Sahel Journal of Life Sciences FUDMA (SAJOLS)
September 2025 Vol. 3(3): 382-389
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: https://doi.org/10.33003/sajols-2025-0303-49

Research Article

Hepatoprotective Effect of n-Butanol fraction of *Lophira lenceolata* (Ochnaceae) Against Carbon Tetrachloride Induced Hepatotoxicity in *Drosophila melanogaster*

***Yusuf Alkali Ibrahim[1] and Aliyu Samaila[2]**

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences Usmanu Danfodiyo University Sokoto, Nigeria
[2]Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmaceutical Sciences Usmanu Danfodiyo University Sokoto, Nigeria
***Corresponding Author's email*: yusuf.alkali@udusok.edu.ng; galilie050@gmail.com**
Phone: +2348069048356

ABSTRACT
This study evaluated *Lophira lanceolata* n-butanol leaf fraction hepatoprotective and antioxidant potentials in the Drosophila melanogaster model. For the acute toxicity study, adult *Drosophila melanogaster* was exposed to *L. lanceolata* butanol fraction at concentrations of 125, 250 and 500 mg/10g feed, and mortality was recorded for a period of seven days. In the hepatotoxicity assay, flies were fed the n-butanol fraction at 125, 250, and 500 mg/10g, with all the groups receiving carbon tetrachloride mixed with the feed, and antioxidant enzyme activities (catalase, superoxide dismutase, and reduced glutathione) were measured. In the oxidative stress assay similar dose pattern was adopted. The adult flies were homogenized, and biochemical assays for hepatic biomarkers and oxidative stress markers were evaluated. A sample of the *Drosophila homogenate* was subjected to mRNA extraction for the expression of the SOD gene. The LC50 was determined to be greater than or equal to 500 mg fraction in 10g of feed. Antioxidant enzyme activities (catalase, superoxide dismutase, and glutathione) significantly increased upon administration of the graded doses of *L. lanceolata* n-butanol fraction. The liver biochemical markers, such as ALT, AST, Albumin, and alkaline phosphatase, were significantly decreased by the n-butanol fraction compared with the CCl4-treated group. These results suggest that *L. lanceolata* possesses antioxidant and hepatoprotective effects on Drosophila flies by free radical scavenging and increasing gene expression of endogenous antioxidant enzymes.

Keywords: Antioxidant; Drosophila melanogaster; Hepatoprotection; Hepatotoxicity; *Lophira lanceolata*; Oxidative stress

Citation: Ibrahim, Y.A., & Samaila, A. (2025). Hepatoprotective Effect of n-Butanol fraction of *Lophira lenceolata* (Ochnaceae) Against Carbon Tetrachloride Induced Hepatotoxicity in *Drosophila melanogaster*. *Sahel Journal of Life Sciences FUDMA*, 3(3): 382-389. DOI: https://doi.org/10.33003/sajols-2025-0303-49

INTRODUCTION

Liver is the largest visceral organ responsible for metabolism of xenobiotics and detoxification of harmful chemical substances (Salamma *et al.,* 2015). It plays a very vital role in the pharmacokinetics parameters such as absorption, distribution, metabolism and elimination of most drugs and several active or inactive metabolites. Hepatic exposure to toxic chemicals and secondary plant metabolites results in the formation of reactive oxygen species (ROS) associated with cellular necrosis and increases level of free radicals leading to lipid peroxidation and depletion in the tissue GSH levels (Cheng *et al.,* 2013). Carbon tetrachloride (CCl4) is one of the most toxic chemicals and commonly used hepatotoxic agents in liver-related disorders' experimental study. In the liver, CCl4 is undergoes metabolism to yield two free radicals: trichloromethyl radical (CCl3) and proxy trichloromethyl radical (OOCCl3) by cytochrome P450 enzymes. It can cause severe damages to the liver, such as fatty changes, centrilobular steatosis, inflammation, apoptosis, and

cell necrosis (Heibatollah *et al.,* 2008). Hence, the main intercellular structural organelle damage by CCl4 is plasma membrane, endoplasmic reticulum, mitochondria, and Golgi apparatus (Reynolds *et al.,* 1963). As a result of damaging the cell membrane of hepatocytes, enzymes release in circulation (Cullen *et al.,* 2005), inducing an increase in tissue lipid peroxidation, oxidative stress, and serum levels of many biochemical markers such as transaminases, alkaline phosphatase, bilirubin, triglycerides, and cholesterol (Shanmugam *et al.,* 2013).

