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Effect of Aqueous Leaves Extract *of Gardenia ternifolia* Plant on Carbon Tetrachloride-Induced Hepatotoxicity in Rats

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Abstract: *The effects of aqueous leaves extract of the Gardenia ternifolia plant (ALEGTP) was investigated against carbon tetrachloride (CCl_4) - induced hepatotoxicity in rats. Thirty albino rats were used for the experiment and grouped into six (n=5); group 1 and 2 were normal and control respectively. Group 3 was pretreated with silymarin while group 4, 5 and 6 were pretreated with 100, 200 and 400 mg/kg body weight of ALEGTP for one week prior to administration of CCl_4. Pretreatment with ALEGTP significantly ($P < 0.05$) decreased the levels of; AST, ALT, ALP, T.CHO, TG and C.BIL. Histopathological view of the liver section of rats treated with ALEGTP also supported the biochemical parameters showing less necrosis in their hepatocytes compared to group that was administered CCl_4 only, revealing features of massive liver necrosis and degeneration of the hepatocytes. Pretreatment with 200 mg/kg body weight of ALEGTP exhibited moderate protective effect by lowering the levels of serum enzymes: The ameliorating effect of ALEGTP was supported by histopathological section of the liver tissue of rats, the micrograph of the groups that were pretreated with the ALEGTP revealed moderate necrosis. From the study carried out it is evident that ALEGTP has properties of amelioration of CCl_4-hepatotoxicity in rats.*

Key words; *Hepatotoxicity, CCl_4, Liver enzymes, non enzyme markers, lipid profile and Gardenia ternifolia plant*

I. Introduction

Liver organ is responsible for metabolism and detoxification of most components that enter the body [1]. It plays pivotal role in regulating many important functions, such as metabolism, secretion, storage and regulation of various physiological processes [2]. Furthermore, it is involved in detoxification of a variety of drugs and xenobiotics and therefore at increased susceptibility to the toxicity from these agents. The liver is frequently abused by poor drug habits, alcohol and widely exposed to environmental toxins, prescribed and over-the-counter drugs, which cause various liver diseases [3]. Other important liver functions include, it storage of minerals and vitamins, it produces bile to digest food, it purifies the blood, it produces a blood clotting agent, it filters poisonous toxins and it produces new proteins [4]. Liver injury or liver dysfunction is a major health problem that challenges not only health care professionals, but also the pharmaceutical industry and drug regulatory agencies. Liver cell injury caused by various toxic chemicals (certain anti-biotic, chemotherapeutic agents, carbon tetrachloride (CCl_4), thioacetamide (TAA), excessive alcohol consumption and microbes are well-studied [5]. Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects [6]. Herbal medicines are in great demand in developed as well as developing countries' for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs [7]

Carbon tetrachloride is a highly toxic chemical agent, the most famous drug used to induce liver damage experimentally. Histopathological sectioning of the liver tissues indicated that CCl_4 induced fibrosis, cirrhosis and hepatocarcinoma [8, 9]. The toxic effect of CCl_4 is attributed to trichloromethyl radical produced during oxidative stress [10]. The number of infiltrated neutrophils, macrophages, Kupffer cells, lymphocytes and natural killer cells are significantly increased after liver injury induced by hepatotoxins such as CCl_4 [11] the activated macrophages are released and contributed to liver fibrosis, inflammation and injury [12]. Once the liver became injured, its efficient treatment with famous chemical drugs is limited [13]. Numerous studies noted that CCl_4 is widely used to induce liver damage because it is metabolized in hepatocytes by cytochrome P450, generating highly reactive carbon-centered trichloromethy radical, leading to initiating a chain of lipid peroxidation and thereby causing liver fibrosis [14, 15],

The plant *Gardenia ternifolia* (Schum and Thonn) belongs to the family *Rubiaceae*. A shrub or small tree of about 5m high is called Gaude in Hausa language or T*reke* in Yungur dielec. The plant is distributed across tropical Africa e.g. Mali, Ivory-coast, Nigeria, Chad, Sudan and Senegal [16]. Various parts of *G. ternifolia* (leaves, stem, bark and roots) have been reported by traditional healers as a remedy against malaria fever. The Leaves and roots is use in treatment of hypertension and skin diseases [17]. According to Alhassane Barké traditionally *G. ternifolia* roots is of purgatives, stomachic, anthelmintic diuretics, and emetics for ascites,

infant diarrhoea, and rickets. Bark of the plant use for healing wound, injury, and tooth decay. Fruits are more or less consumed by livestock in the dry season. Branch's used for fencing and pens for livestock, hardwood, yellowish, for tool handles. Fruits (ash) black dye and soap for poison fishing [18]. A decoction of the roots is used to restore failing strength. When boiled with sorghum flour, the root is used to treat Black Water fever [19]. The macerated root is laxative and vermifuge [20]. It is used in the treatment of stomach aches and kwashiorkor [21]

The aim of this study was to determine the effect of leaf extract of *Gardenia ternifolia* (Schum and Thonn) plant on carbon tetrachloride- induced hepatotoxicity in rats.

