Original Article

Anti-Oxidant and Hepatoprotective Activities of *Ziziphus mucronata* Fruit Extract Against Dimethoate-Induced Toxicity

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Abstract

Objective: The study was carried out to evaluate the hepatoprotective and antioxidant potential of *Ziziphus mucronata (ZM)* fruit extract.

Methods: The different types of fruit extract were prepared by soaking the dry powdered fruit in different solvents followed by rotary evaporation. Each extract was tested for its phenol content and antioxidant activities. An in vivo study was performed in Sprague-Dawley (SD) rats. Thirty adult male SD rats (aged 21 weeks) were divided into six groups of five rats each and treated as follows: The normal control (NC) received distilled water while the dimethoate control (DC) received 6 mg/kg.bw.day-1 dimethoate dissolved in distilled water. The experimental groups E1, E2, E3, and E0 received dimethoate (6 mg/kg.bw) + ZMFM (100 mg/kg.bw-1), dimethoate (6 mg/kg.bw) + ZMFM (200 mg/kg.bw-1), dimethoate (6 mg/kg.bw) + ZMFM (300 mg/kg.bw-1), and ZMFM (300 mg/kg.bw-1) only. Both the normal control and the dimethoate control groups were used to compare the results. After 90 days, rats were sacrificed, blood was collected for biochemical assays, and livers were harvested for histological study.

Results: High phenol content was estimated, and 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) spectrophotometric, thin layer chromatography (TLC) and 2, 2-Azobis-3-ethyl benzothiazoline-6-sulphonic acid (ABTS) assays showed a high antioxidant activity among the extracts. The preventive effects observed in the E1, E2 and E3 groups proved that the extract could prevent dimethoate toxicity by maintaining normal reduced glutathione (GSH), vitamin C and E, superoxide dismutase, catalase, cholineasterase and lipid profiles. The preventive effect was observed to be dose dependent. The EO group showed no extract-induced toxicity. Histological observations agreed with the results obtained in the biochemical studies.

Conclusion: The study demonstrated that ZM methanol fruit extract is capable of attenuating dimethoate-induced toxicity because of its high antioxidant activity.

1. Introduction

The occasional use of pesticides in large amounts may cause environmental pollution and therefore be a

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cause of concern. Residual amounts of organochlorine and organophosphate pesticides have been detected in soil, water reservoirs, vegetables, grains and other food products [1]. Organophosphates induce toxicity in mammals through inhibition of acetylcholinesterase (AchE) and subsequent activation of cholinergic receptors [2]. Oxidative stress as a result of organophosphate exposure has been reported [3], and lipid peroxidation has been revealed to be one of the molecular mechanisms in organophosphate-induced toxicity [4, 5]. The use of natural remedies has been recently observed to be a good means of curbing oxidative stress by boosting the natural antioxidant defence system [4].

In Africa, medicinal plants are used traditionally to treat various ailments. Among the promising medicinal plants, *Ziziphus mucronata* (ZM) in the family Ramnaceae is very common in Botswana, and has a rich historical and religious background. Various parts of the tree are already in use by common people as medicine and as food. The roots, bark and leaves are used in the treatment of arthritis, chest pains and boils. The sweet fruits of the plant are widely eaten in Botswana, and they are dried, ground to powder and cooked as porridge [6]. In Botswana, dimethoate is widely used by the local farmers as a pesticide. A proposal has been made that the fruit might be useful in protecting the population from being affected by dimethoate toxicity. Hence, the present study has been aimed at establishing the antioxidant and hepato-protective properties of ZM fruit extracts.

2. Materials and methods

Dimethoate emulsifiable concentrate was purchased from Agrivet PTY (LTD) Botswana. Other chemicals were of analytical grade and were bought from Sigma-Aldrich (St. Louis, USA). The kits were purchased from Aggape diagnostics, Kerala-India. The ZM fruits were collected around Gaborone. The plant authentication was performed by Mr. M. Muzila, curator, Hebarium Sector of the University of Botswana. Fruits were washed with distilled water and sun dried. The dried fruits were ground with a laboratory grinder to obtain 500 g of powder. The powder was soaked and extracted in hexane, 50% hexane/chloroform, chloroform, 50% chloroform/methanol, methanol and 70% methanol/water for 24 hr. The extract was made solvent free in rotary vaporizer at reduced pressure. The final extract yield was 25% w/w.