Bioactive compounds of medicinal plants showed an important role in managing oxidative stress-induced liver diseases currently and could therefore be a promising strategy to develop drugs with efficacy against these diseases. Numerous studies have shown that plant extracts having antioxidant activities protect against CCl4-induced hepatotoxicity by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity (Dhiman *et al.,* 2013; Duh *et al.,* 2011; Kuo *et al.,* 2010; Quan *et al.,* 2011; Kuo *et al.,* 2015).

Drosophila melanogaster, known popularly as the fruit fly, remains one of the most widely used model organisms for biomedical research. For more than ten decades, the low cost, rapid generation time, and excellent genetic similitude with higher organisms have made the fly indispensable for basic research. The inclusion of several molecular tools has allowed the model system to keep up with the latest advances. Growing body of evidence have disclosed how Drosophila is currently being used, and what directions they think the system is moving from human disease modelling to the dissection of cellular morphogenesis, internal organ disease to behaviour and aging (Kohler, 1994).

Most drugs of plant origin used by medical practitioners are in the form of extract of the whole plant material or part of it. In view of this, local medicinal plants, which show suitable biological effect, could be standardized and similarly utilized. Some of the effects elaborated by extract of plants used in traditional medicine include antiviral, antitumor, antimicrobial, insecticide and central nervous system effect (Sofowora, 1982). World health organization has estimated that perhaps about 80% of more than 4000 million people on earth rely chiefly on traditional medicine for their primary health care needs, and also can safely be presumed that a major part of traditional therapy involves the use of plant extract or their active principles. Such treatments include the administration of infusion boiled parts, as some of the natural drugs are not usually in the form of tablet or pills (WHO, 2019).

Lophira lanceolata is a useful tree in Nigeria whose seeds are eaten. It is known that we can use every part of the plant for pest control, such as food, medicine, and mystical practices. In conventional medicine, the oil is applied to the skin to avoid dryness and is used to treat dermatitis, toothache, and muscle exhaustion (Kalmobe *et al.,* 2017). Children are given the oil as a tonic after mixing it with oatmeal. Women are advised to consume a decoction made from the roots and fresh or dried young leaves to combat menstrual pain, digestive ailments, diarrhoea, dysentery, and malaria. In the West Region of Cameroon, the bark is also used to cure fevers, bacterial infections, and digestive issues. The bark of the root is used for treatment of yellow fever, and they used an infusion made from the bark and leaves as an antitrypanosomal drug (Aliou *et al.,* 2017). The aim of this study is to provide scientific evidence on the use of *Lophira lanceolata* as a hepatoprotective agent in Drosophila melanogaster.

MATERIALS AND METHODS

Preparation of Plant Material

The plant leaves were obtained from Tureta local herbal market and was authenticated at the Department of Pharmacognosy and Ethnomedicine, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The sample was assigned a voucher number (PCG/UDUS/Lophir/003). The leave of the plant material was peeled, shade-dried, and pounded by pestle and mortar into a fine powder. A methanol extraction was performed with the powdered material using the maceration method for seventy-two hours. The resulting mixture was filtered using filter paper. The filtrate was subjected to fractionation using different organic solvent. The n-butanol fraction was filtered and evaporated at 40^{o}C and stored for the experimental studies.

Drosophila Stocks and Culture

Wild-type *Drosophila melanogaster* stocks were obtained from the Centre for Advanced Medical Research and Training (CAMRET). All flies were maintained in an incubator at a controlled temperature of 22–25°C with a 12-hour light/12-hour dark light cycle.

Food Preparation

The diet for the flies consisted of 1000g of prepared food media, which included 100g of corn flour, 20g of yeast, 10g of agar-agar, and 1g of methylparaben with water added to achieve the appropriate consistency. To prepare this media, agar-agar was gradually added to boiling water and stirred continuously for 10 minutes. Moist corn flour was then incorporated and cooked for an additional 10 minutes. Yeast was subsequently added, followed by methylparaben, with stirring

maintained for another 10 minutes. The media was then allowed to cool briefly before being transferred into culture bottles.