II. Materials and Methods

2.1 Collection of plant material

Fresh leaves of *Gardenia ternifolia* plant was collected around Jabbilamba village along Yola-Mubi road Adamawa State with some branches, which was taken to the department of Plant Science, Moddibo Adama University of Technology Yola for authentication.

2.1.1 Plant sample preparation

A leaves of *Gardenia ternifolia* plant collected were wash thoroughly with tap water and rinsed with distilled water twice and allowed to dry under room temperature in the laboratory. The dry material was crushed in to a powdered form using pestle and mortar. The obtained fine powder form was used for phytochemical screening and extraction.

2.1.2 Preparation of aqueous extract

The aqueous extract of leaf of *Gardenia ternifolia* plant was prepared according to the method described by Oluduro and Ade [22]. The powder form of the plant was weighed 200g and soaked in 1000 ml of distilled water and left for 48 hours at room temperature and thereafter filtered using Whatman filter paper. The bulked filtrate was concentrated at 40˚C using water bath, and kept in a desiccator for complete dehydration. The residue was later stored at -4˚C untill when needed. Ten grams of the extract was dissolved in 100 ml of distilled water to obtain stock solution of the extract.

2.3 Phytochemical analysis

The powdered form of *Gardenia ternifolia* leaves was used to screen for the presence of phytochemicals such as carbohydrate, tannins, saponins, anthraquinones, flavonoids, alkaloids, glycosides, terpenes, resins, balsam, phenols and sterols. The phytochemical screening was carried out by using standard methods [23].

2.4 Animals

Thirty (30) albino male rats weighing (140 ± 0.8g) were purchased from Veterinary Research Institute, Vom, Jos, Plateau State and they were housed in well ventilated room in a plastic cage through out the experiment.

2.5 Experimental design

Thirty albino rats were used for the experiment; the animals were divided into six groups with five rats (n=5) in each group. Group 1 was the normal group; the animals were fed on normal diet only. Group 2 was negative control was administered with single dose 1 mg/kg body weight of CCl_4 in 1:1 with olive oil only. Group 3 was positive control, pretreated with 100 mg/kg body weight of silymarin drug for a week. While group 4, 5 and 6 were pretreated with 100, 200 and 400 mg/kg of aqueous leaf extract of *Gardenia ternifolia* for a week. After last treatments on day seven, group 2, 3, 4, 5 and 6 were injected (intraperitonealy) with single dose of carbon tetrachloride (CCl_4) 1mg/kg body weight (1:1 with olive oil) to induce hepatotoxicity in rats. After 48 hours the animals in the entire groups were anaesthetized in slight chloroform and sacrifices obtained blood through cardiac puncture for determination of biochemical parameters. Liver tissue was equally collected for histopatological examination.

2.6 Biochemical analysis

The enzymatic activities of serum aminotransferase ALT, AST were assayed basically by the method of (Reitman S; Frankel [24]. The serum total protein was determined according to the method of; [25]. Serum bilirubin was measured by colorimetric method based on Jendrassik and Grof [26]. Triglyceride was measured base by the method of Reihman [27]. The enzymatic end point method of Richmond was used to estimate total cholesterol (TC). The serum HDL-cholesterol was determined by the method of Hiller [28]. Serum triglyceride

level was determined by the method of Fried Wald *et al.* and Stens and Myers [29, 27]. LDL-cholesterol (lipoprotein) in the serum was obtained by subtracting the value for HDL cholesterol from total cholesterol.

2.7 Statistical analysis

Statistical evaluation of data was done using one way analysis of variances with the aid of SPSS version 20 (USA). Difference between means was considered significantly different at ($p < 0.05$). The results were expressed as mean ± S.E.M.