Thirty male Sprague Dawley (SD) albino rats aged 20-21 weeks were kept in galvanized cages under a controlled temperature of 25℃ and a normal photoperiod (12-h dark, 12-h light). The animals were fed commercial food pellets

(protein: 18%, fat: 6%, fiber: 6%, carbohydrates: 56%, calcium: 0.6% moisture: 10%) and water ad libitum. All procedures with the animals were performed in accordance with the protocols established by the University of Botswana Animal Care Committee (Approval no: UB/IRB/1084). The thirty adult male rats were divided into six groups of 5 rats each and treated as follows:

Group 1 - normal control (NC) received distilled water,

Group 2 - dimethoate control (DC) received 6 mg/kg.bw.day^{-1} dimethoate dissolved in distilled water,

Group 3 - experimental group (E1) received dimethoate (6 mg/kg.bw) + ZMFM (100 mg/kg.bw^{-1}),

Group 4 - experimental group (E2) received dimethoate (6 mg/kg.bw) + ZMFM (200 mg/kg.bw^{-1}),

Group 5 - experimental group (E3) received dimethoate (6 mg/kg.bw) + ZMFM (300 mg/kg.bw^{-1}),

Group 6 - experimental control (EO) received ZMFM (300 mg/kg.bw^{-1}) only.

The experiment was run for 90 days, and all extracts were administered orally with the help of a rubber tube and syringe. At the end of the experiment, rats were sacrificed after having been treated with mild ether anaesthesia.

Blood was collected in heparinized tubes and centrifuged at a rate of 6000 rotations per minute, after which plasma samples were drawn and stored at -70℃ for further analysis. The rat liver tissue was removed and immediately fixed in 10% phosphate-buffered formalin for 24 hr. The tissue was dehydrated in an ethanol series (70%, 80%, 100%, and 100%), cleared in xylene and embedded in paraffin. Five-μm-thick sections were taken by using a rotary microtone, and sections were affixed on clean glass slides by gentle heating. The sections were deparaffinised, stained with haematoxylin and eosin, and mounted with a cover slip by using neutral mounting media (DPX). The slides were observed under a light microscope for any histological changes at x400 [16].

The 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) semi-quantitative assay was performed on thin layer chromatography according to the method described by Yeboah and Majinda [7], and the spectrophotometric measurement of free-radical scavenging activities of the extracts by DPPH was done using the method described by Stoilova et al. [8]. The 2.4.3. 2, 2-Azobis-3-ethyl benzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity. (ABTS) was determined as described by Pellegrini et al. [9], and the total phenol content (TPC) was determined by using the method described by Stoilova et al. [8].

Activities of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) and measurements of the levels of plasma low-density lipoproteins (LDL), high-

density lipoproteins (HDL), total protein (TP), triglyceride (TG) and cholinesterase activity were done using kits from Aggape Diagnostics, India. Thiobarbaturic acid reactive substances (TBARS) were measured by using the method described by Chaturvedi [10], and reduced glutathione (GSH) was measured by using the method described by Ellman [11]. Vitamin C was estimated according to the method described by Omaye et al. [12], and Vitamin E was estimated according to the method described by Martinek [13]. Superoxide dismutase (SOD) was estimated according to Flohe and Otting [14], and the activity of catalase was assayed by using the method described by Bisswanger [15].

Descriptive data analysis and evaluation were done by using Sigma Stat 3.1 software. One-way ANOVA was used to determine the differences among groups. The level of significance was calculated using the Turkey test. Values of $P < 0.05$ were considered significant. The total phenol content was estimated by using a linear regression on the standard curve of the standard (gallic acid).

3. Results

3.1. TLC/DPPH antioxidant activity of the fruit extracts of ZM

Various concentrations (0.1 μg, 0.5 μg,1.0 μg, 5.0 μg, and 10.0 μg) of the fruit extracts from different solvents spotted on the TLC sheet showed high activity as indicated by the yellow color over the purple DPPH background.

