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Effects of *Gardenia Ternifolia* Leaf Extract on antioxidant and hepatic enzymes in sniper-induced toxicity in Albino rats

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Abstract

Aim: This study evaluates the effects of *Gardenia ternifolia* leaf extract on antioxidant and hepatic enzymes in sniper-induced toxicity in albino rats.

Methodology: A total of 42 male albino rats weighing 120g to 150g, grouped into 6 groups of 7rats each. Group 1 (Negative control), Group 2 (Positive control), Group 3 (Therapeutic Low Dose), Group 4 (Therapeutic High Dose), Group 5 (Prophylactic Low Dose) and Group 6 (Prophylactic High Dose). After 4 weeks of treatment, the rats were anaesthetized, sacrificed and blood samples collected. The Liver was also harvested for histological analysis. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) were estimated using a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were carried out using Reitman Frankel method. Alkaline phosphatase (ALP) was carried out using colorimetric phenolphthalein method. The liver tissue was prepared and stained using standard Haematoxylin and Eosin (H & E) staining technique and captured using scope Tek™ device.

Results: The mean SOD and GPx values for the therapeutic and prophylactic groups were significantly higher (P <.05) when compared with the positive control. The mean SOD and GPx values in the therapeutic groups were not significantly different (P >.05) from the negative control. The mean ALT, AST and ALP values for the therapeutic and prophylactic groups were significantly lower (P <.05) than the positive control but significantly higher (P <.05) than the negative control. Histology analysis of liver indicated normal histoarchitecture in the negative control while in the positive control, there were severe cell degeneration, distortion, necrosis and death of the hepatocytes. The Therapeutic Low Dose and Prophylactic Low Dose groups indicated severe degeneration and hypertrophy of hepatocytes. The Therapeutic High Dose and Prophylactic High Dose groups showed less deposit, well delineated radiating hepatocytes and near normal cytoarchitecture.

Conclusion: Administration of Sniper® (dichlorvos) led to an increase in oxidative stress; depletion of antioxidant enzyme levels. It elevated liver enzymes levels, caused histopathological changes and death of cells in the liver. The treatment with leaf extract of *Gardenia ternifolia* ameliorated the effect of dichlorvos toxicity, restored the anti-oxidant and liver enzyme levels especially the high dose extract.

Keywords: Dichlorvos toxicity; *Gardenia ternifolia*; Antioxidant enzymes; Hepatic enzymes; Sniper.

1. Introduction

Sniper also called dichlorvos (2,2-dichlorovinyl dimethyl phosphate), is one of the most commonly used organophosphate insecticides in agriculture, industry and homes usually to control pests [1]. It is used to control

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household pests like houseflies, cockroaches, mosquitoes and others. It is also used in farms to protect crops and animals from insects and weeds that cause diseases [2]. Dichlorvos is highly hazardous and has been classified by the World Health Organization (WHO) as class 1B [3]. Poisoning from sniper is a serious challenge, especially in developing countries and this has led to many deaths annually [4]. Pharmacologically, sniper induces its toxicity by inhibiting neural enzyme acetylcholinesterase. This inhibition leads to the accumulation and subsequent toxicity of acetylcholine. Exposure to sniper whether accidentally or deliberately (such as the case of suicide) leads to toxic effects, with immunological. hepatic, neurological, carcinogenic, renal, respiratory, dermal and other systemic effects [5].

Gardenia ternifolia is a shrub or small tree widely used as a traditional medicine throughout tropical Africa. It is rich in vitamins and minerals [6,7]. Studies reveal that *G. ternifolia* has antimicrobial, anti-inflammatory, antioxidant, anti-plasmodial, anti-sickling and hepatoprotective activities. The leaves are also used to treat syphilis, skin diseases and could serve as antidote to some poisons [8].

Certain phytochemical compounds like alkaloids, anthocyanins, coumarins, flavonoids, phenols, saponins, tannins and terpenoids etc play a role in the characteristics and anti-poison activities of *G. ternifolia* by preventing absorption, binding and neutralizing the poison directly, antagonizing its end-organ effect or conversion to more toxic metabolites [9,10]. They also play roles in various metabolic pathways, interact with receptors to bring about drug-like responses, interfere with the effect of toxic substance through maintenance and modulation of immune function, hence bring about cure and prevention of specific diseases [11]. Most phytochemicals are also hepatoprotective with antioxidant agents which help to neutralize free radicals, ameliorate oxidative stress and prevent many health challenges [12].

