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Journal of Bioscience and Applied Research
www.jbaar.org

Study of the protective role of polyphenol antioxidants from extracted Damiana (*Turnera diffusa* Willd) against chlorpyrifos pesticide-induced toxicity in male rats

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DOI: 10.21608/jbaar.2023.284031

Abstract

Pesticides have been associated with oxidative stress and enhanced reactive oxygen species generation (chlorpyrifos). This study aimed to determine whether extracted damiana (*Turnera diffusa* Willd) may effectively prevent chlorpyrifos-induced toxicity in male rats. Thiobarbituric acid-reactive substances (**TBARS**) plasma levels have been measured in chlorpyrifos-intoxicated animals to quantitatively evaluate lipid peroxidation, and these measurements have shown a marked increase in the plasma levels of these animals. However, rats given damiana alone experienced a decrease in lipid peroxidation and an increase in the majority of the measured parameters. Furthermore, damiana pretreatment of chlorpyrifos-intoxicated rats significantly decreased (lipid peroxidation) in comparison to the control group. While plasma total protein (**TP**), albumin (**A**), urea, acetylcholinesterase (**AChE**) activity, antioxidant enzymes activities of superoxide dismutase (**SOD**), glutathione S-transferase (**GST**), catalase (**CAT**), as well as glutathione content (**GSH**), fell significantly. The plasma levels of total cholesterol (**TC**), low-density lipoprotein-cholesterol (**LDL-c**), and triglycerides (**TG**) examined were considerably more significant in the rats exposed to chlorpyrifos than in the control group, although the level of high-density lipoprotein-cholesterol (**HDL-c**) was lower. So according to our findings, chlorpyrifos pesticide caused renal and hepatic disorders via oxidative stress causing biochemical alterations. Otherwise, damiana showed a possible protective effect against chlorpyrifos-induced toxicity which may be attributed to the antioxidant properties of its polyphenolic compounds and its capacity to scavenge active free radicals.

Keywords: Chlorpyrifos, damiana (*Turnera diffusa* Willd), oxidative stress, male albino rats

Received: October 18, 2022. Accepted: December 20, 2022.
Article in Press: February 4, 2023. Published: March 1, 2023

Introduction

Pesticides are pervasive in the environment and have a considerable negative influence on the economy, the environment, and public health. As a result of their use, food is more readily available, has a longer shelf life, and costs less money. Additionally, pesticides lessen the need for human labor and the associated risk of an accident at work. Large sectors of the population may be exposed to pesticides, including agriculture workers and their families, in addition to the general public, who may be exposed through pesticide use at home or through residues on food (1,2). It is common to find organophosphates (OPs) in household dust and indoor air (3). However, many different chemical pathways are involved in the methods through which pesticides produce damage (4). Free radical production throughout the hazardous process has been hypothesized in several investigations with varied lengths of exposure to organophosphorus pesticides (5). Numerous initial products that serve as more potent oxidative damage propagators are produced as a result of oxidative damage. As their targets, these extremely reactive free radicals can oxidize proteins, lipids, carbohydrates, and nucleic acids. Additionally, the rate of metabolic clearance of OPs in mammals directly correlates with their poisonous efficacy (6). Chlorpyrifos is an insecticide that belongs to the phosphorothioate class of organophosphorus compounds. In the liver, it undergoes metabolic activation to become the equivalent oxygen analog, called oxon (7). Chlorpyrifos-oxon binds to and inhibits **AChE**, causing cholinergic toxicity. The liver is where chlorpyrifos is mostly activated, and the liver and serum are where it is detoxified. Cytochrome P450 and related enzymes catalyze the biotransformation of organophosphorus pesticides (8).

A blend of avermectins called abamectin (ABM) contains roughly 80% avermectin B1a and 20% avermectin B1b (9). B1a and B1b share similar biological and toxicological characteristics (10). In various regions of the world, ABM is employed as an acaricide and insecticide (11). It paralyzes insects by disrupting their neurological systems, acting as a pesticide. ABM is used by homeowners to control fire ants as well as to eradicate insect and mite pests of citrus, pear, and nut tree crops (12). In veterinary medicine, it is given to sheep and cattle to treat nasal bots, lungworms, and gastrointestinal nematodes. It also inhibits chloride channels, so it might have an impact on membrane stability (13). *Turnera diffusa* also referred to as damiana, is a shrub that is indigenous to the Caribbean, Central America, Mexico, South America, and southern Texas in the United States. It is a member of the Passifloraceae family. Damianin, tetraphyllin B, gonzalitosin I, arbutin, tricosan-2-one, acacetin, 1,8-cineole, apigenin, -pinene, -carotene, -pinene, tannins, thymol, and hexacosanol are all present in damiana. The genus Turnera has been reported to contain a total of 22 flavonoids, maltol glucoside, phenolics, seven cyanogenic glycosides, monoterpenoids, sesquiterpenoids, triterpenoids, the polyterpene ficaprenol-11, fatty acids, and caffeine. Damiana extract, as well as the isolated substances pinocembrin and acacetin, have been shown to inhibit aromatase activity (14,15). The current study looked at the anti-toxin properties of extracted damiana (*Turnera diffusa* Willd) throughout 2 weeks of chlorpyrifos toxicity. Rats were administered chlorpyrifos and/or damiana daily for two weeks using a ball-tipped curved intubation needle to accomplish this goal.

Materials and methods

Chemicals

Analytical-grade chemicals were employed throughout the entire study. Chlorpyrifos, also known as [O,O-diethyl-O-(3,5,6-trichloro-2-

pyridyl) phosphorothioate], was purchased with 99% purity from ChemService in the USA. Benseng Food Supplements BV in the Netherlands supplied the leaves of the Mexican plant Damiana (*Turnera diffusa* Willd).

Plant extraction

Turnera diffusa dried, chopped, and sifted leaves (910 grams) were percolated with 3L×3 of MeOH at room temperature. Under less pressure, the solvent evaporated completely, leaving 180 grams of extractives (16).

Animals

At Alexandria University's Faculty of Medicine's animal house, 28 male albino rats weighing 150-170 g were procured. The NIH guidance for laboratory animal welfare's standards of laboratory animal care was followed when handling the animals. For two weeks, the rats were kept in stainless steel-bottomed wire cages with a 12-hour/12-hour light/dark cycle, room temperature, relative humidity of 40-60%, and free access to pellet food, water, and libitum.