III. Results

3.1 Determination Phytochemical

The quantitative determination of phytochemical components of the leaves extract of the *Gardenia ternifolia* plant revealed the presences of; alkaloids flavonoids Glycoside, phenol, saponins, steroids tannin and terpenoids in (Table 1). Table 2 indicates the concentration of quantitative components of phytochemicals in the leaves of *Gardenia ternifolia* plant. The concentration was expressed in percentages. The result indicates that the leaves extract contain high amount of saponins 12.00 ± 0.23% followed by tannins 10.00 ± 0.23%, alkaloids 8.00% ± 0.45%, flavonoids 2.25 ± 0.03% and terpenoids which is 1.28±0.13%.

Table 1 Qualitative determination of phytochemical components in the leaf of *Gardenia ternifolia* plant

Phytochemical	***Present***
Alkaloids	+
Flavonoids	+
Glycoside	+
saponins	+
Steroids	+
tannins	+
terpenoids	+

Key: + = present

Table 2: Quantitative determination of phytochemical components in the leaf of *Gardenia ternifolia* plant

Phytochemical	***Percentages Composition (%)***
Alkaloid	8.00 ± 0.45
Flavonoids	6.80 ± 0.23
Phenols	2.25 ± 0.03
Saponins	12.00 ± 0.23
Tannins	10.00 ± 0.23
Terpenoids	1.28 ± 0.13

Values are means ± SEM

The effect of CCl_4 and pretreatment with ALEGTP on rats is shown in table 3. Administration of carbon tetrachloride only to rats in group 2, it elevated the levels of; alanine amino transferase (ALT) to 168.80 ± 5.22, aspartate amino transferee AST (403.30 ± 21.24) and alkaline phosphatase (ALP) (126.52 ± 6.75) which is significantly ($P < 0.05$) compared with group 1 AST (116.40 ± 2.94), ALT (73.00 ± 2.8), and ALP (113.60 ± 2.94). Pretreatment with 100, 200 and 400 mg/kg body weight of aqueous leaf extract of *Gardenia ternifolia* (ALEGT*)* showed reduction in the levels of AST and ALT significantly ($P < 0.05$) when compared with group 2. Only pretreatment with 200 mg/kg body weight of ALEGT had significant ($P < 0.05$) reduction in the levels of ALP compared to group 2.

Table 4 shows the serum concentration of total bilirubin or indirect (T.BIL), conjugated bilirubin or direct (C.BIL), total protein (TP) and albumin (ALB) of rats pretreated with different concentrations of ALEGT prior to induction of liver damage with carbon tetrachloride. The result indicates that, group 2 rats that were administered with carbon tetrachloride only had elevated levels of T.BIL (2.92 ± 0.14) and C. BIL (1.72 ± 0.12) but lower levels of TP (57.2 ± 5.26) and ALB (31.80 ± 1.92), significantly ($P < 0.05$) compared with group 1. However, pretreatment with 100 mg/kg body weight of silymarin drug to the rats in group 3 decreased the levels of; T. BIL and C.BIL significantly ($P < 0.05$) compared to group 2, but has no significant effect on the level of total protein and albumin. Pretreatment with 100 mg/kg body weight of ALEGT plant had no significant difference compared to groups 2. Pretreatment with 200 mg/kg body weight of the ALEGT had shown the significant reduction in the level of direct bilirubin (1.38 ± 0.058) only compare with group 2.

Result of lipid profile of rats is shown in Table 5. Administration of CCl_4 only to rats in group 2, remarkably elevated the levels of T.CHOL, TG and LDL with decreased level of HDL significantly ($P < 0.05$) compared to group 1. Pretretment of rats with 100, 200 and 400 mg/kg body weight of ALEGT significantly *(P* < 0.05) reduced the level of T.CHOL and TG significantly ($P < 0.05$), but not level LDL and HDL when compared to group 2. Pretreated with 200 mg/kg body weight of ALEGT exhibited significantly ($P < 0.05$)

reduction in the level of lipid profile closer to that of silymarin when compared with group 4 and 6 that were pretreated with 100, and 400 mg/kg body weight of ALEGT respectively.

