

# HEPATO-PROTECTIVE POTENTIAL OF METHANOL EXTRACT OF LEAF OF *ZIZIPHUS MUCRONATA* (ZMLM) AGAINST DIMETHOATE TOXICITY: BIOCHEMICAL AND HISTOLOGICAL APPROACH

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## SUMMARY

**Background:** The leaves of *Ziziphus mucronata* are used locally as food and a health drink; the leaf paste can also be used in the treatment of boils. The root of the plant is usually used in the treatment of a wide range of pains.

**Objective:** The study was carried out to evaluate the hepatoprotective potential of the methanol leaf extract of *Ziziphus mucronata* (ZMLM).

**Method:** The extract was prepared by soaking in 70% methanol/water and rotary evaporation. The phenol content of extract was then estimated. Twenty five adult male Sprague dawley rats (aged 21 weeks) were divided into five groups of five rats each and treated as follows; normal control (NC) received distilled water. Dimethoate control (DC) (received 6 mg/kg.bw.day<sup>-1</sup> dimethoate dissolved in distilled water). Experimental Groups (E1) received dimethoate (6mg/kg.bw) + ZMLM (100 mg/kg.bw<sup>-1</sup>); (E2) received dimethoate (6 mg/kg.bw) + ZMLM (200 mg/kg.bw<sup>-1</sup>) and (E3) received dimethoate (6 mg/kg.bw) + ZMLM (300 mg/kg.bw<sup>-1</sup>). In both the cases a normal control and dimethoate control were kept to compare the results. After 90 days, blood was collected and rats were sacrificed to collect the liver tissue for biochemical assays and histological estimations.

**Results:** The results of E1 did not show much change from the normal control group but was significantly different from the dimethoate control group ( $P \leq 0.05$ ). The preventive effect which was tested in E2 and E3 proved that the extract could almost retain the normal condition in 90 days time. Histological observations also agreed with the results obtained in biochemical parameters.

**Conclusions:** *Ziziphus mucronata* methanol leaf extract possesses a preventive effect against dimethoate induced oxidative stress as observed in male albino Sprague Dawley rats.

**Keywords:** Leaf methanol extract, hepatoprotective, *Ziziphus mucronata*, oxidative stress, phenol content

## INTRODUCTION

Pesticides are very effective in pest control and are sometimes used indiscriminately in large amounts that lead to environmental pollution. Residues of organochlorides and organophosphates have been detected in the soil, water, reservoirs, vegetables, grains and other food products.<sup>1</sup> Organophosphates have been found to inhibit acetylcholinesterase (AChE) and subsequent activation of cholinergic receptors.<sup>2</sup> Oxidative stress caused by lipid peroxidation induced by dimethoate has also been found to be one of the molecular mechanisms involved in organophosphate toxicity.<sup>3,4</sup>

Biotransformation of dimethoate in the cytochrome P-450 results in the production of an oxygen analogue (omethoate). Omethoate is ten times more toxic compared to dimethoate.<sup>5</sup> Oxidative stress is associated with many ailments like atherosclerosis, arthritis and liver diseases.<sup>4</sup> In Africa medicinal plants are traditionally and carefully selected alternatives to curb against various ailments. Among the promising medicinal plants *Ziziphus mucronata* (ZM) in the family Rhamnaceae is a very common tree in Botswana with a rich historical and religious background.

The prominent usage of the plant has been observed among the bushman of the Okavango river bank in Botswana, Kavango and Zulu tribes in southern Africa (Koeven, 2001).<sup>6</sup> The roots bark, and leaves of this plant are used in the treatment of a wide range of ailments, these include arthritis, chest pains, arthritis, boils, liver diseases and gastrointestinal problems.<sup>6</sup> Since dimethoate has also been reported to induce oxidative stress and this tree in Botswana has already been in use to treat the diseases caused by oxidative stress. Therefore, we are proposing that this tree might be useful in preventing and protecting the population from getting affected by dimethoate toxicity.

Dimethoate is locally sold in shops) throughout Botswana and easily available for household usage and for the local farmers. The study was aimed to establish the antioxidant and hepato-protective properties of the methanol extract of the leaf in albino rats.

## MATERIALS AND METHODS

### Chemicals

Dimethoate emulsifiable concentrate was purchased from Agrivet PTY (LTD) Botswana and other chemicals were of analytical grade and were bought from Sigma-Aldrich (St. Louis, USA). The kits were purchased from Aggape diagnostics, Agappe Hills, Dist. Enakulam, and Kerala-683 562 India.

### Experimental Animals

Male Sprague Dawley albino rats aged 20-21 weeks were kept in galvanized cages under controlled conditions of temperature at 25°C 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 animals were performed in accordance with University of Botswana and affiliated institutions animal care committee (Approval no: UB/IRB/1084). The animals were handled carefully to minimize the suffering.