Extracts from 100% methanol, 70% methanol/water, 70% methanol/chloroform, 100% chloroform, 50% hexane/chloroform and 100% hexane changed to yellow on the purple DPPH background. The extent of yellow coloration was comparable to the gallic acid standard (Fig. 1).
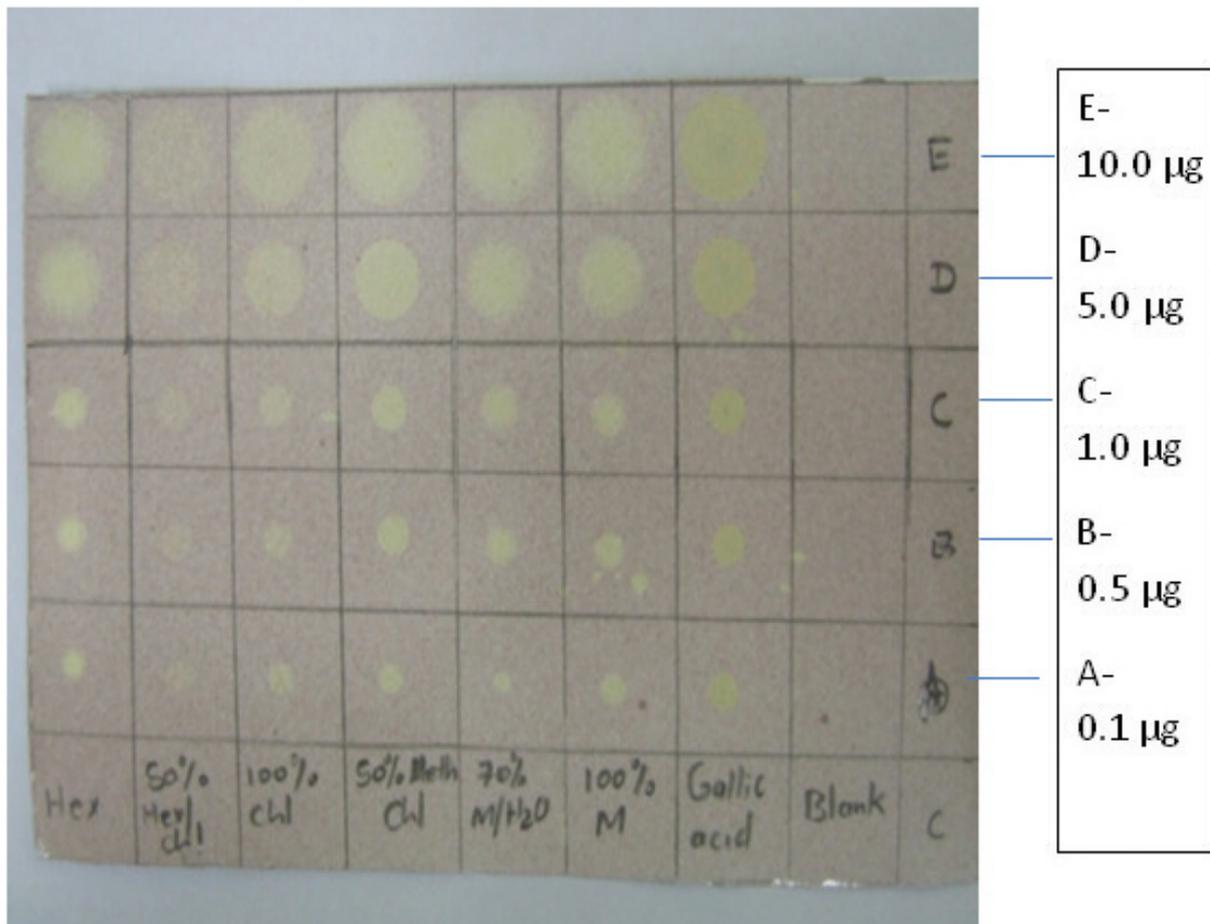

Figure 1 TLC-DPPH assay showing the antioxidant activities of different concentrations of ZMFE spotted on a TLC sheet.

3.2. Percent DPPH inhibition versus concentration for different extracts of ZM and standards observed after 30 min.

ZM fruit extracts showed high antioxidant activity. The increase in the antioxidant activity occurred in a concentration-dependent manner. The percentage inhibition increased when going from low-polarity-solvent to a high-polarity-solvent extracts: namely, 100% methanol, 70% methanol/ chloroform, 100% chloroform, 50% hexane/chloroform, 100% hexane in that order. The standards, gallic acid, displayed 100% DPPH inhibition (Fig 2).

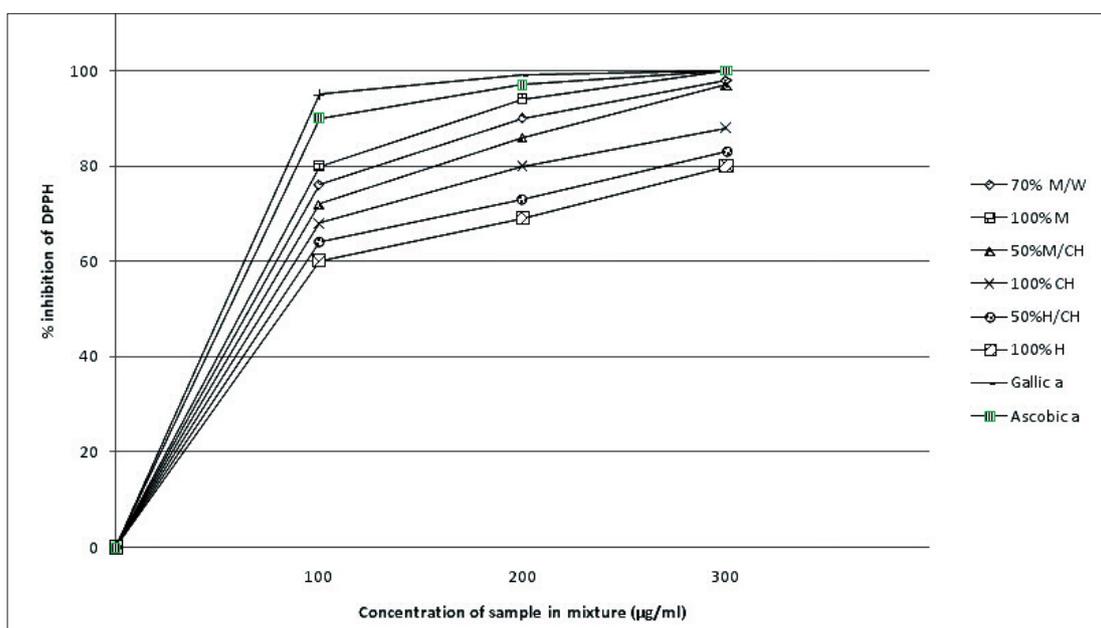### 3.3. Total phenol content of different types of fruit extracts of ZM