Fractionation of the Methanol Extract

Flash chromatography was used to separate the methanol extract into Hexane fraction, Ethyl acetate and Butanol.

Qualitative determination of phytochemicals was conducted using methods described by Trease and Evans (2002).

Acute Toxicity Study

Acute toxicity test was performed for the duration of 5 days. Three (3) different concentrations of the most active fraction (Butanol) were prepared as 100mg, 250mg and 500mg per 10 g diet.

For the experiment, a total of 135 flies (45 flies per group) was starved for 14-16 hours in empty vials and fed on different concentrations of the extract for 30mins. The flies were then observed daily for incidence of mortality, and the survival rate which was determined by counting the number of dead flies per day. The data was analysed and plotted as cumulative mortality and percentage survival after the treatment period. LD50 was determined graphically and the result was used to determine the formulation of the dose for hepatotoxicity studies.

Measurement of locomotor performance (negative geotaxis)

The negative geotaxis assay is used to evaluate the locomotor performance of flies. In brief, after the treatment period for 5 days, ten (10) flies from each group were briefly immobilized in ice and transferred into a clean tube (11 cm in length 3.5 cm in diameter) labelled accordingly. The flies initially were allowed to recover from immobilization for 10 min and thereafter tapped at the bottom of the tubes. Observations were made for total number of flies that crossed the 6-cm line within a period of 6 s and recorded. The results were expressed as percentage of flies that escaped beyond a minimum distance of 6 cm in 6 s during three independent experiments.

Hepatotoxicity Studies

Antioxidant studies were carried out first to establish the fraction with highest activity. The hepatoprotective activity of the most active fraction was evaluated. Hepatotoxicity was induced in the drosophila by feeding it with carbon tetrachloride diet. CCl4 was added to the diet and observed at 24, 48, 72, 96 and 120 hours. The LC50 was calculated to find out the dose for hepatotoxicity studies.

Experimental grouping in Drosophila melanogaster

The flies were randomly distributed into five groups of 30-50 flies each and treated as follows:

Group I: Normal diet only (Treatment naive).
Group II: CCL4 + Diet to serve diseased (Negative control).
Group III: CCL4 + 0.1g/10g diet of butanol fraction
Group IV: CCL4 + 0.2g butanol fraction /10g diet
Group V: CCL4 + 0.3g butanol fraction /10g diet
Group VI: CCL4 + 0.3g silymarin /10g diet (positive control).

Whole-Fly Tissue Homogenate Preparation

After the last dose, the drosophila was fasted overnight for eight hours and on the last hour, groups of 20 flies were homogenized in 1 mL, 0.1 M phosphate buffer, pH 7.0. Following centrifugation (appropriate speed and time to each test) at 48oC, the supernatant was maintained on ice until the respective biochemical assays.

Biochemical Parameter Determination

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by IFCC methods, using the kits instruction manual (AGAPPE Diagnostics Ltd). Alkaline phosphatase activity was determined by the method described in the assay kit instruction manual (TECO diagnostics Ltd., USA). Serum bilirubin levels were determined by the modified TAB method described in the assay kits manual (AGAPPE Diagnostic Ltd). The creatinine and total protein concentrations were determined by the methods described in assay kits instruction manuals (Randox Laboratories Ltd., UK). The urea and albumin concentrations were estimated by the methods described in assay kits manuals (AGAPPE Diagnostics Ltd).

Determination of Reduced Glutathione GSH

The GSH was assayed using the method described by Jallow *et al* (1974). This method based upon production of relatively stable yellow colour when 5'-5' Dithiobis (2-nitrobenzoic acid) (DTNB) is added to sulfhydryl compound. The chromophoric product resulting from reaction of DTNB with reduced glutathione, 2- nitro- 5-thiobenzoic acid is maximally absorbed at 412 nm and the amount of reduced glutathione in sample was proportional to the absorbance at the wave length.

Briefly, 0.4 ml of each sample was added to 0.4 ml of 20 % trichloroacetic acid (TCA) and mixed by gentle swirling motion and centrifuge at 10,000 rpm for 10 minutes at 4^0C (in cooled centrifuge). Measured 0.25 ml of the supernatant was withdrawn and added to 2 ml of 0.6 mM DTNB and final volume of the solution was made up to 3 ml with (0.75 ml) phosphate buffer (0.2 M, pH 8.0). Absorbance was read at 412 nm against black reagent (2 ml of 0.6 mM DTNB+ 1 ml phosphate buffer (0.2 M, pH 8.0) using spectrophotometer. The concentration of

reduced glutathione in the brain tissue is expressed as micromole per gram of protein (umole/g).