Experimental design

Rats were distributed at random into four groups of seven each. Group I (control): For two weeks, maize oil was given orally to control rats using a ball-tipped, curved intubation needle. Group II (Damiana): For two weeks, rats in this group received an oral administration of an aqueous solution containing damiana extract at a dose of 50 mg/Kg Body weight every 24 hours. Group III (Chlorpyrifos): For two weeks, rats were orally administered 1/25 of the LD_{50} (6 mg/kg BW) of chlorpyrifos, which was dissolved in maize oil. The rat LD_{50} for this compound is 135-163 mg/kg (17). Damiana was given to rats in Group IV (Damiana + Chlorpyrifos) orally once a day at a dose of 50 mg/kg BW. The oral dosage of chlorpyrifos (6 mg/kg BW) for two weeks was dissolved in corn oil after 30 minutes.

Blood samples collection

Animals were given diethyl ether anesthesia after the 14^{th} day of the experiment, and blood samples were quickly drawn from the rats' aortas following scarification. The samples were then gathered in heparinized test tubes and put on ice right away. To separate the plasma, the collected blood was centrifuged at 860 xg for 20 min. Until the assessments of the test parameters, the plasma was held at -80 °C.

Oxidative stress markers and antioxidant parameters

Determination of TBARS and GSH

The method of (Esterbauer and Cheeseman, 2004) was used to identify thiobarbituric acid-reactive compounds (**TBARS**) (18). Whereas the method of (Ellman, 1959) was used to estimate the reduced glutathione content (**GSH**) (19).

Determination of antioxidant enzyme activities

The method of Misra and Fridovich (1972) was used to determine superoxide dismutase activity (**SOD**; EC 1.15.1.1) (20). Using the method of Habig *et al.* (1974), the activity of glutathione S-transferase (**GST**; EC 2.5.1.18) was assessed (21). The method of Abei (1984) was used to measure catalase activity (**CAT**; EC 1.11.1.6) (22).

Biochemical parameters

Using commercial diagnostic kits from BioSystems S.A., Barcelona, Spain, and following the method of measurement (Reitman and Frankel, 1957), the activities of plasma aspartate transaminase (**AST**) and alanine transaminase (**ALT**) were assessed. While Ellman's method (1961) was used to assess plasma levels of acetylcholinesterase activity (**AChE**; EC 3.1.1.7) (23). Following commercial diagnostic kits from BioSystems S.A., Barcelona, Spain, we assessed plasma total protein (**TP**) and albumin (**A**) using the techniques described in

Armstrong and Carr (1964) (24), and Doumas et al. (1977) (25), respectively. Utilizing a commercial diagnostic kit and following the technique described in (Patton and Crouch, 1977), plasma urea levels were quantified (26).

Lipid profile assessment

Using commercial diagnostic kits, the plasma samples were examined for total cholesterol (**TC**; cat. no. 11805), triglycerides (**TG**; cat. no. 11828), and high-density lipoprotein-cholesterol (**HDL-c**; cat. no. 11557) following the manufacturer's instructions (BioSystems S.A., Barcelona, Spain). By dividing the **TG** readings by a factor of 5, plasma levels of very low-density lipoprotein-cholesterol (**vLDL-c**) were computed. The following equation was used to calculate low-density lipoprotein-cholesterol (**LDL-c**) plasma levels:

$$\textbf{LDL-c=TC-(HDL-c + vLDL-c)}$$

Statistical analysis

The data were examined using SPSS for Windows (version 17.0). Tukey's multiple comparisons approach was used after a one-way analysis of variance (ANOVA) to determine the significance of differences. Statistics were deemed significant at $P < 0.05$.

Results

Markers of oxidative stress and antioxidant parameters

In male rats treated daily for two weeks with damiana, chlorpyrifos, or a combination of the two, the plasma levels of thiobarbituric acid-reactive substances (**TBARS**), reduced glutathione (**GSH**), and the activities of superoxide dismutase (**SOD**), glutathione S-transferase (**GST**), and catalase (**CAT**) were measured. The results are shown in table 1 and figures 1, 2, 3, 4, and 5. The results showed that, as compared to the control group, treatment with chlorpyrifos alone significantly ($P < 0.05$) reduced the plasma activities of **SOD**, **GST**, **CAT**, and the levels of **GSH** in the rats' plasma and raised the plasma levels of **TBARS**. **TBARS** levels in the plasma were lowered, while treatment with damiana alone significantly ($P < 0.05$) boosted the plasma activities of **SOD**, **GST**, **CAT**, and the levels of **GSH**. Compared to the group that was treated with chlorpyrifos, the group that used both damiana and chlorpyrifos reduced all of the negative effects.

Table (1): Plasma levels of (**TBARS**), (**GSH**), and plasma antioxidant enzyme activities of (**SOD**), (**GST**), and (**CAT**) in male rats given treatments with damiana, chlorpyrifos, and both.

Biomarkers	Experimental groups			
	Control	Damiana	Chlorpyrifos	Damiana+Chlorpyrifos
TBARS (nmol/l)	24.91±1.10	21.5±0.81	32.76±0.81[a]	25.37±1.73[b]
GSH (µg/l)	2.55±0.13	2.84±0.08	1.5±0.26[a]	2.21±0.13[b]
SOD (U/ml)	76.2±3.39	85.81±3.21	54.66±1.32[a]	73.43±1.69[b]
GST (U/ml)	91.94±1.37	101.44±1.69	78.504±2.52[a]	90.8±0.85[b]
CAT (U/ml)	57.43±1.63	69.31±3.34	43.62±2.23[a]	55.86±3.23[b]

The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

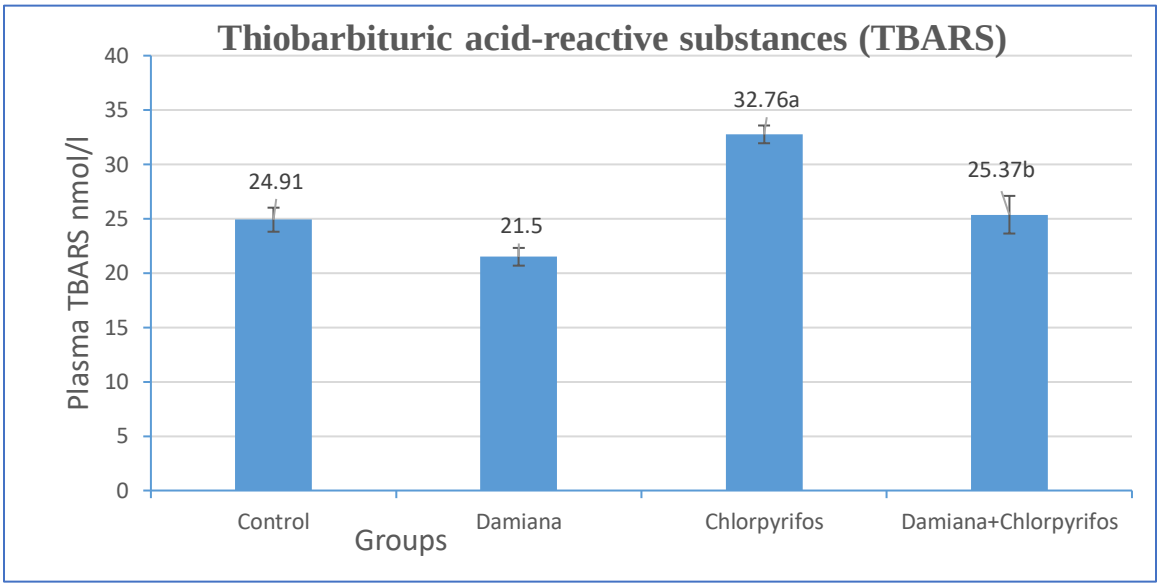

Figure (1): Plasma levels of (**TBARS**) in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with a control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