As presented in Table 1, the phenol content was found to increase from 30 mg GAE/g for hexane extract to 100 mg GAE/g for 50% methanol chloroform, 57 mg GAE/g for chloroform, 48 mg GAE/g for 50% hexane/chloroform and 250 mg GAE/g for 70% methanol extract.

3.4. 3.2.5 In vitro antioxidant activity by ABTS.

Effect of ABTS free radical scavenging activity of *Z. mucronata* was assayed at various concentrations from 2.5, 5, 10, 25, 50 and 100 $\mu g/m\ell$. The method is based on the ability of antioxidant molecules to quench the long lived ABTS radical cation (ABTS).

Significant ABTS scavenging activity was evident in 100% methanol extract 7.21, and a weak scavenging activity was observed in 100% hexane (Table 2).

Figure 2   Percent inhibition of DPPH by different concentrations of *Z. mucronata* fruit extracts after 30 min.

Table 1   Total phenol content of ZM fruit extracts

Fruit extract	TPC (mg GAE/g)
70% methanol	250 ± 4.3
100% methanol	240 ± 3.2
50% methanol/chloroform	100 ± 2.4
Chloroform	57 ± 0.9
50% hexane chloroform	48 ± 1.2
Hexane	30 ± 0.3

* Values are numbers or means ± SDs.

Table 2   In-vitro ABTS free-radical scavenging activity of ZM extracts

Plant part	Test extract	IC$_{50}$ ($\mu g/m\ell$)
Fruit extract	100% methanol	7.21
	50% methanol/chloroform	30.34
	100% chloroform	50.13
	50% hexane/chloroform	60.55
	100% hexane	80.22

The values are means of three replicates ± SDs. Extracts were tested at 2.5, 5, 10, 25, 50 and 100 $\mu g/m\ell$. Reference standard (gallic acid) was tested at 1, 2, 4, 8 and 16 $\mu g/m\ell$.

3.5. Effects ZMFM on plasma TBARS and liver transaminases

Daily administration of dimethoate resulted in elevated levels of TBARS in the plasma of the DC group. A dose-dependent reduction in the levels of plasma TBARS was associated with the ZMFM administration. Compared with the levels in NC rats, liver transaminases, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) were increased significantly ($P < 0.001$) in rats of the DC group. Significant dose-dependent declines in the levels of malonyldialdihyde and the activities of tranaminases were observed in plasma SGOT ($P < 0.001$), SGPT ($P < 0.001$) and ALP ($P < 0.001$) as a result of extract administration. Administration of ZMFM only resulted in no signs of toxicity as evidenced by the levels of TBARS having no significant differences from those of the NC group. SGOT, SGPT and ALP also had no significant differences when compared to the values for the NC group (Table 3).

3.6. Effects ZMFM on plasma reduced glutathione (GSH), vitamin C and E.

The results showed that the plasma levels of reduced GSH, vitamin C and E were reduced significantly ($P < 0.001$) in DC rats compared to the levels in NC rats. When compared with NC rats, the levels were also reduced significantly ($P < 0.001$) in experimental rats (E1-E3) in a dose-dependent manner. Although the activity of SOD was low in the E3 group, it still differed significantly from levels in the DC group ($P < 0.05$). The activities of catalase in the E3 group differed significantly from those in the DC group, but no difference was noted when comparison was made with the NC group. Administration of ZMFM along with dimethoate helped the animals to ameliorate the levels of these non-enzymatic antioxidants (Table 4).