Table 3: Effect of pretreatment with ALEGT on (AST, ALT and ALP) of CCl_4-induced liver damage

Groups	Treatment	AST (U/I)	ALT (U/I)	ALP (U/I)
1	Normal	116.40 ± 2.94	73.00 ± 2.81	113.60 ± 2.94
2	Control(1mg/kg B.wt. of CCl_4)	403.30 ± 21.24 *	168.80 ± 5.22 *	126.52 ± 6.75
3	100mg/kg B.wt. of silymarin + CCl_4	75.66 ± 3.70 ^{a}	57.80 ± 3.18 ^{a}	77.20 ± 5.00 ^{a}
4	100mg/kg B.wt. of (ALEGT) + CCl_4	221.02±5.13a ^{bd}	164.6 ± 4.75 ^{bd}	94.60 ± 4.51 ^{ab}
5	200mg/kg B.wt. of (ALEGT) + CCl_4	129.04 ± 2.02 ^{abc}	94.62 ± 2.74 ^{abc}	86.40 ± 2.89 a d
6	400mg/kg B.wt. of (ALEGT) + CCl_4	211.42 ±2.40 ^{abd}	117.40 ± 4.30 ^{abc}	95.20 ±1.50 ^{bcd}

Values are mean ± SEM (n=5), * - Significantly (P <0.05) higher than group 1, **- Significantly (P < 0.05) lower than group 1 a - Significantly (P < 0.05) lower than group 2, b – Significantly (P <0.05) higher than group3, c- Significantly (P < 0.05) lower than group 4, d- Significantly (P < 0.05) higher than group 5
AST Aspartate aminotransferase, ALT Alanine aminotransferase, ALP Alkaline phosphatase, B.wt. Body weight, ALEGT Aqueous leaves extract of *Gardenia ternifolia*.

Table 4: Effect of pretreatment with ALEGT on T.BIL, C.BIL, TP and ALB of CCl_4-induced liver damage

Groups	Treatment	T.BIL (mmol/l)	C.BIL (mmol/l)	TP (g/L)	ALB (g/L)
1	Normal	1.98 ± 0.21	1.22 ± 0.086	73.8 ± 2.95	40.20 ± 10.43
2	1mg/kgB.wt.of CCl_4	2.92 ± 0.14 *	1.72 ± 0.12 *	57.2 ± 5.26**	31.80 ± 1.92**
3	100mg/kgB.wt. of silymarin + CCl_4	1.90 ± 0.071 ^{a}	1.36 ± 0.07 ^{a}	62.00 ± 2.00	37.80 ± 1.92
4	100mg/kg B.wt.of (ALEGT) + CCl_4	2.86 ± 0.09 ^{b}	1.86 ± 0.15 ^{b}	58.20 ± 8.29	30.60±3.65
5	200mg/kg B.wt.of (ALEGT) + CCl_4	2.42 ± 0.18 ^{b}	1.38 ± 0.058 ^{ac}	74.40 ± 2.30	33.40 ± 1.56
6	400mg/kg B.wt.of (ALEGT) + CCl_4	2.70 ± 015 ^{b}	1.96 ± 0.15 ^{bd}	70.20 ± 3.54	32.00 ± 4.30

Values are mean ± SEM (n=5), * - Significantly (P < 0.05) higher than group I, **significantly (P < 0.05), lower than group 1, a – Significantly (P < 0.05) lower than group 2, b – Significantly (P < 0.05) higher than group 3, c- Significantly (P < 0.05) lower than group 4, d- Significantly (P < 0.05) higher than group 5.
T.BIL Total bilirubin, C.BIL Conjugated bilirubin TP Total protein, ALB Albumin**,** B.wt Body weight, ALEGT Aqueous leaves extract of *Gardenia ternifolia* plant

Table 5: Effect of Pretreatment with ALEGT on lipid profile of carbon tetrachloride-induced liver damage

Groups	Treatment	T.CHOL (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
1	Normal	80.60 ±3.76	56.40 ± 1.21	44.20 ± 0.86	24.40 ± 4.79
2	1mg/kg B.wt. of CCl_4	81.60 ±5.10	68.80 ± 2.54 *	29.60 ± 0.95**	38.84 ± 4.98 *
3	100 mg/kg B.wt. of silymarin + CCl_4	52.00 ± 1.87 [a]	52.40 ± 3.03 [a]	32.20 ± 1.24	9.32 ± 0.79 [a]
4	100 mg/kg B.wt. of (ALEGT) + CCl_4	63.20 ± 066 [a]	57.10 ± 2.25 [a]	30.00 ± 0.89	22.04 ± 1.57
5	200 mg/kg B.wt. of (ALEGT) + CCl_4	60.60 ± 1.78 [a]	53.60 ± 1.2 [a]	31.80 ± 2.13	18.04 ± 1.92
6	400 mg/kg B.wt. of (ALEGT) + CCl_4	65.40 ± 2.73 [a]	62.20 ± 1.85	30.40 ± 1.03	25.56 ± 2.80

Values are the mean ± S.E.M (n=5), * - Significantly *(P* < 0.05) higher than group I, **significantly (*P* < 0.05), lower than group 1,a- Significantly (*P* < 0.05) lower than group 2, b - Significantly (*P* < 0.05) higher than group 3, c- Significantly (*P* < 0.05) lower than group 4, d- Significantly (*P* < 0.05) higher than group 5
T.CHOL Total cholesterol, TG Triglyceride, HDL High density lipoprotein, LDL Low density Lipoprotein, B.wt. Body weight, ALEGT. Aqueous leaves extract of *Gardenia ternifolia* Plant.

