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# EVALUATION OF AMELIORATIVE AND ANTIOXIDANT ACTIVITY OF THE CRUDE METHANOL LEAF EXTRACT OF *BALANITES AEGYPTIACA* IN WISTAR RATS EXPOSED TO CHLORPYRIFOS.

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## ABSTRACT

*Antioxidant Activity of Balanites aegyptiaca in Wistar Rats Exposed to chlorpyrifos was investigated in this study. About 1000 g of the powdered leaves of Balanites aegyptiaca was used for the extraction and methanol was the extraction medium while cold maceration was emplyed. Thirty wistar rats were randomly divided into six groups of five rats each. Rats in group I were neither treated nor exposed to chlorpyrifos. Rats in groups II to VI were exposed to chlorpyrifos at 2 mg/kg daily for seven days. Rats in groups; II, III and IV were treated with the extract at doses; 100, 300 and 500 mg/kg respectively. Rats in group V were treated with ascorbic acid (150 mg/kg). Rats in group VI were administered normal saline. All treatments were through oral route and were for 7 consecutive days. Seven days after, all rats were humanely euthanized by severing their jugular vein, and 5 ml of blood sample was collected from each rat and transferred into plain container devoid of anticoagulant for serum chemistry and antioxidant assay. There was significant (P<0.05) increase in the mean values of liver enzymes (ALT, AST and ALP) in rats exposed to chlorpyrifos and treated with normal saline when compared to all groups. Similarly, a significant (P<0.05) reduction int the mean values of liver enzymes was recorded in rats treated with ascorbic acid when compared to rats treated with 500 mg/kg of the crude methanol leaves extract of Balanites aegyptiaca. A significant (P < 0.05) increase in the serum level of specific antioxidants enzymes (SOD, CAT and GPx) were recorded in rats treated with Balanites aegyptiaca and ascorbic acid when compared to group VI. A significant (P<0.05) increase in the mean values of antioxidants enzymes were recorded in group III, IV and V when compared to group II. In this study, methanol leaves extract of Balanites aegyptiaca significant ameliorative effect.*

**Key Words:** Antioxidants, *Balanites aegyptiaca*, Chlorpyrifos, Liver enzyme, Methanol extract, Wistar rats.

## INTRODUCTION

Plants still remains the primary source of supply of many important drugs and minerals in the orthodox medicine today. Plants are being exploited for their active compound which is responsible for their biological function (Clark, 1996). *Balanites aegyptiaca* is commonly known as the Desert date, 'Aduwa' in Hausa, 'enyi-ndi- mmuo' in Igbo, and 'Tanni' in Fulani (Ugwah *et al.*, 2016). It's a desert plant that is widely distributed in the arid zone of Nigeria. It is also found in other African countries like Senegal and Sudan, as well as in India (Chothani and Vaghasiya, 2011; Pandey, 2012). In Nigeria and Asia, it was reported to be used in a variety of folk medicines (Ugwah *et al.*, 2016). Chlorpyrifos pesticide was reported in 1070s to have immunotoxicity in (Ercegovich, 1973) and Street and Sharma (1975). In addition, Street and Sharma (1975) observed thymic atrophy and reductions in splenic germinal centers in rabbits following exposure to 1.5 mg/kg/day of chlorpyrifos. A number of organophosphate pesticides including chlorpyrifos, malathion, methylparathion and trichlorphon have displayed immunotoxicity in laboratory studies (Casale *et al*. 1983) and noticed suppression of primary humoral immune responses. Because of toxicity associated with Chlorpyrifos and its effectiveness prompted us to embark into this study in order to determine how the toxicity can be ameliorated.

## MATERIALS AND METHODS

### *Location of the Research*

The research was conducted in the Department of Animal Health and Production Binyaminu Usman Polytechnic Hadejia, Jigawa State.

### *Plant Collection and Identification*

Fresh leaves of *Balanites egyptiaca* was collected around the main campus of Binyaminu Usman Polytechnic, Hadejia, Jigawa State, Nigeria. The plant was identified at the herbarium of the department of plant biology, Bayero University Kano, Nigeria with voucher number BUKHAN 359.

### *Extraction of the Plant Material*

About 1000 g of the powdered leaves of *Balanites aegyptiaca* was used for extraction and methanol was employed as the extraction medium and the technique deployed was cold maceration using a 1-litre capacity separating funnel at room temperature for 48 hours. The initial extraction was done with 1.5 litres of methanol and the process was repeated twice using 750 litres of methanol in each extraction. The extracts obtained during the 3 phases of extraction were pooled together and concentrated under reduced pressure at 55 ºc, and then stored in a vacuum dissector. Stock solution of the extract at concentration of 1 g/mL was prepared and stored at 4 ºC for pharmacological test.

### *Experimental Animals*

Thirty wistar rats of either sex weighing between 200 and 220 grams were used. They were purchased from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Bayero University Kano. The rats were housed in locally constructed cages at room temperature. Wood shavings were used as beddings in the cages and changed once every day. The rats were allowed free access to feed (commercially prepared) and water *ad-libitum*.

### *Experimental Treatments*

Thirty wistar rats were randomly divided into six groups of five rats each. Rats in groups two to six were exposed with chlorpyrifos (2 mg/kg) daily and then treated as follows:

Group I- The rats in this group were neither exposed nor treated, therefore served as neutral control group.