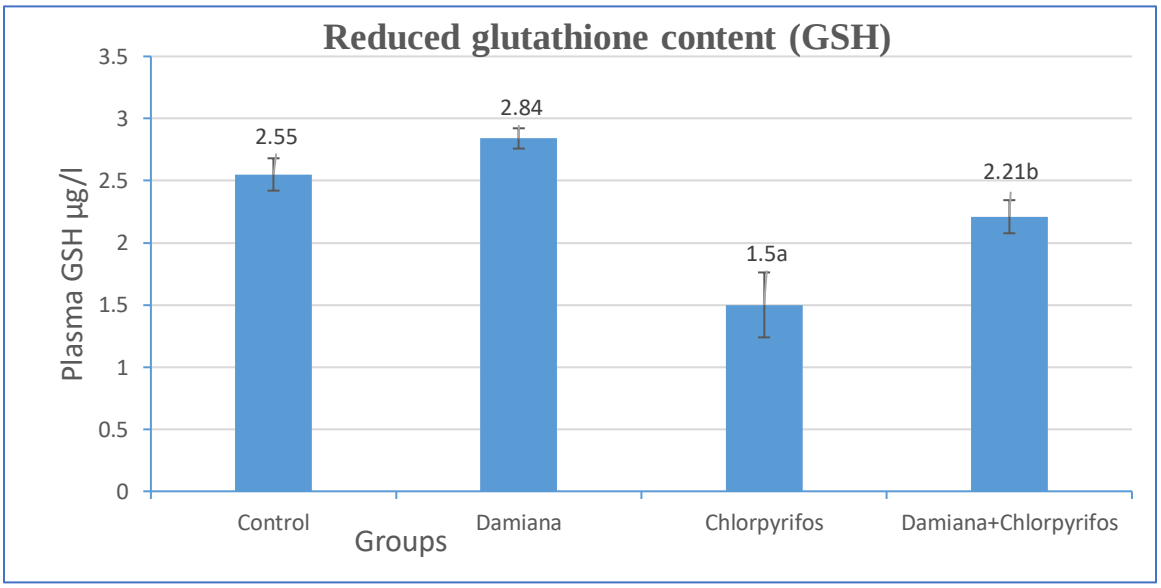

Figure (2): Plasma levels of (**GSH**) in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

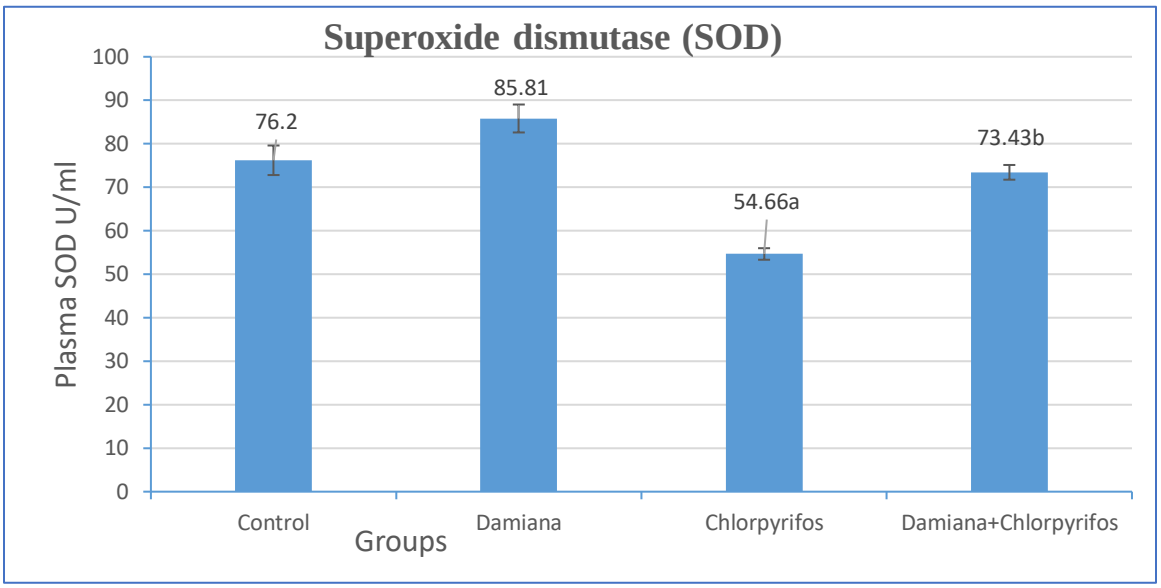

Figure (3): Plasma (**SOD**) activities in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

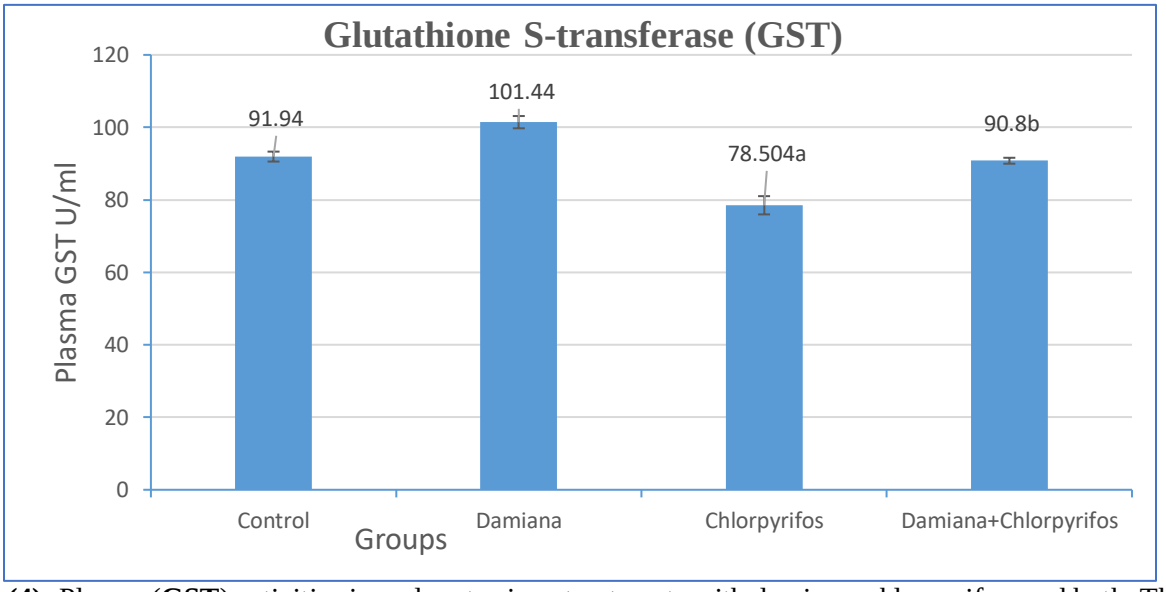

Figure (4): Plasma (**GST**) activities in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