III. Histological Results

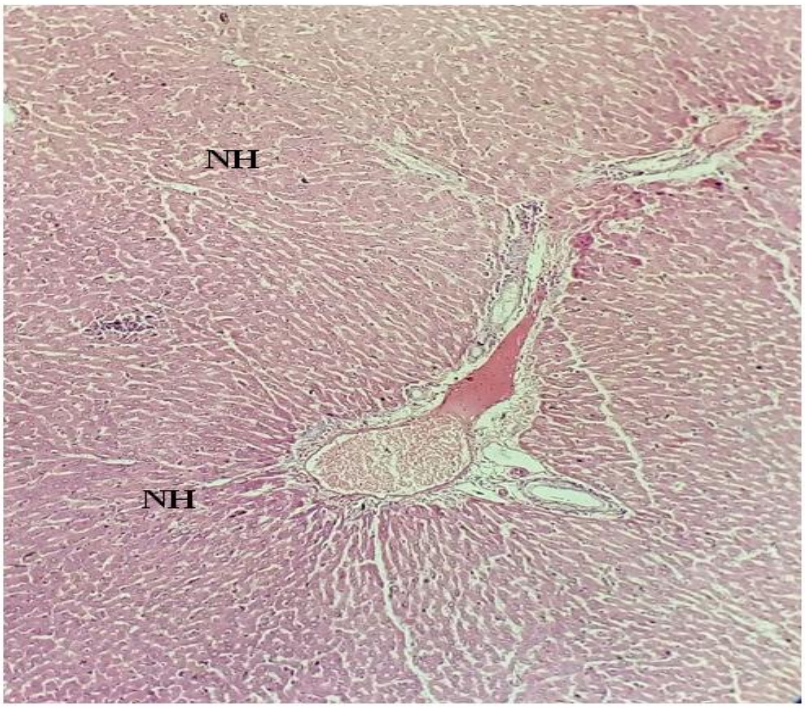

Plate I. Micrograph ((H and E x40) of the liver section of rat in normal group
NH. Indicating normal hepatocytes with well-preserved cytoplasm, prominent nucleus and well brought out central vein (preserved liver architecture).

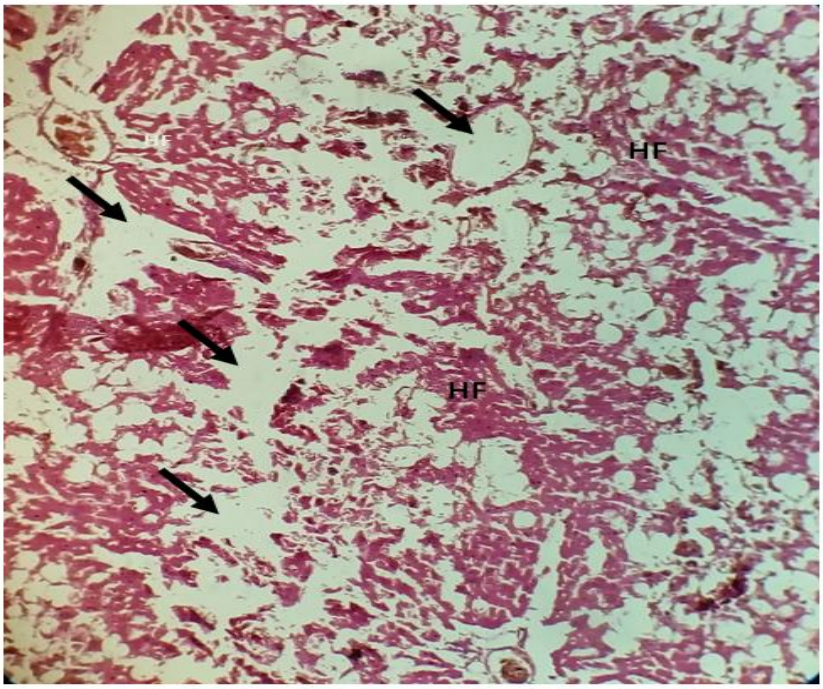

Plate II: Micrograph ((H and E x40) of liver section of the rats administered with CCl_4 only (negative control). HF indicating hepatic fibrosis, black arrow showing ballooning degeneration infiltration