Determination of antioxidant defenses Catalase

The catalase (CAT) activity assay was quantified by measuring the decomposition of H_2O_2 using the catalase activity assay kit (Elabsicence, USA) according to the manufacturer's protocol. Briefly, 1 mL of buffer solution was added to each control tube and a mixture of sample 0.1 mL of haemolymph and 1 mL of buffer solution was added to each sample tube. All tubes were incubated at 37°C for 5 minutes. Next, 0.1 mL of substrate was added to each tube, mixed thoroughly, and incubated at 37°C for precisely 1 minute. The reaction was stopped by adding 1 mL of chromogenic solution and 0.1 mL of clarificant. After standing for 10 minutes at room temperature, absorbance was measured at 405 nm using a microplate reader (Infitek, China). The CAT activity (U/mL) was calculated as: CAT activity(U/mL) = $\Delta A \times 32.51 \times V \times f$

Where, 32 5: reciprocal of the slope; 1: Reaction time; ΔA: Absolute OD (ODControl – ODSample); V: Volume of the sample; and f: Dilution factor of the sample before the test.

Superoxide dismutase (SOD)

The Total Superoxide Dismutase (T-SOD) assay was performed using the Total Superoxide Dismutase (T-SOD) activity assay kit (Elabsicence, USA) according to the manufacturer's protocol. The T-SOD assay was conducted by first preparing sample and control tubes, each containing 1 mL of buffer working solution with either the sample or double distilled water. Then, 0.1 mL of nitrosogenic agent, 0.1 mL of substrate solution, and 0.1 mL of enzyme stock working solution were sequentially added into each tube. The tubes were then vortexed thoroughly and incubated at 37°C for 40 minutes. Following incubation, 2 mL of chromogenic agent was added to each tube, mixed thoroughly, and left to stand at room temperature for 10 minutes. Absorbance was subsequently measured at 550 nm using a microplate reader (Infitek, China). SOD activity was calculated from the optical density values of the sample and control tubes, based on the inhibition percentage formula: T−SOD activity (U/mL) =$i \times 50\% \times V1V2 \times f$

Where, i: inhibition ratio, Inhibition ratio = (ODControl – ODSample/ODControl)×100%; V1: the total volume of the reaction solution; V2: the volume of sample added; f: Dilution factor of the sample before the test.

RNA Isolation, and Real-time PCR Analyses

Total RNA was isolated from the *Drosophila homogenate* (whole fly) by single step guanidinium isothiocyanate/ phenol extraction using PureZol RNA isolation reagent (Invitrogen, Japan) following the manufacturer's instructions, (Luoni *et al.,* 2014). The isolated mRNA of superoxide dismutase enzyme (SOD) was reversely transcribed into complementary deoxyribonucleic acid (cDNA) using RT Reagent Kit (RR037A, Takara Biotechnology, Shiga, Japan). RNA concentration was determined using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Real-time PCR amplification was carried out by Applied Biosystems 7500 Real-time PCR System using the SYBR R RT-PCR Kit (RR820A, Takara Biotechnology, Shiga, Japan). Relative quantification method was used to calculate the difference in gene expression (Livak and Schmittgen, 2001).

Data Analysis

The statistical package for the social sciences (SPSS version 21.0) produced by SPSS Inc., Chicago, USA, was used for statistical analysis. Data were presented as mean ± standard error of mean (SEM), with n = 5. The data were analysed by one-way analysis of variance (ANOVA), followed by least significant difference (LSD) as Post Hoc test for multiple comparisons. Mean differences were considered statistically significant at $p < 0.05$.

RESULTS

Fractionation Yield

The n-butanol extraction fraction yielded greater amount of 4.2 g. Ethyl acetate and n-hexane fractions yielded 2.9 and 1.6 grams respectively (Table 1).

Table 1. Phytochemical secondary metabolites

Test	Result
Alkaloids	+
Flavonoids	+
Phenols	+
Saponins	+
Tannins	+
Cardiac glycosides	+
Steroids	+

Acute toxicity study

All the flies that received treatments survive after seven (7) days of exposure across all the doses (125, 250 and 500 mg per 10g diet) of the three fractions tested (Table 2).