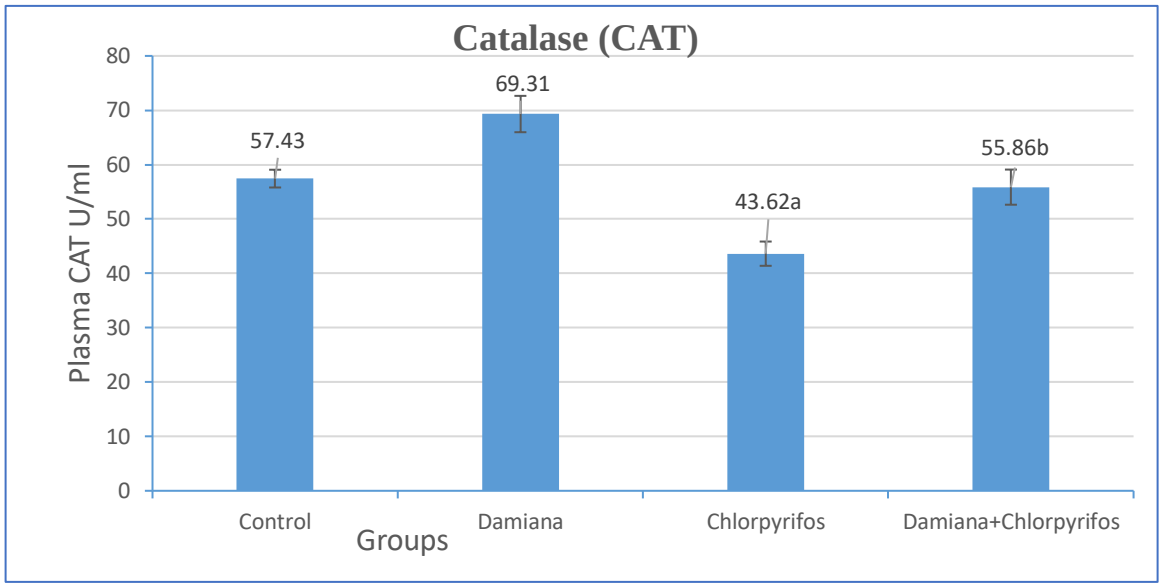

Figure (5): Plasma (**CAT**) activities in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

Biochemical parameters

The mean values of the aspartate transaminase (**AST**), alanine transaminase (**ALT**), and acetylcholinesterase (**AChE**) plasma activities of male rats treated with damiana, chlorpyrifos, and their combination for 14 days were shown in Table 2 and Figures 6, 7, and 8. The plasma **AST**, **ALT**, and **AChE** activities were shown to be considerably ($P < 0.05$) higher in the chlorpyrifos-treated group compared to the control group. However, as compared to the control group, treatment with damiana alone resulted in a substantial ($P < 0.05$) drop in the activity of these enzymes. In comparison to the group treated with chlorpyrifos, the combination group demonstrated a considerable decline in the activity of the enzymes under study.

Table 3 and figures 9, 10, and 11 display the mean plasma total protein (**TP**), albumin (**A**), and urea values following a 14-day experiment. When compared to the control group, treatment with chlorpyrifos alone caused a substantial ($P < 0.05$) drop in the plasma levels of total protein (**TP**) and albumin (**A**), as well as a significant ($P < 0.05$) increase in plasma urea levels. In contrast, treatment with damiana alone resulted in a non-significant rise in plasma levels of total protein (**TP**), albumin (**A**), and urea ($P < 0.05$) when compared to the control group. On the other hand, the combination group's presence of damiana and chlorpyrifos raised the levels of plasma total protein (**TP**), albumin (**A**), and urea. However, these values fell short of those of the control group.

Table (2): Plasma (**AST**), (**ALT**), and (**AChE**) activities in male rats given treatments with damiana, chlorpyrifos, and both.

Enzyme's activities	Experimental groups			
	Control	Damiana	Chlorpyrifos	Damiana+Chlorpyrifos
AST (U/L)	158.26±2.05	157.7±1.64	189.41±2.26[a]	166.19±2.48[b]
ALT (U/L)	89.67±0.89	88.52±1.37	122.36±0.81[a]	100.53±1.21[b]
AChE (U/ml)	1.33±0.089	1.48±0.119	0.88±0.092[a]	1.11±0.082[b]

The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

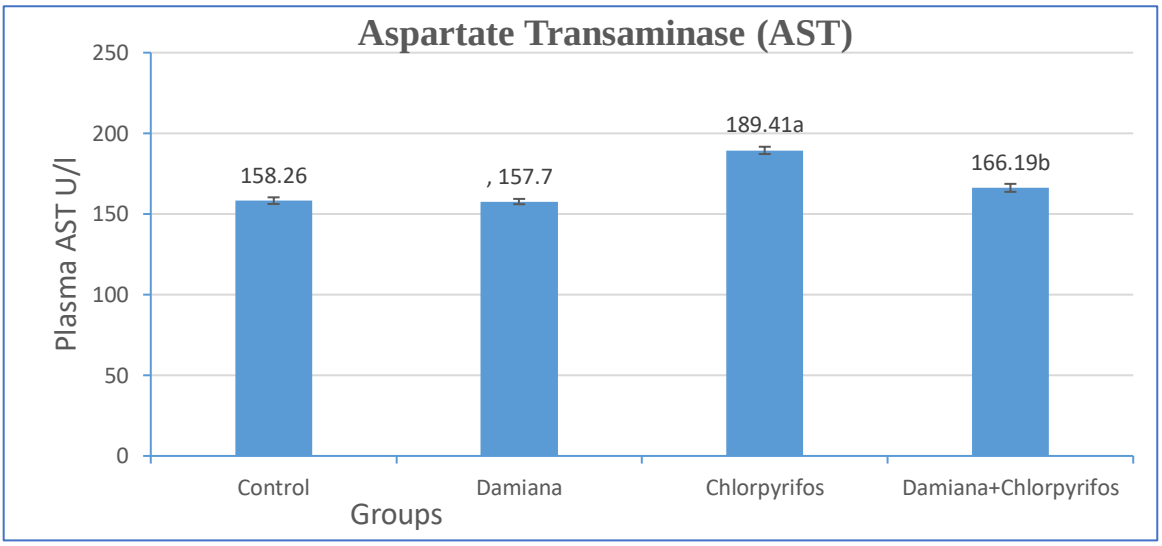

Figure (6): Plasma (**AST**) activities in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