### Plant collection and preparation of the plant extract

The leaves of *Ziziphus mucronata* (Voucher no: 0010) were collected around Gaborone. The plant authentication was performed by Mr. M. Muzila of the University of Botswana Herbarium and the Herbalist Mr J Moshageng also helped in plant identification. They were washed with distilled water and sun dried. The dried plant leaves were crushed with a blender or laboratory grinder to obtain 500g of powder. The powder was soaked and extracted in 70% methanol/water for 24 hours. Using a rotary evaporator the methanol and water were evaporated resulting in a 25% extract yield.

### Experimental design

Twenty five adult male rats (aged 21 weeks) were divided into five groups of five 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<sup>-1</sup> dimethoate dissolved in distilled water
- Group 3 - Experimental Group (E1) received dimethoate (6 mg/kg.bw) + ZMLM (100 mg/kg.bw<sup>-1</sup>)
- Group 4 - Experimental Group (E2) received dimethoate (6 mg/kg.bw) + ZMLM (200 mg/kg.bw<sup>-1</sup>)
- Group 5 - Experimental Group (E3) received dimethoate (6 mg/kg.bw) + ZMLM (300 mg/kg.bw<sup>-1</sup>)

Group 6 - Experimental Group (EO) received only ZMLM (300 mg/kg.bw<sup>-1</sup>)

The experiment was run for 90 days and all the administration was done orally with the help of a rubber tube and syringe. At the end of the experiment, rats were killed after mild ether anaesthesia. Blood was collected in heparinised tubes and centrifuged at the rate of 6000 rotations per minute. Plasma samples were drawn after that and stored at -70°C for further analysis.

### Determination of total phenol content

The total phenol content (TPC) was determined using Folin-Ciocalteu reagent. One milliliter of extract or standard solutions (Gallic acid) (0-500 mg/l) was added to a mixture of 10 ml deionising water and 1.0 ml of Folin - Ciocalteu phenol reagent. After 5 minutes, 2.0 ml of 20% sodium carbonate was added to the mixture. After 1 hour of incubation at room temperature in darkness the absorbance was measured at 750 nm. The TPC was calculated from the linear regression equation of the standard curve, from this equation, the concentration of gallic was determined for each extract and converted to mg of gallic acid equivalents/g of dry extract (mg GAE/g).<sup>7</sup>

### Biochemical measurements

#### Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation in plasma was estimated in terms of thiobarbituric acid reactive substance as described by Sushmakumari et al, (1989).<sup>8</sup> with little modification. Original method describes the measurement of TBARS in tissue and it was adopted for plasma. Plasma or liver homogenate (0.1ml) was treated with 2 ml of TCA-TBA-HCL (TBA 0.37%, 0.25N HCl and 15% TCA) reagent (1:1:1) and incubated in boiling water bath for 10 minutes, the mixture was cooled and mixed with 2ml of freshly prepared 1N NaOH and absorbance measured at 535 nm

#### Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate oxaloacetate pyruvate transaminase (SGPT)

Diagnostic kits for SGOT and SGPT were bought from Aggape Diagnostics, India and manufacture's guidelines were strictly followed. The working reagent 1000 µL and 100µL were mixed and incubated at 37°C for 1 minute at 37 °c. The change in absorbance per 20 sec (OD/20 sec.) during 1 min

$$\text{SGOT activity (U/L)} = (\text{OD/min.}) * 1745 \quad \Delta$$

#### Reduced glutathione (GSH)

Reduced glutathione was measured by the method described by Ellman et al, (1959).<sup>9</sup> Plasma was precipitated with metaphosphoric acid 0.25 ml of

plasma was mixed with 0.5ml of precipitating buffer (1.67g Metaphosphoric acid, 0.2g EDTA, 30g sodium chloride dissolved in 100 ml of double distilled water) and centrifuged. Supernatant was collected and mixed with 2.5ml of 0.3M phosphate buffer (pH 7.2). Colour was developed by adding 100µl of 0.01% 5, 5-dithiobis 2-nitrobenzoic acid (DTNB) and read at 412 nm.

#### **Super oxide dismutase**

Super oxide dismutase was assayed by the method described by Tripathi et al, (2001).<sup>10</sup> Plasma was treated with a mixture of chloroform and ethanol. The reaction mixture consisted of 150µl EDTA (0.5 mmol), 600µl L-methionine (130 mmol) and 300µl NBT (750 umol). The volume of reaction mixture was made up 2.8µl with SOD buffer. Then 200µl of plasma was added except in the control. Finally 200µl of riboflavin was added to start the reaction. All the test tubes were placed under fluorescent lamp except the blank. Absorbance was recorded at 540nm after for 4 minutes. One unit of enzyme activity was calculated as the activity that was required to inhibit the reduction of NBT by 50%.