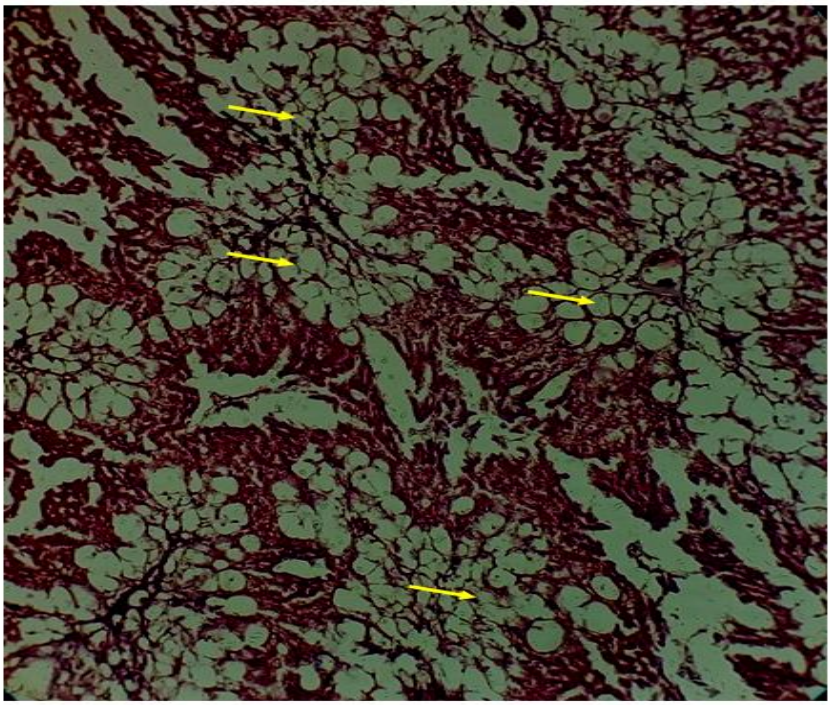

Plate III: Micrograph (H and E x40) of the liver section of rats pretreated with 100 mg/kg body weight of sylmarin drug before administration of CCl_4, yellow arrow indicating cell regeneration

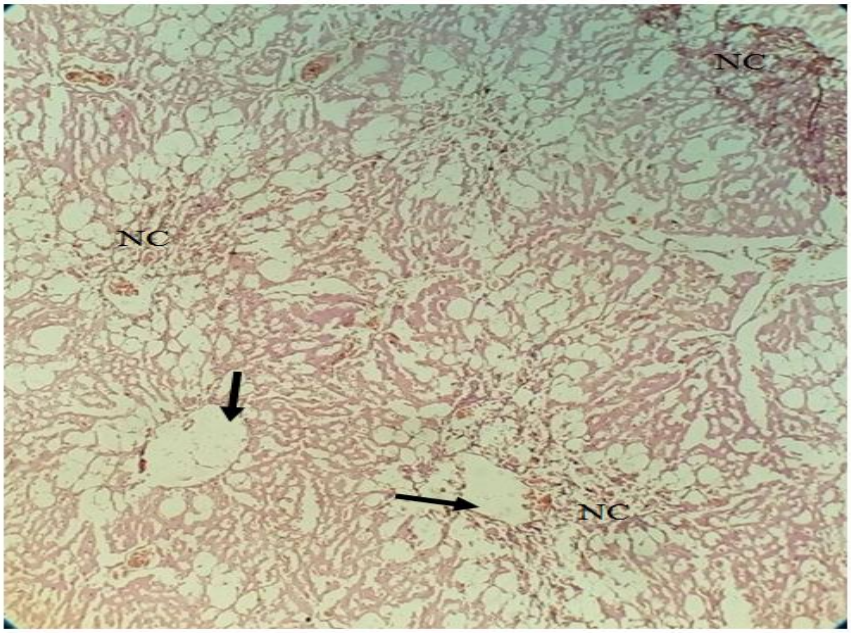

Figure IV: Micrograph (H and E x40) of the liver section of rats pretreated with 100 mg/kg body weight of the leaf extract of *Gardenia ternifolia* plant before administration of CCl_4. NC indicating necrosis of hepatocytes, black arrows showing balloning degeneration infiltration

