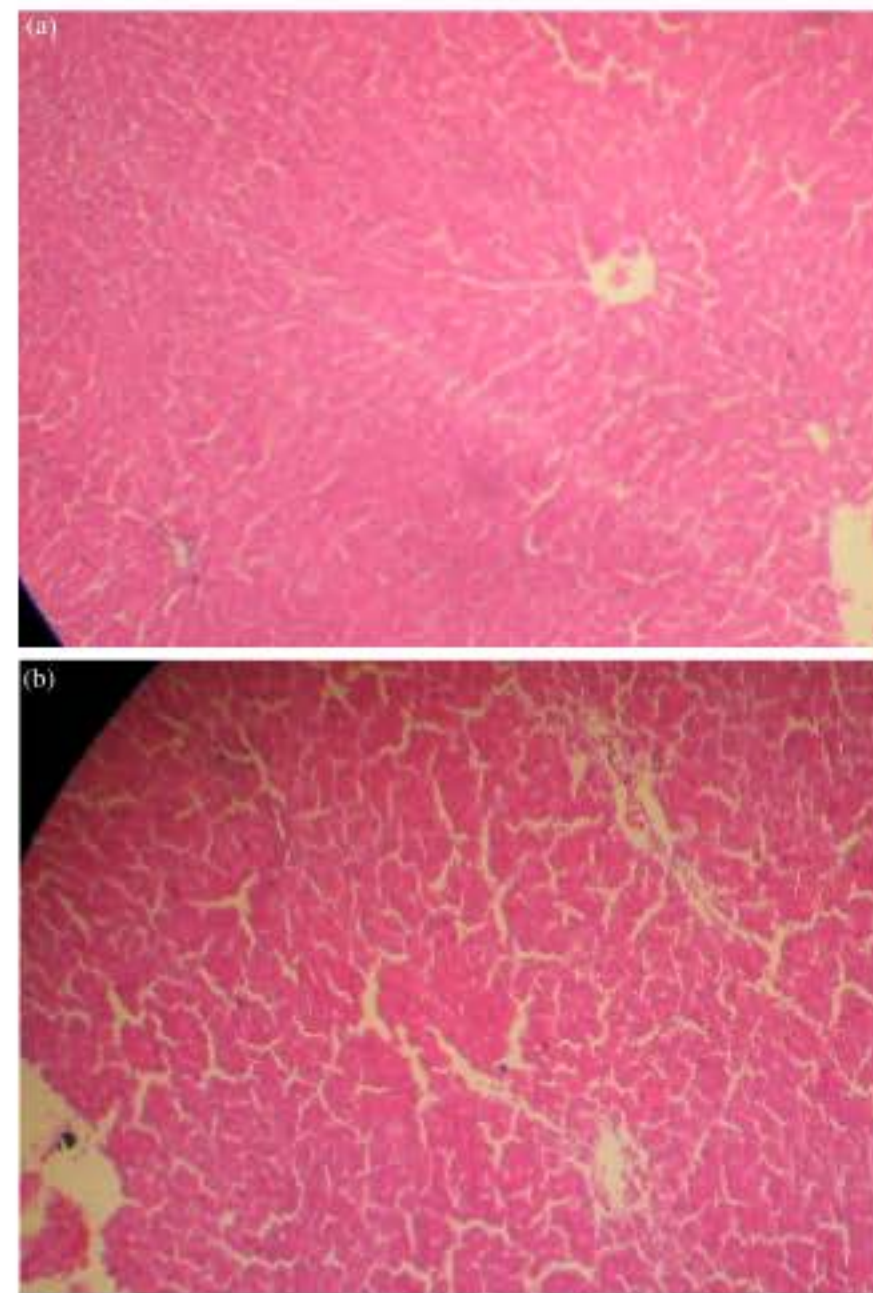


Fig. 1: (a) Photo micrography of a cross section of liver of rats fed with control diet (CD). MAG. x 40 and (b) Photo micrography of a cross section of liver of rats fed with *Gongronema latifolium*-supplemented diet (GLSD). MAG. x 40

weight. In a similar study, another commonly consumed vegetable named *Telfairia occidentalis* increased the weight of animals that consumed it for a long term due to its palatibility (Iweala and Obidoa, 2009). The bitter taste of leaves of *G. latifolium* may prevent its culinary use as a major source of nutrients. Also the increase in the serum protein by GLSD was not statistically significant (p<0.05) eventhough *G. latifolium* has been shown to be a good source of protein and other essential amino acids (Eleyinmi, 2007). The reduction in cholesterol level

