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EVALUATION OF THE AMELIORATIVE EFFECT OF THE CRUDE METHANOL LEAF EXTRACT OF *BALANITES AEGYPTIACA* ON SOME HAEMATOLOGICAL PARAMETERS IN WISTAR RATS EXPOSED TO CHLORPYRIFOS.

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ABSTRACT
Acute toxicity study and phytochemical screening on Balanites aegyptiaca as well as the effect of Balanites aegyptiaca on some haematological parameters in wistar rats were investigated. Following extraction, stock solution of the extract at concentration of 1 g/mL was prepared and stored at 4 ºC for pharmacological test. Phytochemical screening test and acute toxicity study were carried. 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. 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 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 EDTA container for haematology. The results of phytochemical screening revealed the presence of carbohydrates, glycosides, saponins, flavonoids, tannins, alkaloids, anthraquinones and steroids. Geometric mean of 3807.9 mg/kg was calculated from the results of LD_{50}. There were significant ($P< 0.05$) improvement in the mean values of haematological parameters (PCV, RBC count and Hb concentration) in a dose dependent manner in groups; II, III and IV. No significant difference ($P > 0.05$) was recorded in the mean values of haematological parameters between rats in group IV and V. Conversely, there was a significant ($P< 0.05$) decrease in the mean values of haematological parameters in group VI when compared with II, III, IV and V. Thus, methanolic leaf extract of Balanites aegyptiaca contained bioactive compounds that improved haematological parameters in wistar rats exposed to chlorpyrifos.

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

INTRODUCTION
Traditional medicine is a common practice in Nigeria and other parts of the world, plants in the form of crude extracts, decoction, infusion or tincture are used to treat common infection and chronic conditions (Bako *et al.*, 2005), hence ethnobotany and ubiquitous plants provide a rich resources of natural drug research and development. *Balanites aegyptiaca*, a tree that is widely spread in the drier regions of Nigeria (Manji *et al.*, 2013). The aqueous extract of *Balanites aegyptiaca* seed contained balanitin-7, used as anthelmintic agent (Gnoula *et al.*, 2007). The bark aqueous extract of *Balanites aegyptiaca* has been used in treatment of both AIDs and leukaemia (Manji *et al.*, 2013). Blood parameters have been considered as diagnostic guides of the pathological condition and are significant for the assessment of systemic functions and general health of animals (Chaudhary *et al.*, 2015; Prasad *et al.*, 2015). Chlorpyrifos is a highly toxic to animals and affects their growth and reproductive activity (Levin *et al.*, 2004; Sledge *et al.*, 2011) and also damage their lungs and liver (Xing *et al.*, 2012). It was found that chlorpyrifos also causes several other effects including hematological alterations (Alagoa and Ekweozor, 2009), Therefore, based on the various therapeutic effects of *Balanites aegyptiaca* and detrimental effects *of* chlorpyrifos on different tissues and organs of both farmers and animals, the current experiment was conducted to check the *in vivo* activity of the crude methanol leaf extract of *Balanites aegyptiaca* in wistar rats exposed to chlorpyrifos

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 aegyptiaca* were 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 subjected to extraction with methanol by cold maceration using a 1-litre capacity separating funnel at room temperature for 48 hours. Stock solution of the extract at concentration of 1 g/mL was prepared and stored at 4 ºC for pharmacological test.

***Phytochemical screening*:** The methanol leaf extract of the *Balanites aegyptiaca* was subjected to phytochemical screening tests, using methods described by Brain and Turner (1975).

***Safety Determination (LD_{50})*:** The LD_{50} is an indicative of the relative safety of the extract in laboratory animals, it was determined as described by Lorke (1983).
***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.