2. Materials and methods

2.1. Experimental Animals

A total of 42 male albino rats weighing between 120 – 150g were used for the study. The rats were housed in standard cages and allowed access to normal pelleted rat feed and water *ad libitum*. The rats were allowed to acclimatize for a period of 14 days prior to commencement of the study.

2.2. Treatments

2.2.1. Dichlorvos

Sniper (a product of Saro Science Ltd, Akure, Nigeria) was purchased from a chemical store in Port Harcourt, Rivers State. The container of 250ml contains 1000g/liter of 2,2 - dichlorovinyl dimethyl phosphate (DDVP).

2.2.2. Herbal Extract

The *Gardenia ternifolia* leaves were collected from the herbarium of University of Ibadan (113860), washed thoroughly, dried under shade to avoid the direct impact of sunlight. They were cut into pieces and crushed using a clean mill to obtain the powdered form. The obtained fine powder was used for phytochemical screening and extraction using 95% methanol. The filtrate was later stored in the refrigerator until when needed.

2.3. Acute Toxicity Study

Following 14 days of acclimatization, a pilot study was carried out to determine the safe dose of the leaf extract using the fixed dose procedure [13] and LD_{50} of Dichlorvos using Lorke's method [14].

A total of 9 rats were divided into 3 groups of 3 rats each. Each group was given different concentrations of *G. ternifolia* leaf extract. Group 1 received 500 mg/kg of *G. ternifolia*, Group 2 received 1500 mg/kg of *G. ternifolia*, while Group 3 received 3000 mg/kg. The rats were observed for 48hours for signs of toxicity. There was no mortality and no sign of toxicity observed. The plant extract was considered safe and non-toxic up to a dose of 3000 mg/kg body weight. This study therefore adopted 500 mg/kg and 1000 mg/kg of *G. ternifolia* as low and high doses respectively.

A total of 9 rats were divided into 3 groups of 3 rats each. Each group was given different concentrations of Dichlorvos. Group 1 received 80 mg/kg of Dichlorvos, Group 2 received 40 mg/kg of Dichlorvos, while Group 3 received 20 mg/kg. The rats were observed for 48hours for signs of toxicity. All the rats in group 1 were killed by dichlorvos. Signs of toxicity was noted in group 2 and half of the population died. Signs of toxicity were noted in group 3 but no death was recorded.

LD50= $\sqrt{}$Do X D100. Where Do = Highest dose that gave no mortality, D100 = Lowest dose that gave mortality [14]

$\sqrt{20 \text{ X } 40} = \sqrt{800} = 28.3$ mg/Kg

This study However adopted 20 mg/kg of Dichlorvos to induce toxicity in the rats and as LD50 for the study. Volume of dichlorvos administered = Dose/Concentration per ml

2.4. Experimental Design

The rats for the study were grouped into 6 groups of 7 rats each as shown below. Treatments were administered according to the grouping by means of oral gavage for a period of 28 days. At the end of the experiment, the rats were anaesthetized and sacrificed after a six-hours fast. Blood samples were collected via cardiac and liver tissues were also harvested for histological analysis. All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee.

- **Group1 (Negative Control):** Received no treatment and thus served as the negative control.
- **Group 2 (Positive Control):** Received single dose of 20 mg/kg of dichlorvos.
- **Group 3 (Therapeutic Low dose):** Received single dose of 20 mg/kg of dichlorvos. Treated with 500 mg/kg of *G. ternifolia* leaf extract for 28 days.
- **Group 3 (Therapeutic High dose):** Received single dose of 20 mg/kg of dichlorvos. Treated with 1000 mg/kg of *G. ternifolia* leaf extract for 28 days.
- **Group 5 (Prophylactic Low Dose):** Treated with 500 mg/kg of *G. ternifolia* leaf extract for 28 days, followed by a single dose of 20 mg/kg of dichlorvos, then observed for 48 hours before being sacrificed.
- **Group 5 (Prophylactic High Dose):** Treated with 1000 mg/kg of *G. ternifolia* leaf extract for 28 days, followed by a single dose of 20 mg/kg of dichlorvos, then observed for 48 hours before being sacrificed.