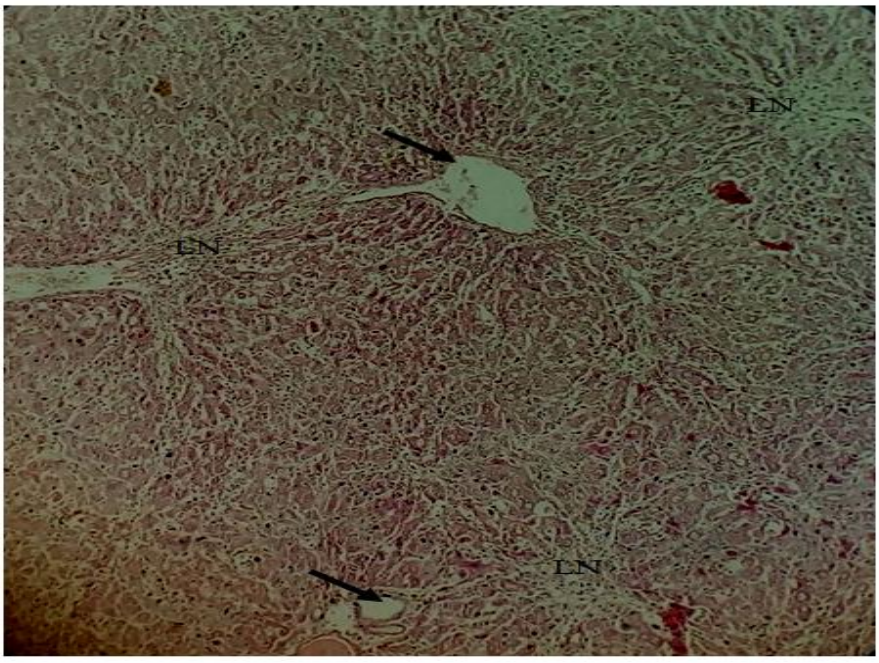

Plate V: Micrograph (H and E x40) of the liver section of rats pretreated with 200 mg/kg body weight of aqueous leaf extract of *Gardenia ternifolia* before administration of CCl_4, NH indicating normal hepatocytes, LN indicating less necrosis, black arrow showing ballooning degeneration infiltration

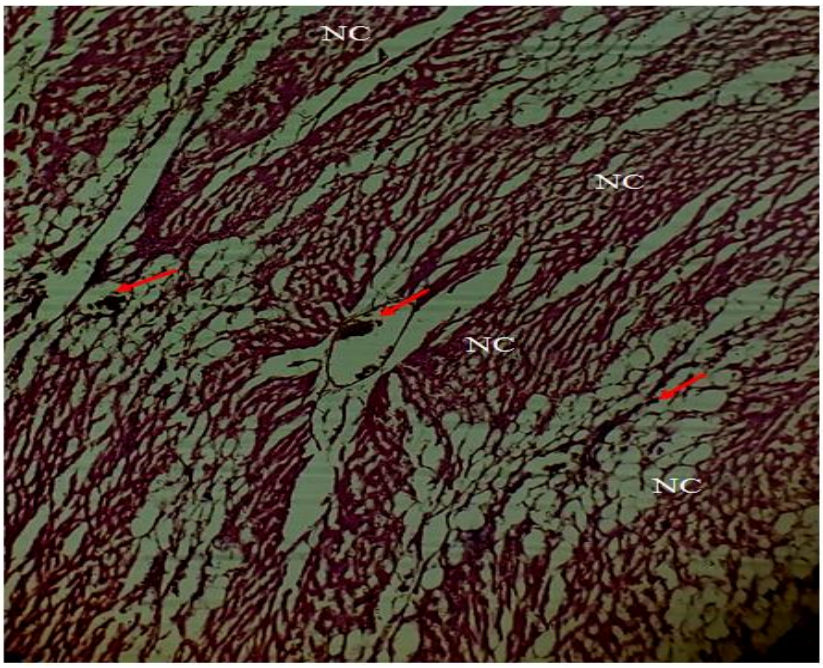

Plate VI. Micrograph (H and E x40) of the liver section of the rats pretreated with 400 mg/kg body weight of the aqueous leaf extract of *Gardenia ternifolia* before administration of CCl_4. NC showing necrosis of the hepatocytes, Red arrows indicating fat vacuole and ballooning degeneration infiltration

IV. Discussion

Carbon tetrachloride is a classic hepatotoxin that causes liver fibrosis, necrosis and cirrhosis sequentially when administrated. It also results in ballooning, inflammatory infiltration of lymphocytes and hydropic degeneration with a clear cytoplasm and vacuolization [30]. Metabolism of CCl_4 is activated by cytochromes to form trichoromethyl radical (CCl_3) which can bind to nucleic acid, protein and lipid, thus impairing crucial cellular processes such as lipid metabolism and resulting in steatosis [15].