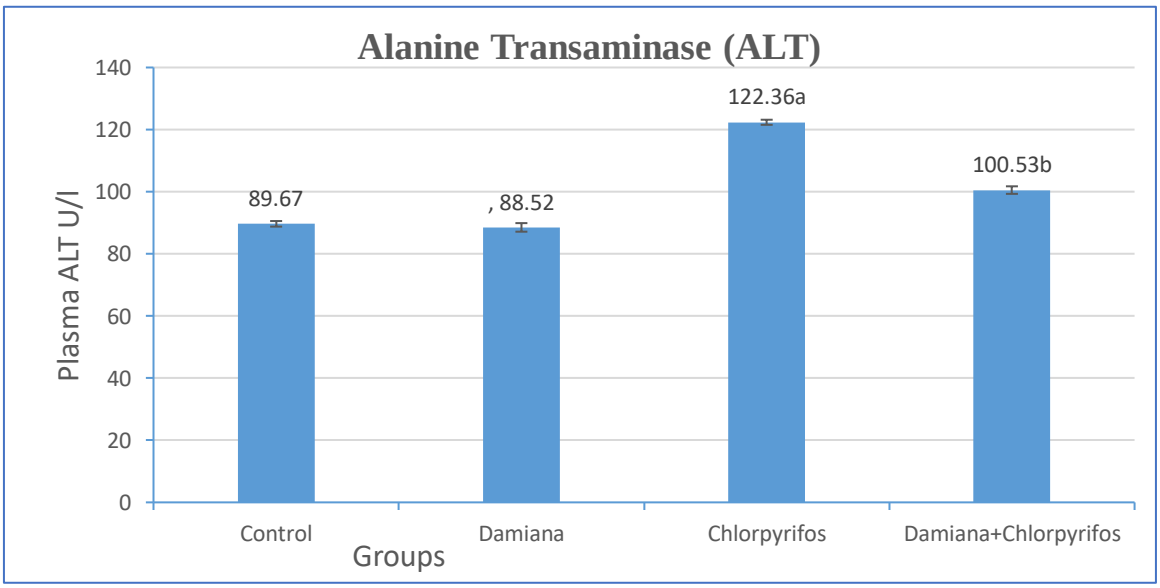

Figure (7): Plasma (**ALT**) activities in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

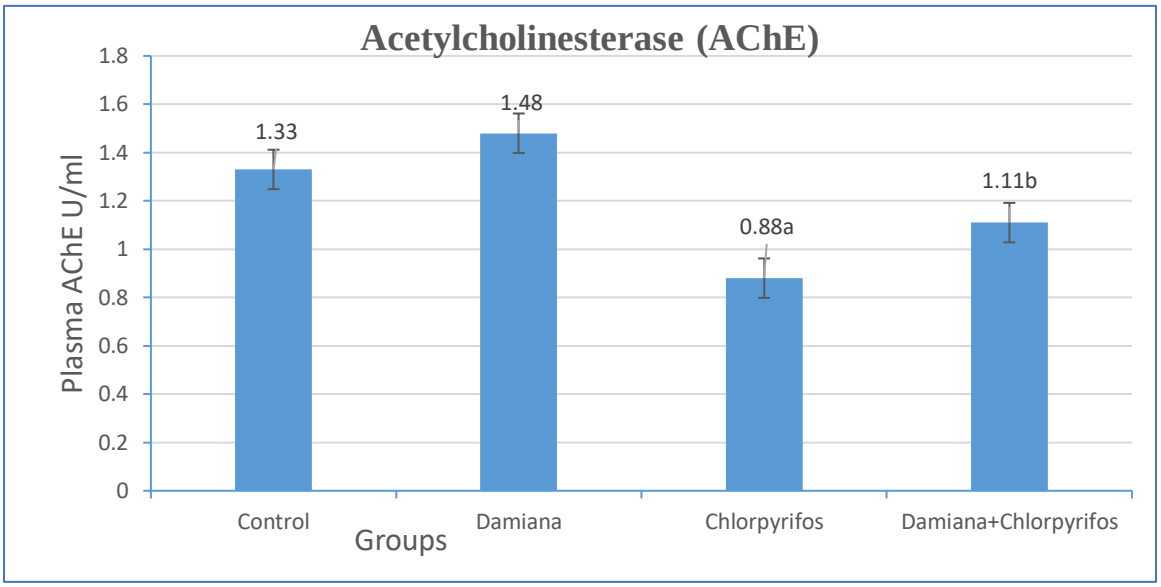

Figure (8): Plasma (**AChE**) activities in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

Table (3): Plasma levels of (**TP**), (**A**), and urea in male rats given treatments with damiana, chlorpyrifos, and both.

Parameter	**Experimental groups**			
	Control	**Damiana**	**Chlorpyrifos**	**Damiana+Chlorpyrifos**
TP (g/dl)	**6.65±0.80**	**6.74±0.90**	**3.15±0.58[a]**	**5.86±0.56[b]**
A (g/dl)	**3.9±0.90**	**4.25±0.69**	**2.32±0.46[a]**	**3.36±0.93[b]**
Urea (mg/dl)	**0.34±0.07**	**0.24±0.08**	**0.87±0.08[a]**	**0.67±0.08[b]**