2.5. Reagents and Biochemical analyses

All reagents were commercially purchased and the manufacturer's standard operating procedures strictly followed. Quality control (QC) samples were run together with the biochemical analysis. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) were analyzed using a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method [15], as described by Elabscience Biotechnology Company limited, China. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were analyzed using Reitman-Frankel method [16], as modified by Randox laboratories limited (UK). The Alkaline phosphatase (ALP) was analyzed using colorimetric phenolphthalein method [17], as modified by Randox laboratories limited (UK). The liver tissue was dissected and processed. Sections were cut at 3µm on rotary microtome. It was stained with the standard haematoxylin and eosin staining technique. The slides were examined and photomicrographs captured with X400 objective lens using the Scope Tek™ device and software v.1.3.

2.6. Statistical Analysis

Data was analysed using SPSS version 23 (Statistical package for Social Science) Differences between groups were compared using one way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Results were considered statistically significant at 95% confidence interval ($p \leq 0.05$). Values are expressed as Mean ± SD.

3. Results and discussion

Table 1 shows the presence of alkaloids, phenols, saponins, quinones, steroids, tannins, terpenoids and anthocyanins in *Gardenia ternifolia* leaf extract. The results show that *Gardenia ternifolia* leaf extract contains the above phytochemicals. This finding agrees with the work of Ngbolua, *et al.* [18], who also reported the presence of these phytochemicals in *G. ternifolia* leaf extract. These secondary metabolites interact with receptors to bring about drug-like responses, interfere with the effect of toxic substance through maintenance and modulation of immune function, hence bring about cure and prevention of specific diseases [11,19].

The table 2 results showed that dichlorvos administration significantly (P <.05) reduced SOD and GPx levels in the positive control compared to the levels of the negative control (NC). The result also shows significantly (P <.05) elevated SOD and GPx levels in both therapeutic and prophylactic groups when compared with the positive control. It also shows that therapeutic groups were not significantly different (P >.05) from the negative control. The mean SOD and GPx values for the therapeutic and prophylactic groups were significantly higher (P <.05) than the positive control thus indicating the therapeutic potentials of the extract of *G. ternifolia*. This is related to the work of Ala *et al.* [20] on the use of methanoic extract of *Morinda lucida* in the prevention and treatment of dichlorvos toxicity. The *Morinda lucida* enhanced antioxidant activity and reduced tissue damage resulting from dichlorvos toxicity.

SOD and GPx are essential antioxidant enzymes that play pivotal roles in shielding cells from oxidative stress. SOD orchestrates the dismutation process, transforming the superoxide (O^2-) radical into hydrogen peroxide (H_2O_2). It functions as the primary defense mechanism against the detrimental effects of oxygen radicals within cells, actively scavenging reactive oxygen radical species. Conversely, GPx assumes a critical role by catalyzing the reduction of H_2O_2, utilizing Glutathione (GSH) as a substrate. These enzymatic activities contribute significantly to safeguarding mammalian cells against oxidative stress [21]

Table 1 Results of Phytochemical Analysis

Phytochemicals	**Observation**
Alkaloids	+ve
Phenols	+ve
Saponins	+ve
Steroids	+ve
Tanins	+ve
Terpenoids	+ve
Oxalate	-ve
Quinones	+ve
Anthocyanins	+ve

+ve- Present, -ve- Absent

Table 2 Antioxidant Enzyme levels of the Rats after Treatment

GROUPS (N=7)	**SOD (ng/ml)**	**GPx (pg/ml)**
G1(Neg. Control)	0.35 ± 0.02^{a}	34.32 ± 1.85^{a}
G2(Pos. Control)	0.19 ± 0.01^{b}	16.09 ± 1.47^{b}
G3(Therapeutic Low Dose)	0.34 ± 0.03^{a}	31.17 ± 1.21^{a}
G4(Therapeutic High Dose)	0.35 ± 0.01^{a}	32.95 ± 2.01^{a}
G5(Prophylactic Low Dose)	0.22 ± 0.01^{c}	24.27 ± 3.68^{c}
G6(Prophylactic High Dose)	0.24 ± 0.01^{c}	25.15 ± 3.55^{c}
P-value	<0.001	<0.001
Summary	S	S

S= Significant, Values with different superscripts are significantly different from each other, while values with similar superscripts are not significantly different from one another.

The significantly elevated SOD and GPx levels in the treated groups indicate antioxidant potentials of the leaf extract. This finding is also in agreement with the work of Dahiru, [22], on the effect of aqueous leaf extract of *Gardenia ternifolia plant* on carbon tetrachloride-induced hepatotoxicity in rats. This finding is also in agreement with the work of Pradeepa *et al*. [23] who reported a similar prophylactic and antioxidant effects of phytochemicals in albino rats. Dejen *et al*. [24] has also reported high curative effect of hydromethanoic extract and fractions of *G. ternifolia* stem barks in infected Mice.