#### **Tocopherol (vitamin-e)**

Tocopherol was estimated using the method of Martinek (1964).<sup>11</sup> The sample (0.5 ml), distilled water as blank (0.5ml) and standard solution (0.5ml) were taken in three centrifuge tubes. To all three tubes 0.5ml the xylene was added. The tubes were stoppered, mixed and centrifuged. The xylene layer (containing the precipitated tocopherol) was then carefully pipetted into clean tubes and mixed with 0.35 ml  $\alpha$ - $\alpha$ ' Dipyrindyl reagent. The extinction of the test and standard against blank were read at 460nm.

In turn, beginning with the blank tube, 0.33ml of ferric chloride solution was added to all the tubes. Optical density of the test and the standard against blank were read at 520nm after 1.5minutes. Amount of tocopherol present in mg/100 ml was calculated using the formula: Mg/100 ml = (Reading unknown at 520nm- Reading unknown at 460nm)\* 0.29/ Reading of standard at 520nm.

#### **Choline asterase activity**

Diagnostic kit for cholinesterase activity was bought from Aggape Diagnostics, India and manufacture's guidelines were strictly followed. A working reagent of 1000µL and 50 µL were mixed and incubated for 2 minutes at 37°C. The absorbance was read after 1, 2 and 3 minutes. The difference in Optical density divided by 60 seconds during the three minutes was determined.

Cholinesterase (U/L) = (OD/60 Sec) \* 22653  $\Delta$

#### **Lipid Profile**

High density Lipoproteins (HDL), Low-density lipoproteins (LDL), Cholesterol (CHOL), Total proteins (TP), and Triglycerides (Trig) were estimated according to Aggape diagnostic kit and the manufacturer's guidelines were strictly followed.

#### **Histological studies**

The rat liver tissue intended for histological studies was removed and immediately fixed in 10% phosphate buffered formalin for at least 24 hours. The tissue was dehydrated in ethanol series (70%, 80%, 100%, and 100%), cleared in xylene and embedded in paraffin. 5 µm thick sections were taken using a rotary microtome and sections were affixed on clean glass slides by gentle heating. The slides were deparaffinised and stained with haematoxylin and eosin and they were mounted with a cover slip using DPX. The slides were observed under a light microscope for any histological changes.<sup>2,13</sup>

#### **Statistical Analysis**

Data were analyzed using Sigma-Stat 3.1. Statistical analyses were performed using one way analysis of variance (ANOVA) followed by Tukey's test for comparison between groups.

## **RESULTS**

#### **Total phenol content of different types of leaf extracts of *Z. mucronata* (ZMLM)**

High phenol content was estimated in the ZMLM. The highest estimated phenol content was 271 mg GAE/g in 100% methanol extract, followed by 260, 120, 60, 45 and 20 mgGAE/g in 70% methanol/water, 70% methanol/chloroform, 100% chloroform, 50% hexane/chloroform and 100% hexane respectively. (Table 1).

**Table 1** Total phenol content of the ZMLM.

| Leaf extract             | TPC (mgGAE/g) |
|--------------------------|---------------|
| 70% methanol             | 260 ± 3.6     |
| 100% methanol            | 271 ± 2.4     |
| 50% methanol/ chloroform | 120 ± 1.6     |
| Chloroform               | 60 ± 0.4      |
| 50% Hexane chloroform    | 45 ± 1.8      |
| Hexane                   | 20 ± 0.2      |

\* Values are the average of three trials ± standard deviation

#### **Effects on plasma TBARS and liver toxicity marker parameters**

Daily administration of dimethoate resulted in elevated levels of TBARS in plasma of the DC (dimethoate control) group.

A dose dependant reduction in the levels of plasma TBARS was associated with the ZMLM administration.

The serum marker enzymes serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) had increased significantly

( $p < 0.05$ ) in rats of group DC when compared with the levels in NC rats.

A significant decline approaching normal activities were observed in the activities of SGOT ( $p < 0.001$ ), SGPT ( $p < 0.001$ ), and ALP ( $p < 0.001$ ) as a result of extract administration. The decrease on the plasma marker parameters was dependant on the dose of the extract, the higher the dose of the extract the more the decrease that occurred. No evident toxicity was observed on feeding ZMLM extract alone, there was no significant difference on the level of TBARS when compared with NC (Table 2).

**Table 2** Effects of ZMLM on plasma marker parameters of dimethoate treated rat.

| Groups | TBARS(nmol/ml)              | SGOT(U/L)                             | SGPT(U/L)               | ALP(U/L)              |
|--------|-----------------------------|---------------------------------------|-------------------------|-----------------------|
| NC     | 0.580 ± 0.013               | 42 ± 5.5                              | 46 ± 2                  | 69 ± 2.1              |
| DC     | 3.868 ± 0.002*              | 339 ± 8.2**                           | 360 ± 5**               | 667 ± 3.0*            |
| E1     | 2.172 ± 0.006*              | 179 ± 9.1**/aa                        | 168 ± 2**/aa            | 228 ± 4**/aa          |
| E2     | 1.200 ± 0.002* <sup>a</sup> | 63 ± 2.7 <sup>aa</sup> / <sup>*</sup> | 59 ± 1**                | 90 ± 3**              |
| E3     | 0.982 ± 0.003* <sup>a</sup> | 54 ± 1.2 <sup>aa</sup>                | 41 ± 6**                | 70 ± 1**              |
| EO     | 0.576 ± 0.004 <sup>a</sup>  | 43 ± 2.1 <sup>aa</sup>                | 44 ± 3.8/ <sup>aa</sup> | 70 ± 3.5 <sup>a</sup> |