The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

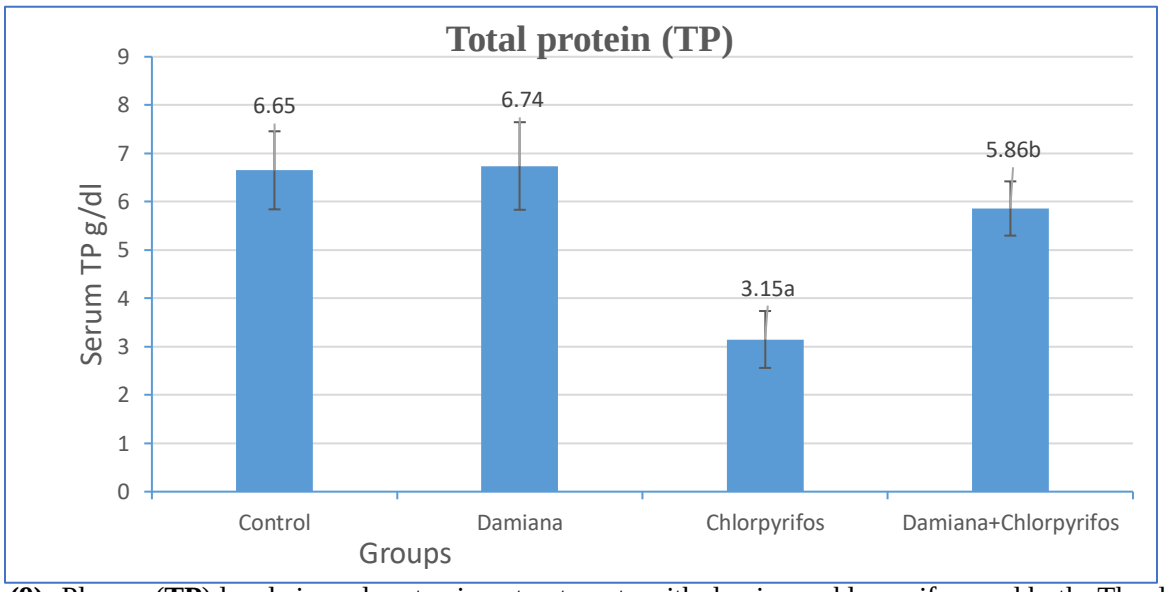

Figure (9): Plasma (**TP**) levels in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

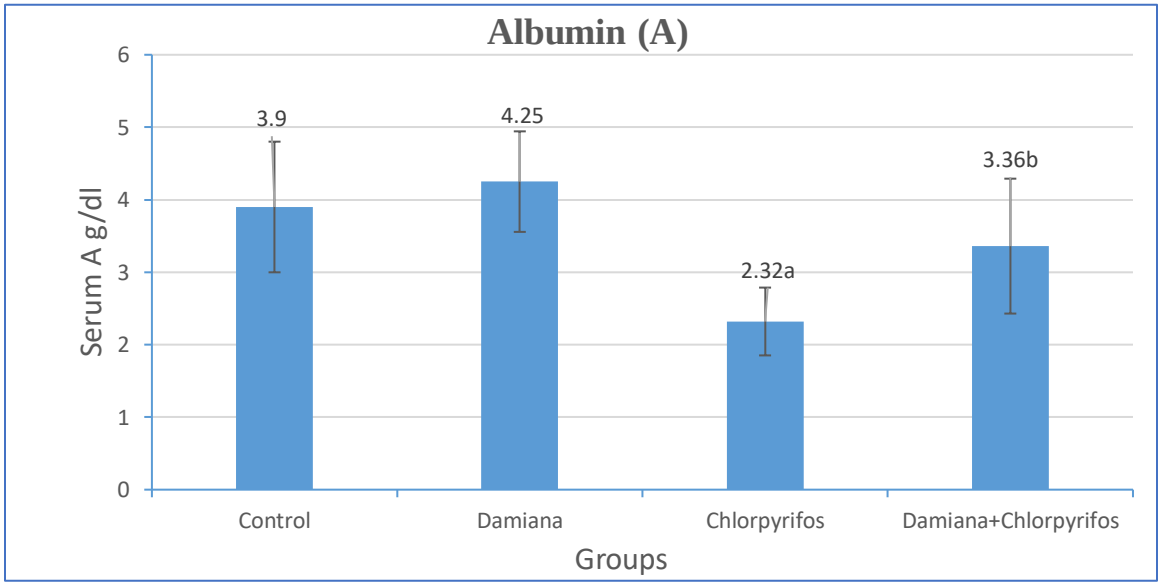

Figure (10): Plasma (**A**) levels in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

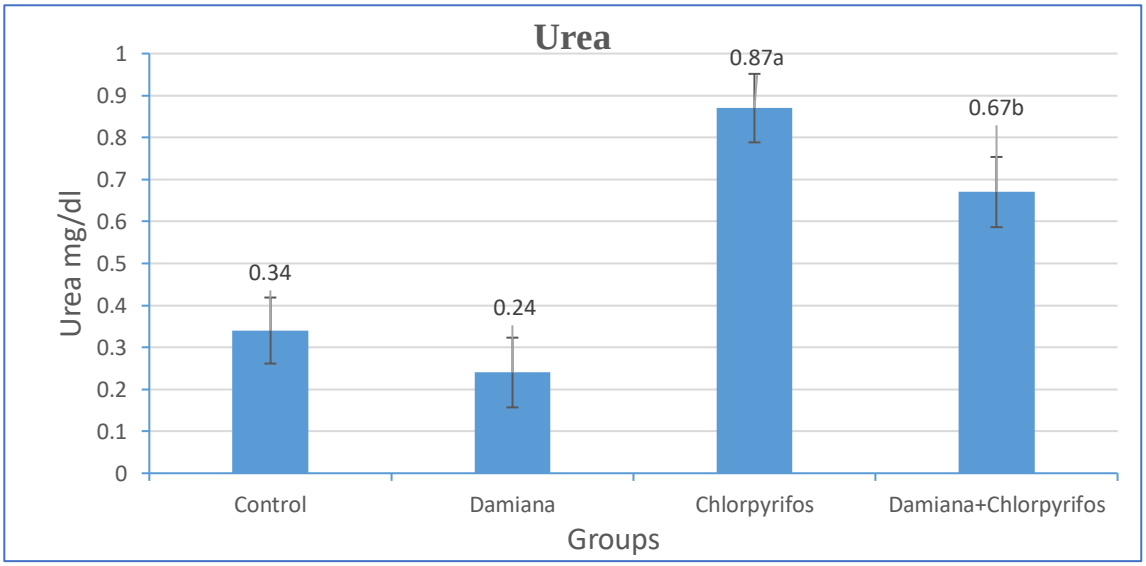

Figure (11): Plasma urea levels in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

Plasma lipid profile assessment

According to table 4 and figures 12, 13, and 14, the plasma lipid profile of male rats revealed significant ($P < 0.05$) rises in total cholesterol (**TC**), **LDL-c**, and triglycerides (**TG**) in the chlorpyrifos-treated group compared to the control group, while the concentration of **HDL-c** decreased significantly ($P < 0.05$) in the chlorpyrifos treated group compared to the control group. However, the lipid profile of the damiana group did not differ significantly ($P > 0.05$) from that of the control rats. As shown in table 4 and images 12, 13, and 14, rats treated with chlorpyrifos and damiana had significantly lower lipid contents than the chlorpyrifos group ($P < 0.05$).

Table (4): Plasma levels of (**TC**), (**LDL-c**), (**HDL-c**), and (**TG**) in male rats given treatments with damiana, chlorpyrifos, and both.