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.
Haematological Analyses: At the end of experiment, rats from each group were euthanized by severing the jugular vein. About 5 ml of blood was collected from each rat in a 10 ml vial containing EDTA as an anti-coagulant and was used to determine the haematological parameters. The packed cell volume (PCV), total erythrocyte count and haemoglobin concentration were determined using methods described by Coles (1986).
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

Extract yield
The percentage yield of the dried methanolic leaf extrct of *Balanites aegyptiaca* was 59.86 %.
Phytochemical screening
The results of phytochemical screening are presented in Table 1.
Table 1: Phytochemical screening test of the crude methanol leaves extract of *Balanites aegyptiaca*

Compound	Test	Observation	Inference
Carbohydrate	Molisch's	violet colour at the interface	+
	Fehling's	brick-red precipitate	+
Glycoside	Fehling solution	brick-red precipitate	+
	Ferric chloride	Grennish precipitate	+
Anthraquinones	Borntrager's	no pinkish-red colouration seen	++
	Modified Borntrager's	no pink colouration seen	+
	Kella-killiani	purple ring colour seen at the interface	+
Cardiac glycoside	Kadde	purple-blue colour	+

	Salkowsk	reddish brown colour	+
Saponins	Frothing	no honey-comb foam persisting for more than 30 min	+++
Steroids	Steroid	Blue colouration	++
Triterpenes	Triterpene	pinkish colouration	-
Flavonoids	Shinoda	orange colouration	-
	Sodium hydroxide	yellow colouration	++++
Tannins	Lead sub-acetate	Greenish-black precipitate	+
	Ferric chloride	blue-black precipitate	+
Alkaloids	Dragendoff	rose-pink colouration seen	+
	Wagner's	whitish precipitate seen	+

Acute toxicity studies

The extract at doses of 10, 100, 1000, 1600, and 2900 and did not produce any sign of toxicity or mortality. But mortality was observed at 5000 mg/kg.

Table 2: Toxic effect of the extract of *Balanites egytiaca* in laboratory wistar rats

Number of wistar rats	Dosage (mg/kg)	Effects
3	10	No apparent clinical sign
3	100	No apparent clinical sign
3	1000	No apparent clinical sign
1	1600	No apparent clinical sign
1	2900	No apparent clinical sign
1	5000	1 Mortality

Key: SO = *Balanites aegyptiaca*

Geometric mean = $\sqrt[2]{5000 \times 2900}$=3807.9

LD50 = 3807.9 mg/kg

Table 3: Effect *Balanites aegyptiaca* on haematological parameters in wistar rats exposed to Chlorpyrifos

		Treatments				
Test	NC	BA (100 mg/kg)	BA (300mg/kg)	BA (500 mg/kg)	AA (150 mg/kg)	CPF (5 ml/kg)
PCV	45.93±1.746	27.07±0.8977	27.90±0.9706	28.88±1.860	28.95±1.814	25.10±0.868
Hb	15.30±0.5854	9.09±0.3040	9.19±0.3459	9.88±0.6174	9.98±0.6030	7.97±0.284
RBC	8.46±0.2587	5.34 ±0.1063	6.89±0.8953	7.75±0.2852	8.27±0.2973	4.11±1.105

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

DISCUSSIONS:

The use of herbs in many traditional medical practice dates back to several generations. Efficacy and safety of these medicinal herbs has been demonstrated and passed unto subsequent generations (Rukangira, 2001). However, scientific evaluation is needed to provide evidence of their safety and efficacy (WHO, 2000). The oral LD_{50} value of

3807.9 mg/kg, obtained for the extract of *Balanites aegyptiaca* falls within the practically non-toxic range (Loomis, 1978). Doses up to 2900 mg/kg were found to be practically safe, causing no any toxic sign or death in the test animals. Phytochemical screening test revealed the presence of carbohydrates, glycosides, saponins, flavonoids, tannins, alkaloids, anthraquinones and steroids in the methanolic leaf extract of *Balanites aegyptiaca*. No anaemia was observed in all groups treated with *Balanites aegyptiaca* following exposure to chlorpyrifos, this perhaps could be due to the presence of phenols and flavonoids that acted as antioxidants, by scavenging free radicals (Calixto *et al.*, 2000; Larkins and Wynn, 2004). Anaemia was observed only in a group exposed to chlorpyrifos and treated with normal saline, and this effect could be due haemolysis of red blood cell membrane by free radical generated during intoxication. The low hematological parameters of PCV, Hb, and RBC counts could be due anaemia as a result of CPF administration.

CONCLUSIONS:

The findings from this study suggest that the methanolic leaf extract of *Balanites aegyptiaca* contains bioactive constituents with documented ethnomedicinal properties. The haematological parameters in the present study showed significant changes under chlorpyrifos exposure. The results obtained, therefore justify the use of *Balanites aegyptiaca* in traditional treatment of disease conditions.

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