Values are expressed as mean ± SEM n=5 for each treatment ( $p < 0.05$ ,  $p < 0.001$ ) compared with normal control and dimethoate control ( $p < 0.05$ ,  $p < 0.001$ ) respectively. E1 (100 mg/kg.bw extract + DM), E2 (200 mg/kg.bw + DM), E3 (300 mg/kg.bw extract + DM), EO (300 mg/kg.bw extract only).

#### Effects on plasma enzymatic antioxidants (SOD, catalase activities)

Effects of ZMLM on plasma SOD and catalase are presented in (Table 3). The result showed that the activities of SOD ( $p < 0.001$ ) and catalase ( $p < 0.05$ ) enhanced significantly in DC rats as compared to the normal control (NC). Significant decrease in the activities of SOD ( $p < 0.001$ ) and catalase ( $p < 0.001$ ) were noticed in experimental groups in a dose dependent manner. The highest and significant reduction ( $p < 0.05$  for SOD and  $p < 0.001$  for catalase) was noticed in Group E3 when compared to DC. In this group, the level of activities is almost parallel to group NC and no significant difference was noticed after its comparison with NC. Group E1 showed similar results like DC and it differed significantly with NC.

**Table 3** Effects of ZMLM on plasma enzymatic antioxidants (SOD, catalase) in dimethoate treated rats.

| Groups | SOD (U/mg)                | Catalase (Umg <sup>-1</sup> ) |
|--------|---------------------------|-------------------------------|
| NC     | 6.90 ± 0.13               | 503.6 ± 2.01                  |
| DC     | 12.39 ± 0.21**            | 830.6 ± 3.71**                |
| E1     | 11.12 ± 0.04**            | 780.2 ± 1.31**                |
| E2     | 7.12 ± 0.06 <sup>aa</sup> | 657.9 ± 0.27 <sup>aa</sup>    |
| E3     | 6.12 ± 0.06 <sup>aa</sup> | 607.2 ± 0.93 <sup>aa</sup>    |

\*Values are expressed as mean ± SEM n=5 for each treatment ( $p < 0.005$ ,  $p < 0.001$ ) compared with normal control and dimethoate control ( $p < 0.005$ ,  $p < 0.001$ ).

#### Effects on Plasma Non Enzymatic Antioxidants-Reduced Glutathione, Vitamin C and Vitamin E

Effects of ZMLM on plasma non enzymatic antioxidants against dimethoate administration are presented in (Table 4).

**Table 4** Effects of ZMLM on plasma non enzymatic anti-oxidants (GSH, Vit C and Vit E) in dimethoate treated rats

| Groups | Vitamin C(mg/ml)            | Vitamin E(mg/ml)          | GSH (mg/ml)                 |
|--------|-----------------------------|---------------------------|-----------------------------|
| NC     | 6.13 ± 0.99                 | 6.28 ± 0.06               | 60.0 ± 1.8                  |
| DC     | 0.99 ± 0.00**               | 2.06 ± 0.00**             | 31.0 ± 2.1**                |
| E1     | 1.50 ± 0.09**               | 2.09 ± 0.11*              | 34.0 ± 1.2**                |
| E2     | 2.67 ± 0.07**/ <sup>a</sup> | 3.31 ± 0.03*              | 37.0 ± 1.3**                |
| E3     | 3.50 ± 0.04**/ <sup>a</sup> | 4.33 ± 0.00* <sup>a</sup> | 49.0 ± 0.23**/ <sup>a</sup> |

\*Values are expressed as mean ± SEM n=5 for each treatment ( $p < 0.05$ ,  $p < 0.001$ ) compared with normal control and dimethoate control ( $p < 0.05$ ,  $p < 0.001$ ) respectively

The results showed that plasma levels of GSH, Vitamin C and Vitamin E reduced significantly ( $p < 0.001$ ) in DC rats as compared to the levels in NC. When compared with NC, their levels were also reduced significantly ( $p < 0.001$ ) in experimental rats (E1-E3) in a dose dependant manner. Although levels of these anti-oxidants were low in experimental groups and they also differ significantly with levels in DC ( $p < 0.05$ ), ZMLM administration along with dimethoate had helped these animals to ameliorate the levels of these non enzymatic anti-oxidants.