Parameter	Experimental groups			
	Control	Damiana	Chlorpyrifos	Damiana+Chlorpyrifos
TC (mg/dL)	108.2± 1.82	100.57± 0.79	138.5± 0.81[a]	122.47± 0.86[b]
LDL-c (mg/dL)	41.2 ± 1.1	40.64 ± 0.90	74.44 ± 0.96	52.83 ± 1.22
HDL-c (mg/dL)	36.84 ± 1	35.78 ± 1.11	29 ± 0.81[a]	33 ± 0.90[b]
TG (mg/dL)	127.60 ± 6.33	128.13 ± 5.12	142.71 ± 5.31[a]	131.92 ± 3.65[b]

The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with control group. [b] Significant difference ($P < 0.05$) in comparison with chlorpyrifos group.

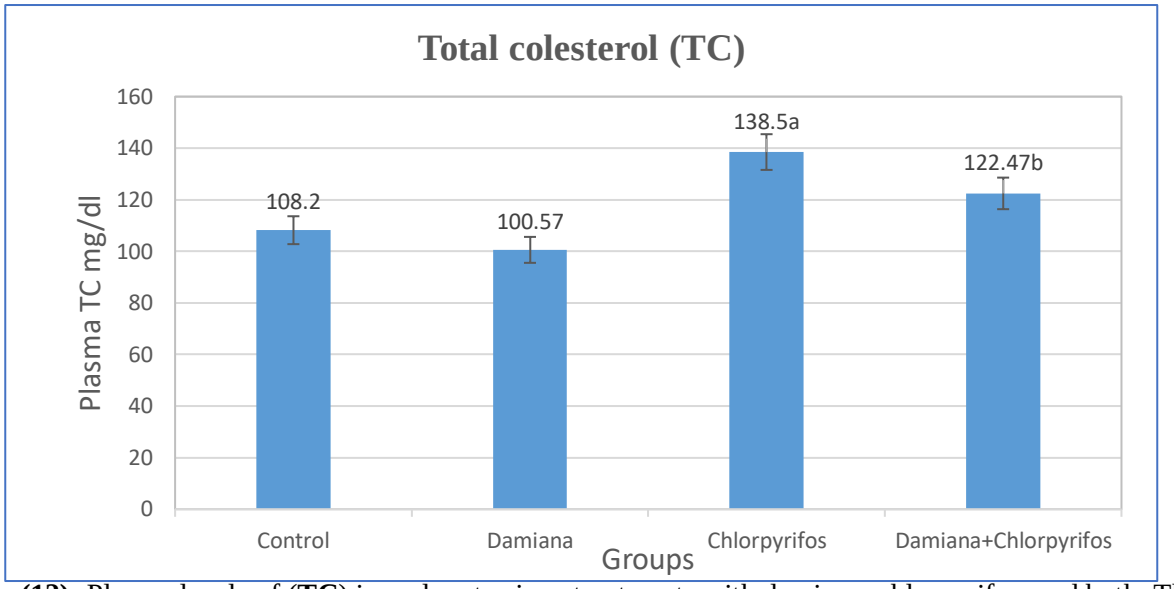

Figure (12): Plasma levels of (**TC**) in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

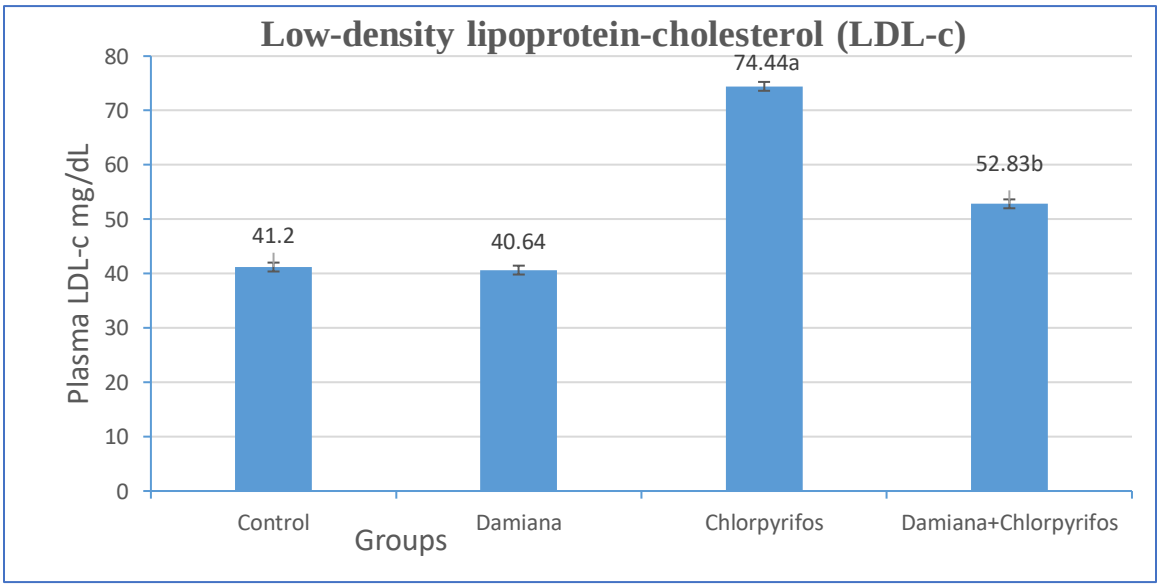

Figure (13): Plasma levels of (**LDL-c**) in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

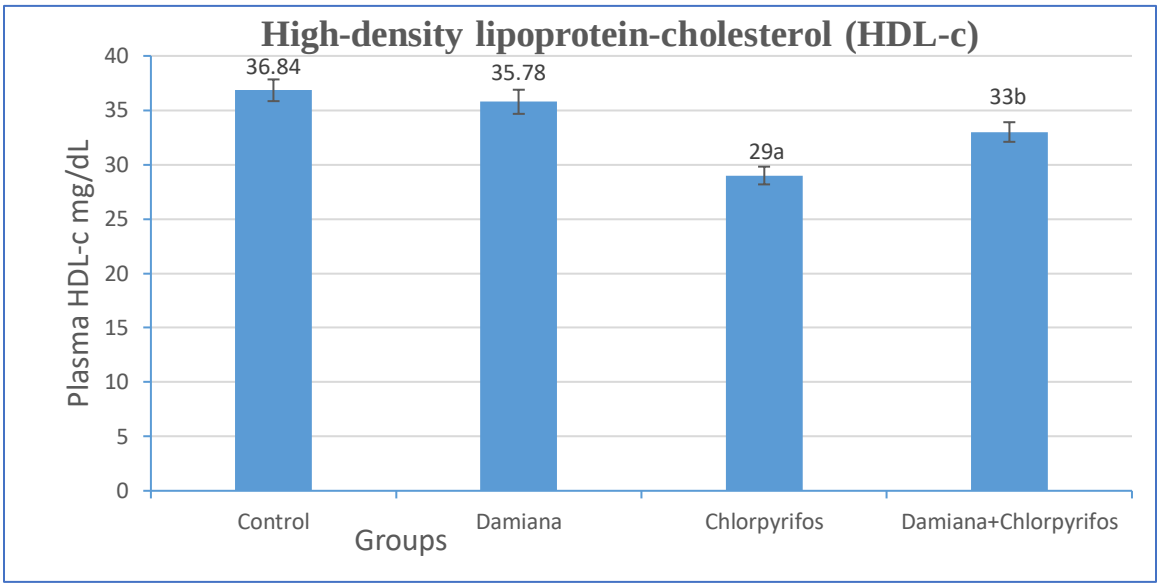

Figure (14): Plasma levels of (**HDL-c**) in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