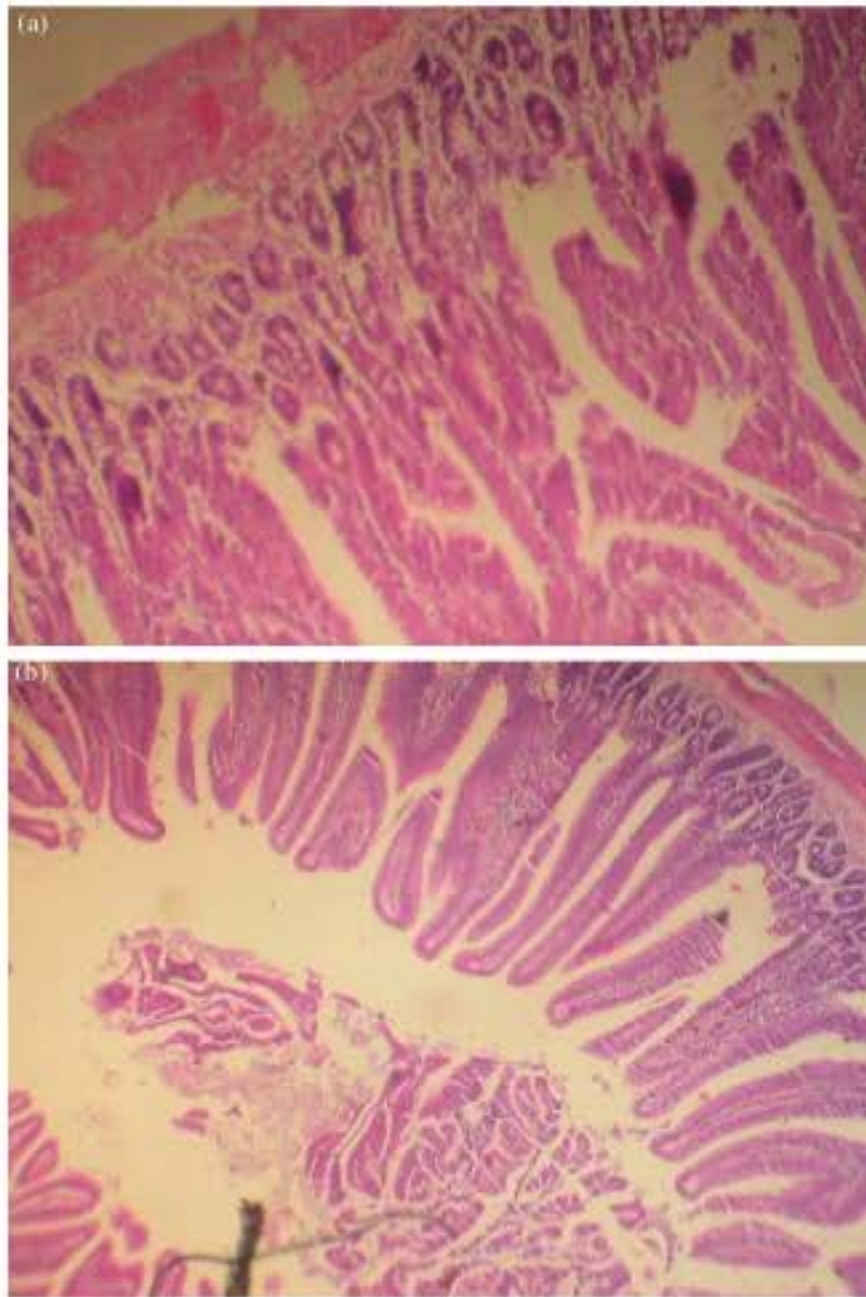


Fig. 2: (a) Photo micrography of a cross section of intestine of rats fed with control diet (CD). MAG. x 40 and (b) Photo micrography of a cross section of intestine of rats fed with *Gongronema latifolium*-supplemented diet (GLSD). MAG. x 40