Effect of *Lophira lenceolata* fractions on Geotactic Behaviour

The effect of *Lophira lanceolata* on geotactic behavior is reveals significant ($p<0.05$) increase in geotactic behavior as shown in Table 3.

Effect of methanol leaf fractions of *Lophira lanceolata* on DPPH radical scavenging activity

The fractions of lophira lenceolata show significant free radical scavenging activity with the n-butanol fraction showing highest activity as dsplayed in Table 4.

Table 2. Acute toxicity studies of Lophira lanceolata in drosophila models (Mortality)

Fractions	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
n-butanol							
500 mg	0	0	0	0	0	0	0
250 mg	0	0	0	0	0	0	0
125 mg	0	0	0	0	0	0	0
Diet only	0	0	0	0	0	0	0

The LC_{50} of the n-butanol fraction is greater or equal to 500 mg

Table 3. Effect of *Lophira lanceolata* on geotactic behavior is displayed in the table 3 below

Fractions/doses	**Locomotion of the flies**
n-Butanol fraction	
125 mg	6.20±0.60
250 mg	8.80±1.30*
500 mg	9.11±0.90*
Diet	7.32±1.10

Data expressed as mean± standard error of mean, *= significance p<0.05, dunnet post-hoc test

Table 4. Effect of methanol leaf fractions of *Lophira lanceolata* on DPPH radical scavenging activity

Concentrations	n-Butanol (%inhibition)	n-Hexane(%inhibition)	Ethyl acetate(%inhibition)
0.2	53	29	31
0.4	56	33	36
0.6	67	36	42
0.8	71	43	46
1.0	83	48	57

Effect of n-Butanol leaf fraction of *Lophira lanceolata* on Liver Biochemical Markers of Drosophila Melanogaster

The results reveal elevated level of liver enzymes, aspartate transaminase, alanine transaminase, alkaline phosphatase and other markers such as bilirubin and total proteins in the carbon tetrachloride treated group (Diet+CCL_4 group) and these same parameters were significantly p<0.05 reduced by administration of graded doses of n-butanol fraction of *Lophira lanceolata* with the drosophila melanogaster diet as displayed in Table 5.

Effect of n-Butanol leaf fraction of *Lophira lanceolata* on Antioxidants enzymes in Drosophila Melanogaster homogenate

The n-butanol fraction has significantly p<0.05 increased the level of antioxidant enzymes in *Drosophila homogenate* across all the doses employed (Table 6).

Effect of n-Butanol leaf fraction of *Lophira lanceolata* on Gene expression of superoxide dismutase in Drosophila Melanogaster homogenate

The n-butanol fraction of *Lophira lanceolata* has significantly increased the expression of superoxide dismutase gene (Figure 1).

Table 5. Effect of n-Butanol fraction on liver biochemical markers in Drosophila melanogaster

Treatment	**ALT**	**AST**	**ALP**	**BL**	**TP**	**AL**
Diet	1.85±0.15	13.22±0.73	27.18±1.50	0.61±0.02	1.10±0.21	0.81±0.03
Diet+CCL_4	5.72±1.60	27.84±2.50	87.75±3.95	1.32±0.04	2.81±0.31	1.40±0.50
CCL_4+125 mg fraction	4.61±1.10	28.12±1.60	87.10±2.81	1.21±0.21	2.43±0.30	1.21±0.60
CCL_4+250 mg fraction	3.85±1.20	21.50±1.10*	65.21±1.80	1.10±0.51*	1.85±0.55*	1.10±0.71*
CCL_4+500 mg fraction	1.50±0.80*	15.21±1.20*	25.40±1.52*	0.85±0.21*	0.91±0.22*	0.75±0.30*
CCL_4+0.3 mg silamyrin	1.65±0.81*	12.51±1.10*	14.50±1.65*	0.65±0.10*	0.82±0.20*	0.62±0.11*

Data expressed as mean ± standard error of mean, *= significance p<0.05, dunnet post-hoc test, CCL= carbontetrachloride

Table 6. Effect of n-Butanol fraction on Antioxidants enzymes in Drosophila melanogaster