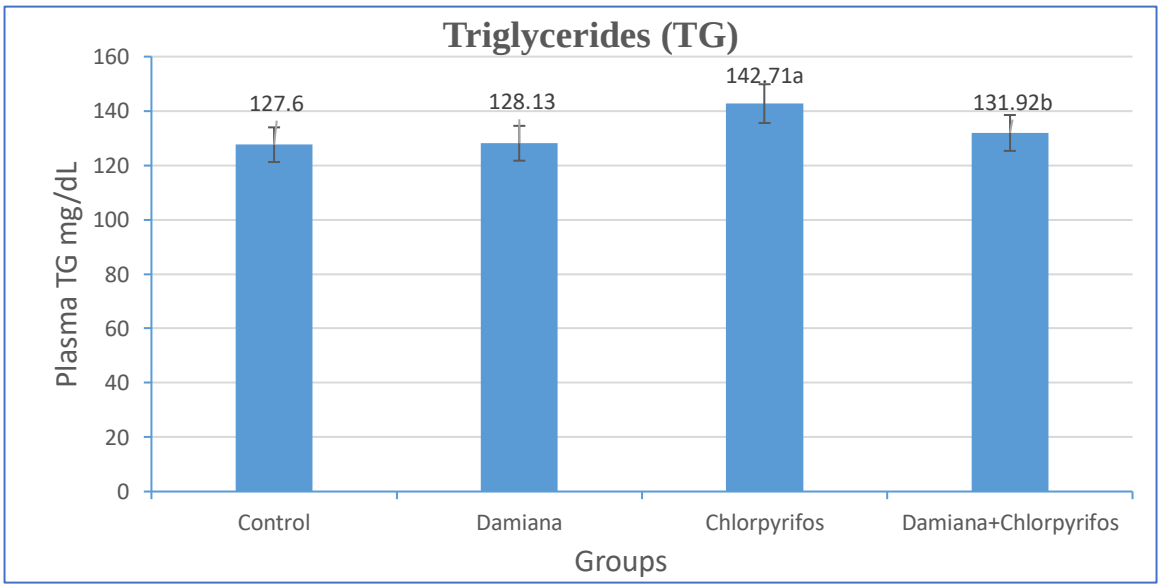

Figure (14): Plasma levels of (**TG**) in male rats given treatments with damiana, chlorpyrifos, and both. The data are shown as mean ± SE, with n equal to 7. [a] Significant difference group ($P < 0.05$) in comparison with the control group. [b] Significant difference ($P < 0.05$) in comparison with the chlorpyrifos group.

Discussion

Results gained through experiments demonstrated that treatment with chlorpyrifos increased plasma levels of thiobarbituric acid-reactive substances (**TBARS**), a marker of increased lipid peroxidation, and decreased plasma levels of superoxide dismutase (**SOD**), glutathione S-transferase (**GST**), catalase (**CAT**), and glutathione content (**GSH**). On the other hand, rats treated with damiana alone had lower plasma **TBARS** concentrations and higher plasma antioxidant enzyme activity. Also, when compared to the chlorpyrifos group, these biomarkers were much better in rats that had been given both damiana and chlorpyrifos. Furthermore, plasma activities of aminotransferases enzymes (**AST**, and **ALT**), and acetylcholinesterase (**AChE**) decreased significantly under therapy with chlorpyrifos. Plasma **AST**, **ALT**, and **AChE** activities were increased after treatment with damiana alone. Rats given both damiana and chlorpyrifos also exhibited a protective effect against the latter.

In the rats' group given chlorpyrifos treatment, there was a decrease in plasma levels of total protein (**TP**) and albumin (**A**). This decline could be the result of increased nephrosis loss or decreased protein synthesis. Additionally, the use of chlorpyrifos resulted in a considerable rise in plasma urea levels. This rise in urea levels is regarded as a strong indicator of nephrotoxicity. However, therapy with damiana alone resulted in a considerable reduction in urea levels. Chlorpyrifos' harmful effects were significantly reduced in rats treated with both damiana and chlorpyrifos. Rats treated with chlorpyrifos had significantly higher plasma levels of total cholesterol (**TC**), **LDL-c**, and triglycerides (**TG**) than control rats for these lipid profile measures. In comparison to the control group, the rats exposed to chlorpyrifos had considerably lower levels of **HDL-c**. In comparison to rats exposed to chlorpyrifos alone, the rats treated with both damiana and chlorpyrifos had significantly lower levels of each lipid profile metric, except for **HDL-c**, which had significantly greater levels.

In conclusion, it is evident that, in addition to the antioxidant defense system, chlorpyrifos caused noticeable detrimental effects on liver and kidney biomarkers. As indicators for the negative effects of chlorpyrifos exposure, estimation of lipid peroxidation, enzymatic and nonenzymatic antioxidants, as well as biochemical parameters, may be used. These biomarkers were shown to be altered from their normal values, which indicates biochemical impairment and may be related to chlorpyrifos' potential impacts on male rats. Its crucial role as an antioxidant may be the reason why using damiana with chlorpyrifos reduced and relieved its harmful effects on most of the examined parameters. In summary, damiana therapy alone enhanced the antioxidant status of rats and may be effective as an antioxidant against insecticide-induced environmental stress.

Conclusion

Finally, we demonstrated that *in vivo* exposure to the chlorpyrifos pesticide caused negative effects on the renal and hepatic functions via oxidative stress causing biochemical alterations. Additionally, our findings demonstrated that the toxic effects of chlorpyrifos on the liver and kidney functions might be mitigated by utilizing a damiana extract rich in polyphenols as an antioxidant to protect healthy tissues and lessen chlorpyrifos toxicity. Finally, to reduce chlorpyrifos' toxicity to the liver and kidneys, we advise utilizing a damiana extract as a preventive agent.

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