Table 3 Effect of ZMFM on serum marker parameters of dimethoate-treated rat

Groups	TBARS (nmol/ml)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
NC	0.690 ± 0.003	46.0 ± 2.8	48.0 ± 0.7	50.0 ± 2.
DC	4.868 ± 0.275**	349.0 ± 3.2**	330.0 ± 0.9**	670.0 ± 1.4**
E1	3.172 ± 0.008**	190.0 ± 2.8**	175.0 ± 1.7**	240.0 ± 2.6**
E2	1.900 ± 0.002**/a	65.0 ± 0.6aa	60.0 ± 1.6 aa	90.0 ± 1.6*/ aa
E3	0.992 ± 0.007*/aa	48.0 ± 1.2aa	43.0 ± 2.1aa	70.0 ± 2.3*/ aa
EO	0.644 ± 0.001a	45. ± 1.1aa	46.0 ± 0.1aa	49.0 ± 0.1a

Values are expressed as means ± SEMs, n = 5 for each treatment, ($P < 0.05$*, $P < 0.001$**) compared with normal control (NC) and ($P < 0.05$a, $P < 0.001$aa) compared with dimethoate control (DC). E1 (100 mg/kg.bw + DM), E2 (200 mg/ kg.bw extract + DM), E3 (300 mg/kg.bw + DM), and EO (300 mg/kg.bw).

Table 4 Effect of ZMFM on GSH, vitamin C and vitamin E of dimethoate-treated rats

Groups	Vitamin E (mg/ml)	Vitamin C (mg/ml)	GSH (mg/ml)
NC	5.00 ± 0.11	6.28 ± 0.02	55.0 ± 0.1
DC	1.00 ± 0.00*	2.03 ± 0.15*	20.0 ± 0.3*
E1	1.35 ± 0.01*	2.10 ± 0.21*	30.0 ± 0.4*/a
E2	1.39 ± 0.02*	3.00 ± 0.00*	37.0 ± 0.1*/a
E3	1.45 ± 0.10*	3.01 ± 0.01*	40.0 ± 0.6*/aa

Values are expressed as means ± SEMs, n = 5 for each treatment, ($P < 0.05$*, $P < 0.001$**) compared with normal control (NC) and ($P < 0.05$a, $P < 0.001$aa) compared with dimethoate control (DC). E1 (100 mg/kg.bw + DM), E2 (200 mg/ kg.bw extract + DM), and E3 (300 mg/kg.bw + DM).

3.7. Effects ZMFM on plasma SOD and catalase activities.

The results showed that the activities of SOD and catalase were enhanced significantly ($P < 0.05$) in DC rats as compared to NC rats, and significant decreases in the activities of SOD and catalase were noticed in the experimental groups and were dose dependent. The highest significant reductions ($P < 0.05$ for SOD and $P < 0.001$) for catalase were noticed in the E3 group when compared to the DC group. The SOD and the catalase in the E3 group showed significant differences from the values for both the DC group and the NC group ($P < 0.05$) (Table 5).

3.8. Effects of ZMFM on lipid profiles and total protein

Dimethoate administration was shown to significantly lower HDL levels ($P < 0.001$) and total protein ($P < 0.001$) in the DC group. ZMFM administration along with dimethoate successfully restored the HDL levels in the E2 and the E3 groups, and they differed significantly ($P < 0.05$) from the DC group. On the other hand, dimethoate

significantly increased the levels of low-density lipoprotein, cholesterol and triglyceride ($P < 0.001$) as compared to the levels in the NC group. The LDL, cholesterol, triglyceride ($P < 0.001$) and total protein ($P < 0.05$) levels were found to decline significantly in the E3 group as compared to the DC group. Levels of total protein and triglyceride differed significantly from those in the NC group, as did the levels LDL and cholesterol ($P < 0.05$). In the E2 group, a decline was noticed, and it showed a significant difference from the NC group. A similar response was noted for the E1group, but the changes were less significant than they were for the E2 group (Table 6).

3.9. Effect of ZMFM on cholineasterase activity

The cholinesterase activity declined ($P < 0.005$) in dimethoate-treated rats when compared to the normal control rats. There was a gradual and significant ($P < 0.005$) increase in the cholinesterase activity during ZMFM administration. The increment in cholinesterase activity occurred as a result of increasing ZMFM dosage (Table 7).

3.10. Effect of ZMFM on Histology

ZMFM administration revealed a preventive effect on the hepatocytes when compared to the dimethoate control (DC). The DC control group showed evident hepatic damage as shown by widening sinusoids, loss of both hepatocellular membrane and hepatocytes when compared to the normal control group. The preventive effect was more evident with increasing dosage of the ZMFM extract. The preventive effect was shown by reduced necrosis in ZMFM-fed rats when compared to dimethoate-fed rats (Fig. 3).