Treatments	SOD(U/mL)	GSH (GSH/L)	CAT(U/mL)
Diet	42.12±1.02	95.10±2.10	125.40±8.55
Diet+CCL_4	14.66±2.35	22.50±3.72	35.60±11.54
CCL_4+125 mg fraction	22.65±5.12*	34.75±2.20	45.78±7.25
CCL_4+250 mg fraction	29.85±2.20*	55.26±3.16*	85.66±7.50*
CCL_4+500 mg fraction	41.26±4.10*	87.28±1.20*	116.25±8.40*
CCL_4+0.3 mg silamyrin	45.21±1.60*	97.41±3.10*	122.60±9.25*

Data expressed as mean± standard error of mean, *= significance p<0.05, dunnet post-hoc test, CCL= carbontetrachloride

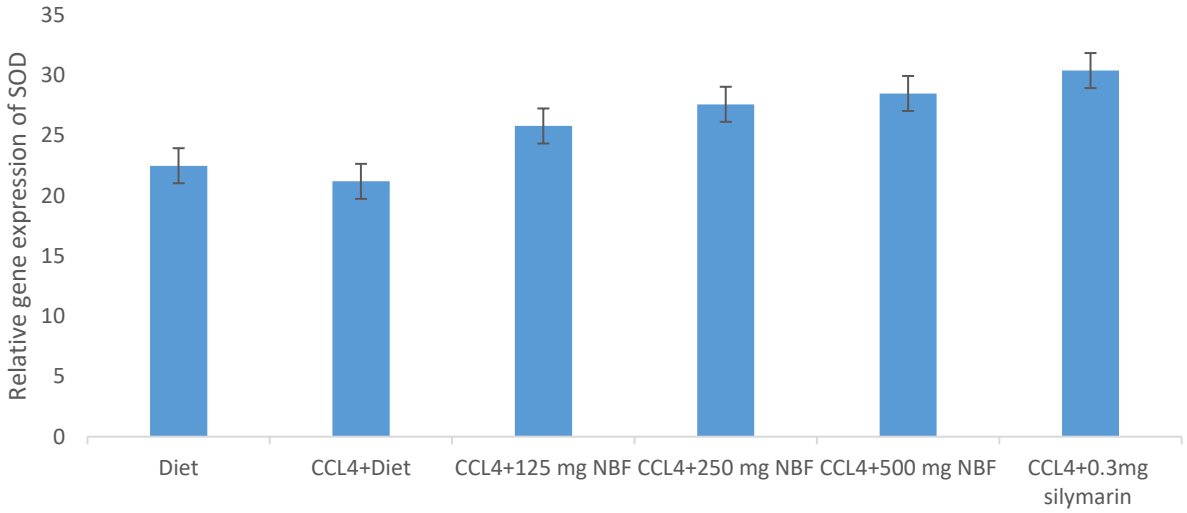

Figure 1. Effect of *Lophira lanceolata* n-butanol fraction on SOD gene expression in Drosophila melanogaster homogenate

CCL4 = Carbon tetrachloride, NBF= n-butanol fraction

DISCUSSION

Acute toxicity outcome indicates no mortality across all the dose exposed with n-butanol fraction in drosophila flies, this inferred relative safety of the n-butanol fraction. The presence of the secondary metabolites in the fraction is a great potential that these metabolites are responsible for the observed hepatoprotective effect in the drosophila flies.

The alterations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities are biochemical indicators of hepatic cells injuries (Atawodi *et al.,* 2014). The significant increase in serum AST, ALP and ALT activities, after exposure of flies to carbon tetrachloride are indicators of liver injuries, attributed to the toxic effect this chemical. Previous studies have shown that increases in serum AST and ALT activities reflect hepatocellular membrane damage and leakage (El-Hosseiny *et al.,* 2016; Atawodi *et al.,* 2014). The significant increase in alkaline phosphatase (ALP) activity may also indicate liver impairments and cholestasis, due to the toxic effect of carbon tetrachloride. ALP activity increases as a result of biliary obstructions or cholestasis; thus, it is a biomarker of hepatobiliary disease (El-Hosseiny *et al.,* 2016). The alteration in serum bilirubin levels is a biochemical indicator of the changes in morphological integrity of hepatobiliary tract, as a sign of liver damage (Sasidharan *et al.,* 2010; Alqasoumi, 2014). Elevated levels of bilirubins may be due to excessive haemolysis or biliary tract obstruction (Alqasoumi, 2014). The significant increase in serum total bilirubin may indicate impaired hepatic excretory function, also an indicator of liver dysfunction, attributed to the toxic effect of carbon tetrachloride. These findings are corroborated with El-Hosseiny et al. Who found significant elevation in the levels of total bilirubin, after exposure of animals to a toxic drug. Bilirubin is the excretory product formed by catabolism of heme in haemoglobin, thus hyperbilirubinemia is usually caused by excessive destruction of heme and blockage of biliary tract; which results in severe inhibition of conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes. The significant reduction of AST, ALT, ALP and bilirubin levels after treatment of carbon