#### **Effects on Plasma Lipid Profiles and Total Protein**

Results of ZMLM administration along with dimethoate on plasma lipid profile are presented in (Table 5). It shows that dimethoate administration had significantly lowered HDL levels ( $p < 0.001$ ) in DC groups. ZMLM administration had almost retained normal HDL levels in all experimental groups and they differ significantly ( $p < 0.001$ ) from DC. On the other hand dimethoate had significantly increased the levels of low density lipoprotein (LDL), cholesterol, total

protein and triglyceride ( $p < 0.001$ ) as compared to the levels in NC group.

The LDL, Cholesterol, Total Protein and Triglyceride levels were found to decline significantly ( $p < 0.05$ ) in E3 as compared to DC and there is no significant difference was noted from NC. In E2, although a decline was noticed but still there existed a significant difference from NC. Similar response was noted for E1 but the changes were less significant than E2.

#### **Effects on Acetyl-Cholinesterase Activity**

The cholinesterase activity declined significantly ( $p < 0.05$ ) in DC when compared to NC. There was a significant ( $p < 0.05$ ) increase in cholinesterase activity in all the experimental groups. In E1 although ZMLM administration increased the activities of cholinesterase a significant difference was noted from NC and not from DC. In E2 and E3, preventive effect was more pronounced with a significant difference from NC and DC. Difference from DC indicated an ability of the extract to retain normal Cholinesterase activity at higher dosages (Table 6).

**Table 5** Effects of ZMLM on plasma lipid and lipoprotein profiles in dimethoate treated rats.

| Groups | HDL(mg/dL)                 | LDL(mg/dL)     | CHOL(mg/dL)                  | TP(gm/dL)                | TG(gm/dL)                 |
|--------|----------------------------|----------------|------------------------------|--------------------------|---------------------------|
| NC     | 56.09 ± 1.32               | 15.00 ± 2.23   | 10.20 ± 1.25                 | 8.00 ± 1.03              | 61.00 ± 4.21              |
| DC     | 12.80 ± 1.14**             | 150.87 ± 3.1** | 124.40 ± 9.10**              | 2.00 ± 0.04**            | 230.00 ± 9.31**           |
| E1     | 42.31 ± 1.30 <sup>aa</sup> | 78.87 ± 5.83*  | 98.19 ± 9.34*                | 3.40 ± 0.01*             | 100.76 ± 8.27*            |
| E2     | 55.00 ± 2.44 <sup>aa</sup> | 40.23 ± 4.76*  | 37.16 ± 2.91 <sup>*/aa</sup> | 6.80 ± 0.11 <sup>a</sup> | 70.29 ± 5.12 <sup>a</sup> |
| E3     | 61.00 ± 3.14 <sup>aa</sup> | 20.55 ± 5.19*  | 17.76 ± 3.17 <sup>a</sup>    | 7.1 ± 0.33*              | 58.65 ± 7.68 <sup>a</sup> |

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

**Table 6** Effects of ZMLM on cholinesterase activity In dimethoated treated rats

| Experimental groups | Cholinesterase activity(Umg <sup>-1</sup> ) |
|---------------------|---|
| NC                  | 24200 ± 16                                  |
| DC                  | 5647 ± 18*                                  |
| E1                  | 8213 ± 26*                                  |
| E2                  | 13280 ± 14 <sup>*/a</sup>                   |
| E3                  | 17420 ± 13 <sup>*/a</sup>                   |

Values are expressed as mean ± SEM n=5 for each treatment ( $p < 0.05$ ,  $p^{**} < 0.001$ ) compared with normal control and dimethoate control ( $p^a < 0.05$ ,  $p^{aa} < 0.001$ ) respectively.

#### **Effects of Dimethoate on General Behaviour and Health**

In this study, no death was observed in any groups of rat. Rats in NC and E1-E3 groups did not show any

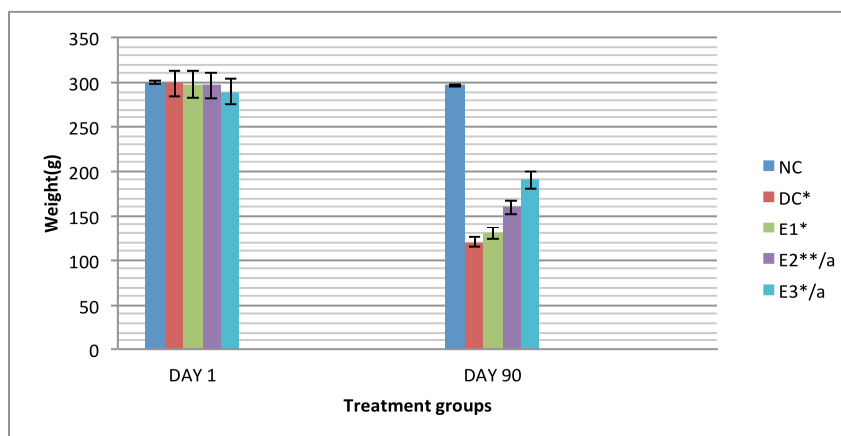
sign of toxicity. Rats in DC showed reduced weight and were generally weak.