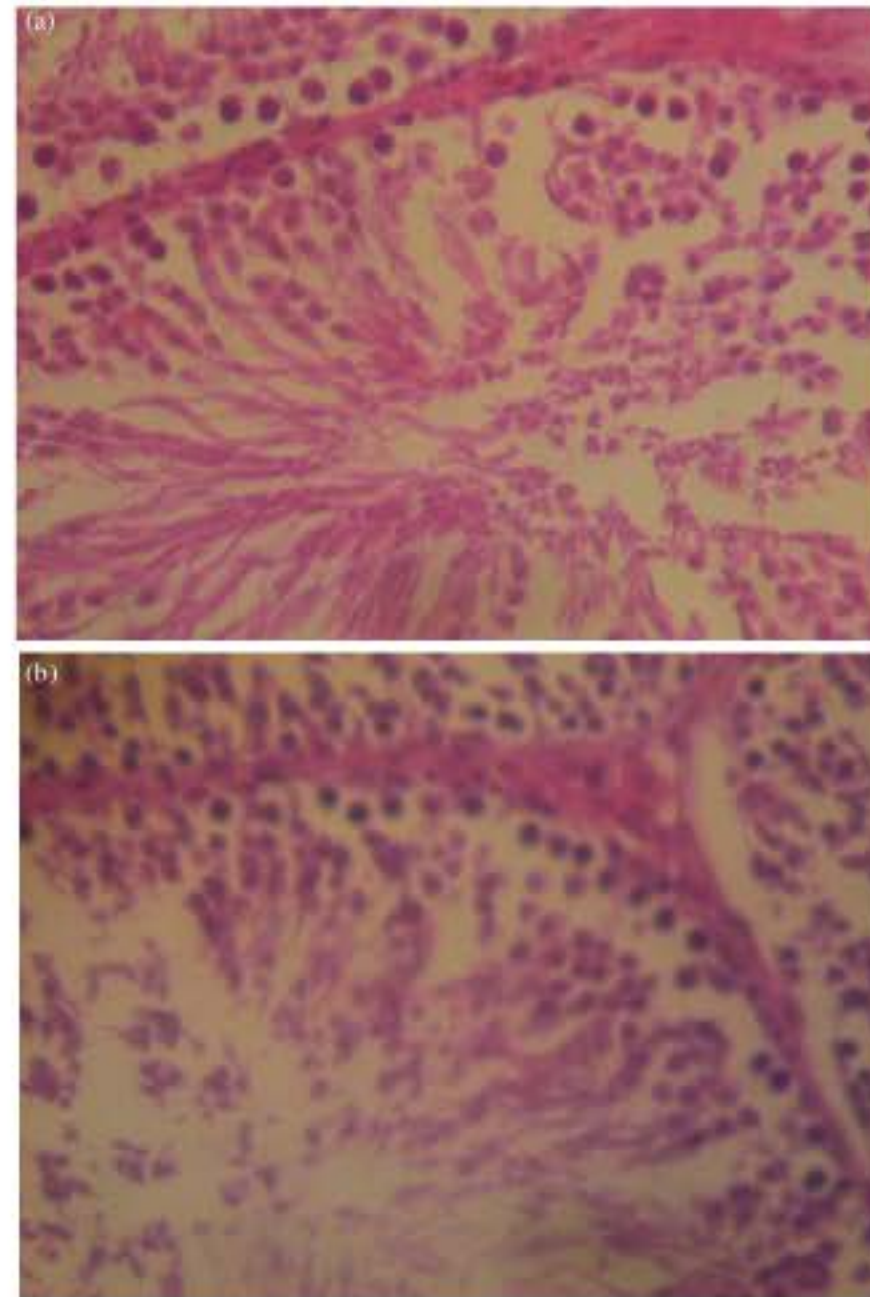


Fig. 3: (a) Photo micrography of a cross section of testes of rats fed with control diet (CD). MAG. x 8 and (b) Photo micrography of a cross section of testes of rats fed with *Gongronema latifolium*-supplemented diet (GLSD). MAG. x 8

by GLSD is similar to the finding of Ugochukwu *et al.* (2003). Decrease in cholesterol level is important in the metabolism of diabetics as well as in heart related ailments (Cho *et al.*, 2002). The reduction in lipid peroxidation represented as the level of malondialdehyde (MDA) is attributed to the inactivation of free radicals by antioxidant phytochemicals especially saponins and flavonoids present in most medicinal plant foods such as *Gongronema latifolium* (Gupta *et al.*, 2002; Hernández *et al.*, 2000). *Gongromena latifolium* extracts have been found to reduce the levels of lipid peroxides in diabetic rats (Nwanjo *et al.*, 2006). The decreased lipid peroxidation is accentuated by the increased activity in SOD in the animals fed GLSD which was similarly reported by Ugochukwu and Babady (2002). SOD is the major enzyme involved in scavenging of reactive oxygen species implicated in lipid peroxidation (Mahdi, 2002; Marklund *et al.*, 1982). The reduction in lipid peroxidation and increase in the activity of SOD confirms these processes as one of the mechanisms by which