tetrachloride -exposed flies with n-butanol fraction of *L. lanceolata* may indicate a protective effect of this plant extract against carbon tetrachloride -induced hepatic injuries. The n-butanol fraction may have stabilized hepatocyte membrane integrity against carbon tetrachloride lethality, thereby preventing elevation of these biomarkers of hepatic injuries in drosophila melanogaster. Some plant extracts have earlier been reported to produce protective effects against drug-induced liver injuries in animals by reduction of ALT, AST, ALP and bilirubin levels (El-Hosseiny *et al.,* 2016).

The significant increase in the values of liver biochemical parameters by treatment of carbon tetrachloride exposed flies with graded doses of n-butanol fraction of L. lanceolata, may suggest that this plant extract fraction has protective effect against carbon tetrachloride -induced liver injuries in drosophila melanogaster. These findings are in agreement with Adejor et al, who found that the protective effect of plant extract prevented significant alteration in organ to body weight ratio of animals. Thus, the prevention of marked decrease in liver serum biochemical parameters in the current study, may suggest that L. lanceolata fraction protected the liver against carbon tetrachloride -induced injuries and necrosis in fruit flies.

Antioxidants act as a defence mechanism that protect against pathologic effects of oxidative reaction produced by reactive oxygen species (ROS) in a biological system (Jayachitra and Krithiga, 2010). Overproduction of ROS and/or inadequate antioxidants has been implicated in the pathogenesis and complications of some disease conditions like diabetes, hepatitis, Alzheimer's disease, cancer, atherosclerosis, arthritis, neurodegenerative disease, and metabolic disorders (Khalaf *et al.,* 2010; Patel *et al.,* 2010). Chemical substance including carbon tetrachloride that are proven to be toxic to internal organs do produces their lethality via oxidative stress pathway, either by increasing the concentration of free radicals or by decreasing the level of endogenous antioxidant enzymes such as superoxide dismutase reduced glutathione and catalase. The plant L. lanceolata have been reported show antioxidant activity in animal models. Liver remains one of the most vulnerable organs to free radical oxidative carnage. Antioxidants have been reported to prevent oxidative damage caused by ROS by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers (Büyükokuroğlu *et al.,* 2001). Oxidative stress is characterized by imbalance between oxidant producing systems and antioxidant defense mechanisms, resulting in excessive formation of reactive oxygen species (ROS). Excessive accumulation of ROS can damage bio molecules, including lipids, proteins and nucleic acids. Thiol groups are important members of the antioxidant team and have been shown to destroy ROS and other free radicals by enzymatic and non-enzymatic mechanisms (Jones *et al.,* 2000). Lophira lanceolata n-butanol fraction revealed significant increase in antioxidant enzymes, inferring valuable invo antioxidant activity which could be the pathway of its hepatoprotective effect observed in this study with drosophila model. The n-butanol fraction has increased the expression of superoxide dismutase gene, indicating possible mechanism of action of this extract via increasing the expression of endogenous antioxidant enzymes.

CONCLUSION

The findings of this study have shown that n-Butanol fraction of *L. lanceolata* leaf may have protective effect against carbon tetrachloride-induced hepatic injury in *drosophila melanogaster*, which may be attributed to the phytochemicals present in it. And the possible mechanism of this hepatoprotective activity might be via increased expression of antioxidant gene, superoxide dismutase. This gives credence to the use of this plant extract in traditional medicine for the treatment of diseases in humans.

FUNDING

This work was supported by the TETFund Institution Based Research (IBR) grant (TETFund/DR&D/CE/UNI/SOKOTO/IBR/2024/Batch-9/VOL)

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