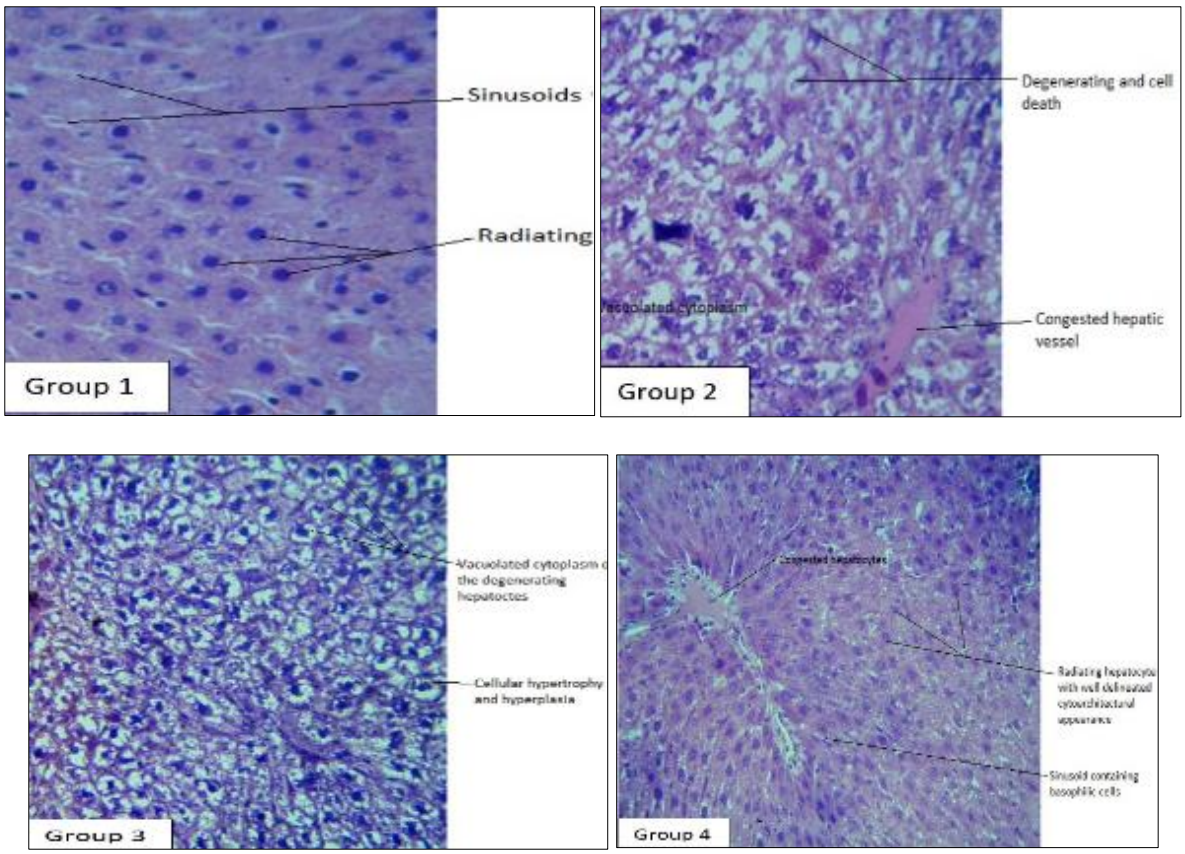
Table 3 The Results of Liver Enzyme parameters of Rats after Treatment

GROUPS (N=7)	**ALT(IU/L)**	**AST(IU/L)**	**ALP(IU/L)**
G1(Neg. Control)	9.03 ± 0.98^{a}	8.34 ± 13.17^{a}	113.36 ± 7.09^{a}
G2(Pos. Control)	117.29 ± 1.20^{b}	105.65 ± 3.28^{b}	233.61 ± 5.43^{b}

G3(Therapeutic Low Dose)	26.44±0.46^{c}	29.36±12.30^{c}	163.61±3.80^{c}
G4(Therapeutic High Dose)	18.53±1.76^{d}	16.06±1.89^{d}	131.21±14.85^{d}
G5(Prophylactic Low Dose)	28.15±2.14^{c}	33.14±9.33^{c}	165.57±2.94^{c}
G6(Prophylactic High Dose)	19.38±2.23^{d}	21.08±11.93^{d}	147.84±6.59^{d}
P-value	<0.001	0.006	<0.001
Summary	S	S	S

S= Significant, Values with different superscripts are significantly different from each other, while values with similar superscripts are not significantly different from one another.