Group II- Rats in this group received 100 mg/kg of *Balanites aegyptiaca per os* for 7 days.

Group III Rats in this group received 300 mg/kg of *Balanites aegyptiaca per os* for 7 days.

Group IV- Rats in this group received 500 mg/kg of *Balanites aegyptiaca per os* for 7 days.

Group V- Rats in this group received 150 mg/kg of Ascorbic acid *per os* for 7 days.

Group VI- Rats in this group received 5 mL/kg of *normal saline per os* for 7 days.

Serum level of liver enzymes

### *Liver Enzymes*

Serum level of alanine amino transferase (ALT), aspartate amino trasferase (AST) and alkaline phosphatase (ALP) were determined using chemical auto-analyzer (Bayer® Clinical Chemistry Analyzer, Germany).

### *Antioxidant Enzymes*

**Determination of Serum Glutathione Peroxidase (GPx) Specific Activity**

Modified method of Paglia and Valentine (1967) was used to determine glutathione peroxidase (GPx) activity spectrophotometrically

### *Determination of serum superoxide dismutase (SOD) specific activity*

The specific activity of SOD will be measured using the method described by Misra and Fridovich (1972) as modified by Rabideau (2001).

***Determination of serum catalase (CAT) activity***

The specific activity of catalase in the serum collected from each bird will be determined using method described by Beers and Sizer (1952).

***Statistical Analysis***

The statistical analysis was carried out using SPSS 20 software package. The results obtained are presented as mean ± standard deviation (M±SD). A one-way analysis of variance (ANOVA) was used for the data analysis. Significant differences between groups were detected using Bonferroni test at ($p \leq 0.05$).

## RESULTS

**Table 1:** Mean ± SEM of liver enzymes in wistar rats exposed to Chlorpyrifos and treated with the crude methanolic leaf extract of *Balanites aegyptiaca*

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| Test | NC | BA (100 mg/kg) | BA (300mg/kg) | BA (500 mg/kg) | AA (150 mg/kg) | CPF (5 ml/kg |
| ALT(µ/L) | 19.33±1.256 | 38.79±3.177 | 28.33±2.551 | 28.33±2.642 | 27.95±1.408 | 38.67±3.136 |
| AST(µ/L) | 49.83±3.102 | 75.53±4.173 | 56.33±4.566 | 54.36±3.903 | 53.33±3.566 | 80.2±5.139 |
| ALP(µ/L) | 80.69±2.643 | 103.4±5.876 | 87.72±6.164 | 85±5.216 | 83.27±5.703 | 108.5±7.707 |

Keys: NC= Neutral Control. BA=*Balanites aegyptiaca.* AA= Ascorbic Acid. CPF=Chlorpyrifos.

Table 2: Mean ± SEM of serum biomarkers of oxidative stress in wistar rats exposed to Chlorpyrifos and treated with the crude methanolic leaf extract of *Balanites aegyptiaca*

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| Test | NC | BA (100 mg/kg) | BA (300mg/kg) | BA (500 mg/kg) | AA (150 mg/kg) | CPF (5 ml/kg |
| SOD(µg/ml) | 69.67±4.302 | 48.33±3.442[a] | 71.17±4.615 | 75.83±4.936 | 75.31±3.992 | 46.67±3.211 |
| CAT(µg/ml) | 72.67±3.474 | 46.5±3.085 | 73.50.5±6.29 | 75.33±2.362 | 77.17±3.790 | 48.33±3.981 |
| GPx(µg/ml) | 32.33±1.358 | 24.15±1.478 | 30±2.989 | 30.33±1.145 | 33.5±1.803 | 24.67±1.544 |

Keys: NC= Neutral Control. BA=*Balanites aegyptiaca.* AA= Ascorbic Acid. CPF=Chlorpyrifos.

## DISCUSSION

Liver enzymes (AST, ALT and ALP reduced significantly ($P<0.05$) in rats treated with crude methanolic leaf extract of *Balanites aegyptiaca* in dose dependent manner. There was significant ($P<0.05$) increase in the mean values of liver enzymes in rats exposed to chlorpyrifos and treated with normal saline when compared to the other groups. Similarly, a significant ($P<0.05$) reduction in the mean values of liver enzymes was recorded in rats treated with ascorbic acid when compared to rats treated with 500 mg/kg of the crude methanolic leaf extract of *Balanites aegyptiaca.* Awobode (2006), reported that liver enzymes were found in the serum and the level of their activity can be determined, and that depression or elevation of a particular enzyme indicate the presence of damage to specific tissue or organ. The elevated serum levels of AST and ALT may be attributed to hepatocellular damage as reported by Kobo, (2012), and

Enwezor and Sakey (2005). A significant ($P < 0.05$) increase in the serum level of specific antioxidants enzymes were recorded in rats treated with *Balanites aegyptiaca* and ascorbic acid when compared to rats exposed to chlorpyrifos and treated with normal saline, this could be due to the presence of flavonoid, saponin and alkaloid present in plants and the finding of this research agrees with the report of Mandal *et al* (2007) in rats subjected to chemically induced hepatocarcinogenesis where the authors reported the ability of flavonoid, saponin and alkaloid to stimulate the immune systems in an immunosuppressed animals. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are enzymatic antioxidants that mop-up free radicals.

## CONCLUSIONS

It was concluded that treatment with *Balanites aegyptiaca* in wistar rats exposed to chlorpyrifos for seven consecutive days have an ameliorative effect.

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