#### **Effect on Weight**

The weight was significantly ( $p < 0.05$ ) decreased in dimethoate-administered rats when compared to the normal control. A significant ( $p < 0.005$ ) increase in weight was observed in ZMLM extract administered rats when compared to dimethoate fed rats (Figure 1).

#### **Effects on Histology**

Table 7 summarises the histological findings of the liver. It revealed a preventive effect on the hepatocytes when compared to dimethoate control (NC). Dimethoate control group showed evident hepatic damage as shown by widening sinusoids, loss of hepatocellular membrane when compared to normal control.



**Figure 1** Weight changes on the rats fed dimethoate and graded doses of the whole leaf extract.

NC-normal control, DC-dimethoate control, E1-100 mg/kg.bw, E2-200, E3-300 mg/kg.bw.

\*Values are expressed as mean  $\pm$ SEM n=5 for each treatment ( $p < 0.05$ ,  $p^{**} < 0.001$ ) compared with normal control and dimethoate control ( $p_a < 0.05$ ,  $p_{aa} < 0.001$ ) respectively

The preventive effect was more evident with an increasing dosage of the ZMLM extract. The preventive effect was evident from reduced necrosis in ZMLM fed rats when compared to dimethoate fed rats, with a lot of necrosis and infiltration.

**Table 7** Effects of ZMLM on histology of liver in dimethoate treated rats (Findings of light micrographs stained with eosin and haematoxylin at 400X)

| GROUP | Histological Findings  |
|-------|--|
| NC    | Normal and preserved hepatic cells with normal sinusoids   |
| DC    | Lots of fatty changes, widened sinusoids and reduced number of hepatocytes, necrosis, and tissue degeneration of the lymphocytes |
| E1    | Most of the hepatic cells were well preserved with no marked necrosis  |
| E2    | The hepatic cells were well preserved with no massive necrosis, when compared to normal control                                  |

## DISCUSSION

Dimethoate contributes a significant risk to human as well as animal population which are exposed to it by any means. This study aimed to establish the protective role of *Ziziphus mucronata* leaf extract, which is a very common health drink in Botswana, in protecting liver against dimethoate toxicity. 6mg/kg.bw dosage was chosen in this study because it was found to induce toxicity by changing the antioxidant status in rats.<sup>3</sup>

Medicinal plants are sources of diverse nutrients, many of which display antioxidant properties which are capable of protecting the body against both cellular oxidation reactions and pathogens.<sup>14</sup> Dimethoate has

been found to be metabolically activated by cytochrome P-450 mediated oxidative desulphurization, where it is converted into its oxygen analogue (omethoate).<sup>15</sup> Omethoate has been indicated to be ten times toxic compared to dimethoate, it is this dimethoate oxygen analogue that has been associated to dimethoate induced oxidative stress.

Dimethoate has been found to be carcinogenic in *in vitro* studies but no results were obtained from *in vivo* studies.<sup>16</sup> Dimethoate mediated oxidative stress is responsible for the inhibition of cholinesterase activity.<sup>17</sup> According to Shaheen et al, (2006),<sup>2</sup> dimethoate sub chronic exposure has lead to a change in the total antioxidant status in the liver, brain and kidney of rats. After dimethoate exposure there was an increase in lipid peroxidation, catalase, and glutathione reductase and superoxide dismutase activities.<sup>1</sup>

Cholinesterase activity is a common biomarker to test Organophosphate (OP) toxicity. In this study a decrease in the activity of this enzyme was noticed in DC group which is in accordance with the previous reports.<sup>3</sup> Dimethoate inhibits the activity of the enzyme cholinesterase that hydrolyse acetylcholine into acetyl and choline and hence the returning of cholinergic neurones to normal is prevented. Amelioration of enzyme activity was observed in E2 and E3 which were administered ZMLM along with dimethoate (Table 6)

Dimethoate intoxication has been demonstrated to induce hepatic lipid peroxidation in rats.<sup>18</sup> In the present study, administration of dimethoate for 90 days had raised the levels of TBARS in DC group hence confirming the previous reports on dimethoate toxicity.

Administration of ZMLM along with dimethoate had suppressed the lipid peroxidation significantly in E3. Results of DC and E1 do not differ significantly in terms of their plasma TBARS levels. This demonstrates that ZMLM fails to reduce the rate of lipid peroxidation induced by dimethoate when administered at low doses. (Table 2)

High rate of lipid peroxidation in hepatic tissues causes severe damage to liver cells and leakage of transaminases like SGOT, SGPT and ALP into the circulation. SGOT and SGPT are transaminases localized in hepatic cells and they leak into the circulation after hepatic cell damage and thus show high levels in the blood.<sup>19</sup> In the present study high levels of plasma SGOT, SGPT and ALP in DC indicates liver cell damage. Liver is central place for protein synthesis and damage to liver reduces the rate of protein synthesis and hence the levels of protein in the circulation.