In the present study it had been shown that serum hepatic biomarkers such as; Aspartate amino transferase (AST), Alanine amino transferee (ALT) and alkaline phosphatase (ALP) activities were greatly elevated significantly ($P < 0.05$) in rats treated with the CCl_4 compare with group1 that fed on normal diet only. CCl_4 also elevated both conjugate (direct) and total (indirect) bilirubin levels, but significantly ($P < 0.05$) reduced albumin (Hypoalbuminemia) and total protein indicating poor liver functions or impaired synthesis [31]. The increases in serum levels of hepatic markers have been attributed to the liver injury, because these enzymes are placed in cytoplasmic area of the cell and are released into circulation during cellular damage [32, 33]. Or the liver injury could be attributing from inflammatory responses originating from CCl_4-derived free radical formation in the liver and activation of non-parenchyma cells that release a variety of inflammatory cytokines in response to several radical species. Administration of leaf extract of *Gardenia terniforia in different concentration* to various groups and other group with sylmarin drug (as a reference) consecutively for seven days before treatment with CCl_4, appears to prevent hepatic damage due to had decreases in the levels of liver enzymes markers of AST, ALT, ALP and both direct and indirect bilirubin with increases the level of plasma albumin and total proteins considerably. The increase in albumin and total protein shows a good hepatoprotection particularly at a dose of 200 mg/kg body weight, which enhances or recovers the damage induced by CCl_4. The decreases in the level of liver enzymes markers may be attributed to the decrease in lipid peroxidation processes and increase in the plasma protein thiols activities.

CCl_4 treatment could have affected lipid metabolism in the liver of the rats basically (triglyceride and cholesterol levels). This is evidenced from the significantly ($P < 0.05$) increase in the levels of; cholesterol (CHOL), triglyceride (TG) and low density lipoprotein (LDL) with decrease level of high density lipoprotein (HDL), which also correlate with the earlier report by Muller *et al*. [34] stated that CCl_4 intoxication is similar to hepatitis in case of the triglycerides catabolism. This situation could be also attributed to the reduction of lipase activity, which could lead to decrease in triglyceride hydrolysis [28]. On the other hand it can be assumed that hypercholesterolemia in CCl_4 intoxicated rats resulted from damage of hepatic parenchyma cells that led to disturbance of lipid metabolism in liver [35]. However, from the present study, pre-treatments with the leaf extract of *Gardenia ternifolia* had significantly ($P < 0.05$) reduced the levels of triglyceride and cholesterol, which is comparable with sylmarin drug.

Histopatological view of the liver section of the rats also supported biochemical parameters of the group pretreated with the aqueous leaf extract of *Gardenia ternifolia* plant revealing a feature of hepatic cell with less necrosis particularly with 200 mg/kg body weight. This may be attributed to the phytochemical component present in the leaf extract of *Gardenia ternifolia* plant.

V. Conclusion

From the study carried out, it is clear that; carbon tetrachloride (CCl_4) has ability of causing liver damage in rats when administered. This was clearly by increases in enzyme markers; AST, ALT, ALP and non serum enzymes; bilirubin and lipid profile parameters while lowering the levels of TP and Albumin, which indicates the severity of hepatopathy. The effect was also confirmed on Histopatological view of the liver section of the rats administered with CCl_4 only. However administration of the leaves extract of *Gardenia ternifolia* plant for seven days prior to induction of liver damage with CCl_4, resulted in significantly ($p< 0.05$) reduction in enzymes markers; (AST), (ALT), (ALP) and bilirubin while significantly ($p < 0.05$) in the concentration of the plasma albumin and the total protein. Histopatological viewed of the liver section of the rats also revealed the feature of hepatic cell with less necrosis particularly with group that pretreated with 200 mg/kg body weight of aqueous leaf extract of *Gardenia ternifolia* compared with the group that was administered with CCl_4 only without pretreatment. *Gardenia ternifolia* offers more amelioration on preventing the severity of damage to the liver with CCl_4. The observed liver protection by ALEGTP could be due to the observed presence of some phytochemical.

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