Table 5 Effect of ZMFM on antioxidant enzymes: SOD and catalase in plasma from dimethoate-treated rats

Groups	SOD (U/mg)	Catalase (Umg⁻¹)
NC	5.821 ± 0.012	403.6 ± 3.67
DC	$14.210 \pm 0.029^*$	$731.6 \pm 2.52^{**}$
E1	$12.100 \pm 0.021^{**}$/ᵃ	$580.2 \pm 2.30^*$/ᵃ
E2	$9.170 \pm 0.050^{**}$/ᵃᵃ	457.9 ± 2.33ᵃᵃ
E3	$7.840 \pm 0.018^*$/ᵃ	407.2 ± 4.30ᵃᵃ

Values are expressed as means $\pm$ SEMs, n = 5 for each treatment ($P < 0.05^*$, $P < 0.001^{**}$) compared with normal control (NC) and ($P < 0.05$ᵃ, $P < 0.001$ᵃᵃ) compared with dimethoate control (DC). E1 (100 mg/kg.bw + DM), E2 (200 mg/ kg.bw extract + DM), and E3 (300 mg/kg.bw + DM).

4. Discussion

Exposure to various organic compounds including a number of environmental pollutants and drugs can cause

Table 6 Effect of ZMFM on lipid and lipoprotein profiles in serum from dimethoate-treated rats

Groups	HDL (mg/dL)	LDL (mg/dL)	Cho (mg/dL)	Tp (gm/dL)	Trig(mg/dL)
NC	59.00 ± 1.00	9.00 ± 3.00	110.00 ± 1.78	8.10 ± 0.12	61.0 ± 7.8
DC	$20.00 \pm 0.37^{**}$	$150.00 \pm 12.20^{**}$	$162.00 \pm 9.15^{**}$	$2.00 \pm 0.02^*$	$120.0 \pm 6.3^{**}$
E1	$38.00 \pm 0.37^{**}$	$67.00 \pm 9.41^{**}$/ᵃᵃ	$158.00 \pm 8.39^{**}$/ᵃᵃ	$3.40 \pm 0.13^*$	$90.0 \pm 9.4^*$
E2	47.00 ± 0.45ᵃ	$30.00 \pm 2.13^{**}$/ᵃᵃ	$118.00 \pm 6.24^*$/ᵃᵃ	6.50 ± 0.07ᵃ	76.0 ± 7.6ᵃᵃ
E3	52.00 ± 2.74ᵃ	$22.00 \pm 4.19^*$/ᵃᵃ	$96.00 \pm 5.31^*$/ᵃᵃ	7.00 ± 0.01ᵃ	69.0 ± 5.3ᵃᵃ

Values are expressed as means $\pm$ SEMs, n = 5 for each treatment ($P < 0.05^*$, $P < 0.001^{**}$) compared with normal control and ($P < 0.05$ᵃ, $P < 0.001$ᵃᵃ) when compared with dimethoate control. E1 (100 mg/kg.bw + DM), E2 (200 mg/ kg.bw extract + DM), and E3 (300 mg/kg.bw + DM).

cellular damages through metabolic activation of those compounds by highly reactive substances such as reactive oxygen species (ROS) [17, 18]. Dimethoate is one of the organophosphates frequently used in agriculture, and it has been reported to generate oxidative stress and impose toxicity [19].

Organophosphates have been found to inhibit cholinesterase (AchE) and subsequent activation of cholinergic receptors [20]. The use of antioxidants like botanicals is an effective means to fight against the deleterious effects of oxidative stress. Botanicals are natural sources of antioxidants because of their richness in phenol, flavonoids and other anti-oxidants. In the present study, various extracts of ZM were found to contain significant amounts of phenols. In the present study, the total phenol content was seen to increase when going from the less polar solvent extracts to the more polar solvent extracts. The highest concentration was present in the 70% methanol/water extract.

ZM fruit extracts exhibited high antioxidant activity on the TLC/DPPH antioxidant assay (Fig 1). The TLC-DPPH assay was confirmed by the DPPH spectrophotometric assay(Fig. 2) and ABTS (Table 2) assays. The methanol fruit extract had the highest DPPH scavenging activity (96 shown %)and exhibited $7.21\mu g/ml$-Inhibiting concentration

(IC_{50}) on ABTS Other solvents showed lower antioxidant activities; hence, the highest antioxidant activity by the methanol extracts best represents the fruits antioxidant potential.