Low levels of total protein in DC could be due to liver damage by oxidative stress. Results of E2 and E3 demonstrate low levels of SGOT, SGPT and ALP and high levels of total proteins. This translates to the protection rendered by ZMLM at the dose of 200 and 300 mg/kg body weight in E2 and E3 respectively. Thus administration of ZMLM had significantly inhibited the oxidative stress generated by dimethoate metabolism and rendered protection in E2 and E3.

Administration of only 300 mg/kg.bw ZMLM extract resulted in no signs of toxicity. This was evident from the level of TBARS that had no significant difference with NC. SGOT, SGPT and ALP activities also showed no significant differences when compared to NC (Table 2). The results are consistent with previous findings by Taylor et al, (2003),<sup>20</sup> reporting no toxicity on *Ziziphus mucronata* leaf, root and twig.

Anti-oxidants are the defense system in the body to fight the toxicity associated with free radicals. Super oxide dismutase and catalase provide the first line of enzymatic anti-oxidant defense system against free radicals. The increased activity of superoxide dismutase and catalase reflects an activation of the compensatory mechanism through the effect of pesticide on progenitor cells, and its extent depends on the magnitude of oxidative stress and hence, on the dose of stressor. In the current study, there was an increase in the activities of catalase and SOD in DC group.

The elevated SOD activity is due to an adaptive response to the generated free radicals.<sup>21</sup> Under normal physiological conditions a delicate balance exists

between the rate of formation of H<sub>2</sub>O<sub>2</sub> via dismutation of O<sub>2</sub> by SOD activity and the rate of H<sub>2</sub>O<sub>2</sub> removal by Catalase.<sup>1</sup> The SOD activity was observed to rise significantly in DC compared to NC. The increase is because of an adaptive response due to dimethoate generated free radicals. A decrease in the SOD and catalase activities was observed in E2 and E3 where dimethoate was administered along with ZMLM. It appears that ZMLM takes over the work of scavenging free radicals and hence the normal levels of both enzymatic anti-oxidants were maintained. This is confirmed by E2 and E3 which show a significant difference with DC and with no significant differences with NC (Table 3).

Glutathione is important non enzymatic anti-oxidant in neutralizing the reactive intermediates and radical species generated during oxidative stress.<sup>22</sup> The depletion of GSH may be a result of it being used up in quenching Omethoate (an oxygen analogue generated from dimethoate biotransformation) radical. An oral feeding of ZMLM along with dimethoate seem to scavenge or interfere with free radical formation, thus it may have lead to normal production and hence the restoration of GSH levels (Table 4).

Vitamin E is most important lipid soluble non enzymatic anti-oxidant which inhibits lipid peroxidation reaction and regenerates glutathione and vitamin C. It thus protects all the membranous structures. In our study, the decreased level of Vitamin C in plasma of DC could be explained by depletion of GSH because glutathione recycles this vitamin for further usage. ZMLM might have possibly played a role in scavenging the ROS, thus allowing the recovery of Vitamin E which was possibly used up in scavenging omethoate radical (Table 4).

Apart from oxidative stress generated in DC, there was also a significant reduction in the level of plasma HDL along with increment in the levels of triglyceride, LDL and total cholesterol was noted in DC group. Free radicals enhance the oxidation of light density lipoprotein (LDL) and oxidized LDL affects many biological processes involved in atherogenesis. ZMLM has been successful in elevating the levels of HDL and Vitamin E (Table 5). High HDL levels competes with LDL receptors site on the smooth muscle cells of arterial wall and inhibits the uptake of LDL and thus prevents the organism from the risk of atherogenesis.

Vitamin E also inhibits the peroxidative damage and atherogenesis by protecting endothelial cells and inhibiting the oxidation of lipoproteins. High levels of Triglyceride can accumulate in cardiac tissue because of its high uptake by cardiac muscle cells and lead to the

development of cardiomyopathy. Administration of ZMLM along with dimethoate successfully prevents these effects by lowering the levels of triglyceride in E2 and E3 rats. Extract administration significantly prevented weight loss in E2 and E3 when they are compared to DM (Table 5).

To support the biochemical findings, histological studies of liver tissue were also performed. It revealed that lipid peroxidation induced by dimethoate has caused a significant change in liver histological features like widened sinusoids and reduced number of hepatocytes, necrosis and tissue degeneration. Supplementation of dimethoate administration with ZMLM in experimental groups has preserved the normal structure of liver with no massive necrosis. The structure of E2 and E3 were almost similar to the normal structure of liver in NC (Table 7).

The results of this study clearly show that ZMLM renders protection against oxidative stress induced by dimethoate metabolism. There could be many reasons behind this, one being the richness of different leaf extracts in phenol content (Table 1). Phenols are well known to inhibit lipid peroxidation and reduce oxidative stress. Therefore there is a possibility that the protective effects of ZMLM could be because of its phenol content.