*G. latifolium* produces its medicinal actions. The significant reduction in the liver enzymes tested namely AST, ALT and ALP by GLSD clearly showed that *G. latifolium* is not toxic to the liver but hepatoprotective. Liver damage is assessed by the determination of serum levels of its enzymes such as ALT and AST (Dobbs *et al.*, 2003). High levels of AST and ALT indicate liver damage, cardiac infarction and muscle injury. However, ALT is more specific to the liver and is thus a better parameter for detecting liver injury. Serum ALP level on the other hand, is related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis of the enzyme, in presence of increasing biliary pressure (Moss and Butterworth, 1974). Generally an increase in these liver enzymes indicates injury or toxicity to the organ (Ghadi, 2000). Several African medicinal plants have been shown to have hepatoprotective effects (George and Chaturvedi, 2008; Chaturvedi *et al.*, 2007; Roy *et al.*, 2006; Chattopadhyay, 2003). Hepatoprotection is possibly due to flavonoids which exerts a membrane-stabilizing action

that protects the liver cells from injury (Hahn *et al.*, 1968; Perrissoud, 1986). A good liver function is inextricably linked to a good detoxification and antioxidant system, which is indispensable in the ensuring optimum physiological function and prevention of diseases. The consumption of GLSD increased the level of GST in the animals. GST enzyme and other detoxification enzymes are usually increased by plant foods and medicinal plants (Banerjee *et al.*, 1996; Aruna and Sivaramakrishnan, 1990). The increase in GST is attributed to the presence of phytochemicals that are bifunctional inducers of phase I and II enzymes especially flavonoids and anthocyanidins in plant foods such as *G. latifolium* (Birt *et al.*, 2001; Ren *et al.*, 2003; Ross and Kasum, 2002; Kusamran *et al.*, 1998; Mulcahy *et al.*, 1997). Similarly the water extract of some plants have been reported to enhance GST activity (Saha and Das, 2003; Derbersac *et al.*, 2001). The induction of these enzymes can occur through up regulation of the corresponding genes by interacting with the antioxidant response elements (AREs) that transcriptionally regulate these genes (Ferguson, 2001). Other phytochemicals may induce GST by acting as ligands to receptors involved in the activation of transcription of genes that code for them (Prochaska and Talahay, 1988). The ability of certain phytochemicals to induce GST is due to their possession of a chemical signal in the form of an electrophilic center that is able to react with sulfhydryl groups through oxidoreduction or alkylation (Prester *et al.*, 1993). Increase in GST activity translates to an increased capacity to conjugate and excrete toxic intermediates that can cause diseases (Pantuck *et al.*, 1984). Hemoglobin levels were increased significantly in all the animals fed GLSD which is a direct consequence of the increased serum total protein. Hemoglobin synthesis is also increased by consumption of plant foods due to their high content of minerals and vitamins that may stimulate synthesis of hemoglobin. In traditional Nigerian folklore medicine, aqueous extracts of some leafy vegetables are employed in the treatment of anemia (Alada, 2000). Most phytochemical constituents of plant food affect the immune system (Kubena and McMurray, 1996). Antioxidant phytochemicals are known to protect lymphocytes and reduce their destruction (Duthie *et al.*, 1996). Flavonoids are known to inhibit platelet aggregation (Formica and Regelson, 1995). Phytosterols modulate immune function through their effect on T-helper cells and natural killer cells (Bouic *et al.*, 1999; Middleton and Knadaswami, 1993). In this study, the level of White Blood Cells (WBC) was used as an index of immune function. There were significant increases in WBC levels in the animals fed GLSD whose content of phytosterols and flavonoids may

have possibly influenced processes involved in the production of white blood cells. The distinct histological changes seen in the liver and intestines of animals fed GLSD showed activities by the plant. The changes in the liver histology seen as a distorted architecture complements the observed reduction in the liver enzymes by GLSD discussed earlier. A histological change in the intestines especially on the villi which is the major site for absorption of food substances indicates that GLSD was highly digested and absorbed. This is an important factor ensuring availability of components of *G. latifolium* for its effective culinary and medicinal use.

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