Dimethoate is known to inhibit cholinesterase activity in the target tissues [21]. It accumulates acetylcholine and prevents the smooth transmission of nerve functions, leading to convulsions and death. Dimethoate inhibits the

Table 7 Effects of ZMFM on plasma cholinesterase activity on dim-ethoat-treated rats

Experimental groups	Cholinesterase activity (Umg^{-1})
NC	30200 ± 28
DC	$6657 \pm 34*$
E1	$9914 \pm 40*$
E2	$15262 \pm 12*/^{a}$
E3	$21620 \pm 26*/^{a}$

Values are expressed as means $\pm$ SEMs, n = 5 for each treatment, ($P < 0.05*$, $P < 0.001**$) compared with normal control and ($P < 0.05^{a}$, $P < 0.001^{aa}$) compared with dimethoate control. E1 (100 mg/kg.bw + DM), E2 (200 mg/ kg.bw extract + DM), and E3 (300 mg/kg.bw + DM).
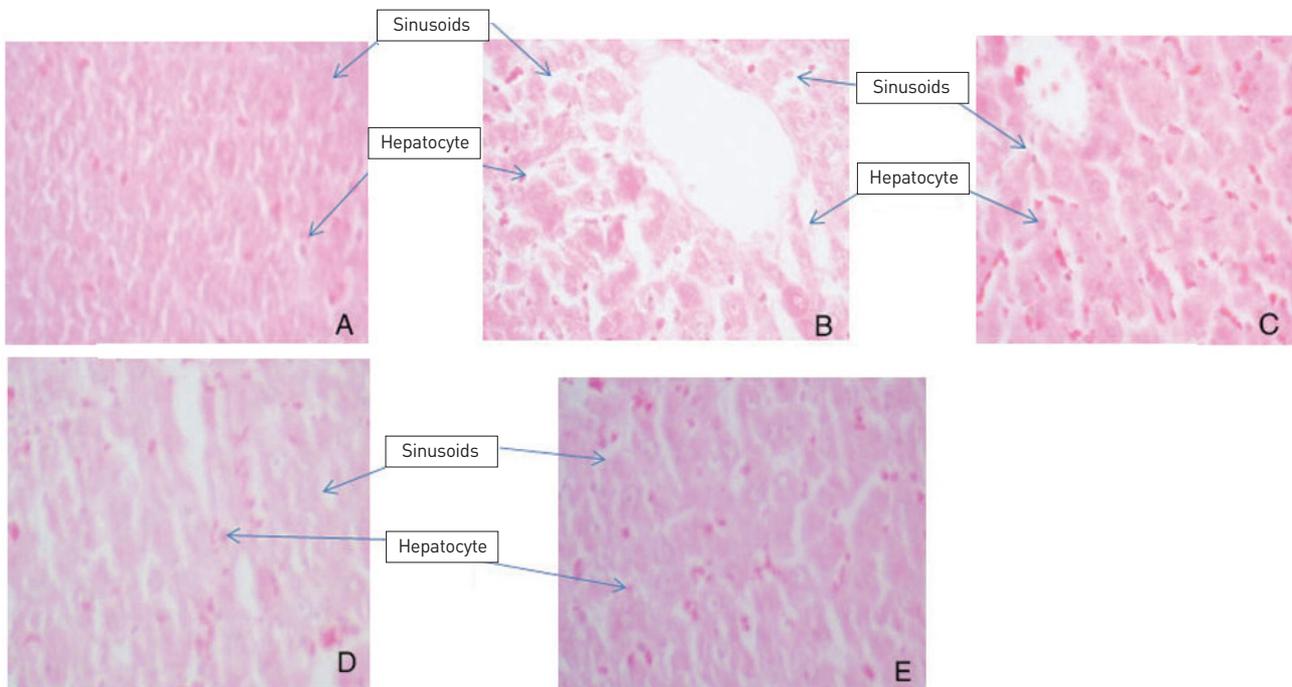

Figure 3 (A) Light microphotograph of haematoxylin and eosin-stained and formalin-fixed liver of the NC group. Normal sinusoids and intact hepatocytes with well-preserved nucli. (B) Light microphotograph of haematoxylin and eosin-stained and formalin-fixed liver of DC group. Many fatty changes and widened sinusoids. (C) Light microphotograph of haematoxylin and eosin-stained and formalin-fixed livers of the E1 group. The hepatic cells were preserved with no massive necrosis. (D) Light microphotograph of haematoxylin and eosin-stained and formalin-fixed liver in the E2 group. Most tissue was well preserved with no massive fatty changes, necrosis, and sinusoids not too wide, almost resembling the normal hepatocytes of the normal control. (E) Light microphotograph of haematoxylin and eosin-stained and formalin-fixed livers of the E3 group. The hepatocytes are well preserved with no distinguishable damage to the cell membranes (x400).

activity of the enzyme cholinesterase that hydrolyses acytylcholine into acetyl and choline and, hence, prevents the return of cholinergic neurons to normal. The liver is the primary organ involved in xenobiotic metabolism and is a major target organ for chemicals and drugs. In the present study, oral administration of dimethoate to rats caused hepatic damage, as evidenced by the elevations in the hepatospecific enzyme activities, as well as by the severe alterations in different liver marker parameters. TBARS, SGOT, SGPT and ALP were significantly increased ($P < 0.05$) in the dimethoate control (DC) group. Administration of ZMFM (Table 3) significantly ($P < 0.005$) lowered the levels of TBARS, SGOT, SGPT, and ALP when coupled with dimethoate during its administration, thus demonstrating a dose-dependent preventive effect of the extract.