The results show significantly elevated (*P* <.05) ALT, AST and ALP levels in the positive control group compared to the negative control and treatment groups. The mean AST, ALT and ALP values for the therapeutic and prophylactic groups were significantly lower (*P* <.05) than the positive control group (*P* <0.05), but significantly higher (p<0.05) than the negative control. It also shows that the liver enzymes in the therapeutic low dose and prophylactic low dose groups were significantly higher (*P* <.05) when compared with the therapeutic high dose and the prophylactic high dose groups. The mean value of high dose groups was significantly lower (*P* <.05) than low dose groups, indicating the high doses of the leaf extract were more effective in ameliorating liver function. Phytochemicals in herbal substances have been reported to possess hepatoprotective and anti-inflammatory potentials [25]. This finding is also in agreement with the work of Dahiru, [22], on the effect of aqueous leaf extract of *Gardenia ternifolia plant* on carbon tetrachloride-induced hepathotoxicity in rats. Some authors have also reported that phytochemicals in herbals attenuate inflammation in experimental animals [26].



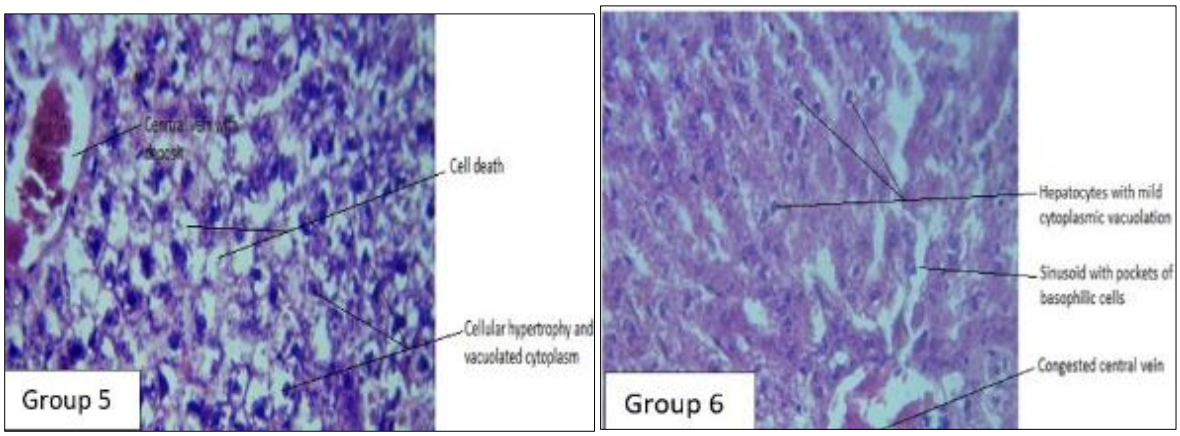


Figure 1 Photomicrograph (X 400) of H&E-stained histologic sections of the liver of the negative control and treatment groups

Histology analysis of liver indicated normal histoarchitecture in the negative control while in the positive control, there is severe degeneration, distortion, necrosis and death of the hepatocytes. The low dose therapeutic and prophylactic group indicated severe degeneration and hypertrophy of hepatocytes. The high dose therapeutic and prophylactic groups showed less deposit, well delineated radiating hepatocytes and near normal cytoarchitecture. The ameliorative changes in the tissues of the treated rats are in line with the works of Nancy *et al.* [27], where the extract of *Moringa oleifera* ameliorated the histological changes caused by lead toxicity.

4. Conclusion

Administration of Sniper® (dichlorvos) led to an increase in oxidative stress; depletion of antioxidant enzyme levels. It elevated liver enzymes levels, caused histopathological changes and death of cells in the liver. Treatment with leaf extract of *Gardenia ternifolia* indicated anti-oxidant and hepatoprotective potentials. The high dose extract of *Gardenia ternifolia* ameliorated the condition caused by dichlorvos toxicity better than the low dose, hence was more effective. Care should be taken in the use/handling of Sniper®, to prevent toxicity from accidental or deliberate exposures.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have affirmed the absence of any competing interests.

Statement of ethical approval

All animal experiments were carried out following ethical norms approved by the Institutional Ethical Committee.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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