## CONCLUSION

*Ziziphus mucronata* methanol leaf extract possess a preventive effect against dimethoate induced oxidative stress in male albino Sprague dawley rats.

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## REFERENCE

1. Attia A.M. and Nasr H.M. Dimethoate induced changes in biochemical parameters of experimental rat serum and its neutralization by black seed (*Nigella Sativa* L.) oil *Slovak J Anim. Sci.*, 2009 2: 87-94.
2. Shaheen F.A.T., Benomran F.A., Hassan A.L. and Daldrup T. (2006). Distribution of dimethoate in the body after a fatal organophosphate intoxication. *Forensic Sci. Int.*, 2006; 170:129-132
3. Sharma Y., Bashir S., Irshad M.M., Nag T.C. and Dogra T.D. Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. *J. Toxicol.*, 2005; 215: 173-181.
4. Halliwell B. and Gutteridge J.M.C. Free radicals in Biology and Medicine 4<sup>th</sup> ed. *Oxford University press Inc, New York: USA; 2007*
5. WHO. Pesticide residues in food- joint FAO/WHO meeting on pesticide residues, *Federal Institute for first Assessment, Berlin, Germany IPCS-INCHEM; 2003*
6. Koeven E. Medicinal and poisonous and edible plants in Namibia. Windhoek: *Namibia: Klaus Hess Publishers/verlag; 2001*
7. Stoilova I., Krastanov A., Stoyanova A., Denev P. and Gargova S. Antioxidant activity of a ginger extracts (*Zingiber Offinale*). *Food Chem.*, 2007; 102: 764-770.
8. Sushmakumari S., Jaydeep A., Kumar J.S. and Manon V.P. Effect of carnitine on malonyldialdehyde, taurine and glutathione levels in heart of rats subjected to myocardial stress by isoproterenol. *Indian J Exp Biol.*, 1989; 27: 134-137.
9. Ellman G.C. Tissue Sulphyryl group. *Arch. Biochem. Biophys.*, 1959; 82: 70-77.
10. Tripathi Y.B., Upadhyay A.K. and Chaturvedi P. Antioxidant properties of Smilax china. *Ind J Exp Biol.*, 2001; 39: 1176-1179.
11. Martinek R.G. Method for the determination of vitamin E (total tocopherols) in serum. *Clin Chem.*, 1964; 10:1078-1086
12. Banks W.J. Applied Veterinary Histology. Missouri: U.S.A; Mosby-Year book Inc; 1993
13. Collins T. Acute and chronic inflammation In: Textbook of Robins pathologic basis of diseases Cotran, R.S., Kumar V., Collins T. (Eds), 6<sup>th</sup> edition, Philadelphia: USA: W.B Saunders company; 1999
14. Souri E.G., Amin A.A., Sharifodi A. and Farsam H. (2004). Antioxidative activity of sixty plants from Iran, *Ir. J. Phamac Res.*, 2004; 3: 55-59.
15. Wiaderkiewicz R., Zasadouski A., Czadowski A. P., Wiaderkiwicz A. and Czajkowska B. Effect of parantel and dimethoate administration on rat liver cytochrome P450 system. *Bull vet Ist Pulawy.*, 2005; 50, 253-257.
16. WHO/IPS. Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. *Environmental health Criteria*. Geneva; 180: 110-112; 1996
17. Bai Y., Zhou L. and Wang J. Organophosphorus pesticides in market foods in Shaanxai area, China. *Food Chem.*, 2006; 98: 240-242
18. Sayim F. Dimethoate- induced biochemical and histopathological changes in the liver of rats. *Exp toxicol pathol.*, 2007; 59: 1-10.
19. Himmerich H., Anghelescu I., Klawe C. and Szegeedi A. Vitamin B12 and hepatic enzyme serum levels correlate in male alcohol dependent patients. *Alcohol Alcohol.*, 2001; 36: 26-28.



20. Taylor J.L.S., Elgorashi E., Maes A., Gorp U.V., Kimpe N.D., Staden J. V. and Verschaeve L. Investigating the safety of plants used in South African Traditional Medicine: Testing for Genotoxicity in the Micronucleus And Alkaline Comet Assays, *Environ and Mol Mutagen.*, 2003; 42: 144-154
21. Koner B.C., Banerjee B.D. and Ray A. Organochlorine pesticide-induced oxidative stress and immune suppression in rats. *Indian J Exp Biol.*, 1998; 36: 395-298.
22. Kim S.H., Yang Y.P., Sung S.H., Kim C.J., Kim J.W. and Kim Y.C. Hepatoprotective dibenzylbutyrolactone ligands of *Torreya nucifera* against CCl<sub>4</sub>- induced toxicity in primary cultured rat hepatocytes. *Biochem. Pharm.*, 2003; 40: 1865-1876 ☪