The levels of GSH, vitamin C and vitamin E were observed to decline ($P < 0.05$) in dimethoate-treated rats. Administration of the ZMFM (Table 4) resulted in a gradual recovery of the non-enzymatic antioxidants in a dose-dependent manner. Glutathione is important in neutralizing the reactive intermediates and radical species generated during oxidative stress [22]. The depletion of GSH may be a result of its being used up in quenching omethoate radicals. An oral feeding of the extract seems to scavenge free radicals or interfere with free radical formation in vivo. Administration of the extract is, thus, demonstrated to prevent GSH depletion, hence leading to normal GSH production.

Vitamin E is thought to be an important chain-breaking antioxidant, is the major soluble antioxidant in all cellular membranes, and protects against lipid peroxidation [23]. ZMFM may possibly play a role in scavenging the reactive oxygen species (ROS). This thus allows the recovery of vitamin E which was used up in scavenging dimethoate-induced radicals.

The observed significant ($P < 0.05$) decline in the activity of SOD in the experimental groups was possibly a result of the extracts that were administered to the rats. This decrease may be a possible indication that the extracts alleviate dimethoate effects on progenitor cells and possibly reduce the activation of compensatory mechanisms. This may possibly occur when the ZM extract scavenges the free radicals produced as a result of dimethoate administration. The preventive effect may also occur through the blockage of the mechanisms leading to biotransformation of dimethoate to its destructive oxygen analogue (omethoate).

The observed elevated catalase activity may be a result of an adaptive response to the generated free radicals [3]. This directly indicates the failure of the total antioxidant defense system as shown by lipid peroxidation. The superoxide ion generated is, therefore, accounted for by the enhanced SOD and is converted to H_2O_2 or glutathione peroxidase. A decrease in the catalase activity suggests the possibility that ZMFM scavenges the free radicals before they necessitate a natural adaptive response by the enzyme. The study, thus suggests the ability of ZMFM to alleviate dimethoate-induced oxidative stress.

Elevated low-density lipoprotein (LDL), cholesterol (Cho) and triglyceride (Trig) levels and decreased high-density lipoprotein (HDL) and total protein levels were observed in dimethoate-treated rats, when compared to normal control rats, after 90 days of treatment. Administration of ZMFM extract resulted in significant ($P < 0.05$) suppressions in LDL, cho and trig levels when compared to normal control rats. The extracts brought about significant ($P < 0.005$) increases in the HDL and the total proteins levels when compared to the dimethoate control group. The increases in the total protein and HDL levels in the extract-treated rats possibly suggests the ability of the extracts to slow down dimethoate-damaging effects on the liver.

LDLs are known to carry cholesterol to the peripheral tissues where it can be deposited and increase the risk of atherosclerotic attack and peripheral vascular disease. LDL accumulation is, thus, very bad for the body [24]. The orally-administered extract offers more benefits by increasing the level of HDLs in rat plasma. Unlike the LDLs, HDLs hasten the removal of cholesterol from peripheral tissues to the liver for catabolism and excretion [1, 25].

The histological studies of the liver sections presented in Fig. 3 provide supportive evidence for the protective effects of ZMFM. The liver sections of normal control animals showed normal hepatic cells, each with preserved and prominent hepatocytes. The liver sections of dimethoate-intoxicated rats showed loss of hepatocytes and loss of cellular boundaries and widened sinusoids; ZMFM attenuated the degenerative effects of dimethoate toxicity. This was observed in the reduced intensity in liver-tissue degenerative changes. Here, less necrosis, limited loss of cell boundaries and reduced loss of intact hepatocytes were observed. The preventive effect of the extracts increased with increasing dosage of the extracts.

5. Conclusion

In conclusion, ZMFM extract could protect against oxidative stress by decreasing lipid peroxidation and enhancing the effects of enzymatic and non-enzymatic antioxidants. It also maintained the lipid profile and protected the liver from oxidative damage. These

protective effects are possibly because of the high antioxidant potential of ZMFM.

Acknowledgments

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