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Evaluation of Acute Toxicity of Water Extract of *Azadirachta indica* Leaves and Seeds in Rats

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Abstract: This study *'in vivo'* was applied on rats *"Rattus norvegicus"* to determine the acute toxicity of water extracts of *Azadirachta indica* leaves and seeds during 48 hours and the 50% lethal dose (LD_{50}) values were calculated. Different doses of *A. indica* water extracts of leaves and seeds were injected to the rats (*Rattus norvegicus*) and the percentage of death was recorded during 48 hours. The present study, found that the percentage of death in all treated rats with *A. indica* leaves and seeds water extracts were increased by doses increased ($R^2 = 0.9$). Rats injected with higher doses of water extract of *A. indica* leaves (0.1 and 0.092 g mL^{-1}) and seeds (0.2 g mL^{-1}) showed 100% death. The LD_{50} of water extract of *A. indica* leaves and seeds were 6.2, 9.4 mL kg^{-1}, respectively. Based on these results, it may be concluded that doses of water extract of *A. indica* leaves and seeds injected to rats showed significant acute toxicity.

Key words: *Azadirachta indica*, rats, acute toxicity, LD_{50}

INTRODUCTION

Neem (*Azadirachta indica*) is a multipurpose medicinal plant having traditionally extensively used to cure various human diseases. Every part of neem (leaves, bark, fruit, flowers, oil, and gum) have been reported as an associate to various remedial properties such as, diabetes (Alam *et al*., 1990), hypertension (Biswas *et al*., 2002), heart disease (Singh *et al*., 1990), cancer (Kumar *et al*., 2010), ulcers (Bandyopadhyay *et al*., 2004) skin disorders (Garg *et al*., 1993; Karmakar and Chatterjee, 1994), antimicrobial effects (Sai Ram *et al*., 2000), antiviral activity (Badam *et al*., 1999), antibacterial agent (Das *et al*., 1999), reduction of paracetamol-induced liver damage (Bhanwara *et al*., 2000), enhancer of hepatic glutathione and glutathione-dependent enzymes (Arivazhagan *et al*., 2000) and etc.

The toxicological effects of *A. indica* were studied. However, previous research on acute oral toxicity found that albino rats fed technical grade azadirachtin (the main active ingredient of neem) ranged from greater than 3,540 mg kg^{-1} to greater than 5,000 mg kg^{-1} (Meister, 1999; EPA, 1993; Thomson, 1992). Additionally, Martineau (1994) reported that, oral toxicity LD_{50} and LC_{50} of the formulated product Azatin-EC (single-dose) fed to rats was 4,241 mg k^{-1} while, the 4 h acute inhalation LC_{50} was 2.18 mg kg^{-1}. Thus, this study was aimed to determine the acute toxicity and lethal dose (LD_{50}) in rats (*Rattus norvegicus*) injected with water extract of *A. indica* leaves and seeds during 48 h.

MATERIALS AND METHODS

Plant material: Fresh leaves and seeds of *Azadirachta indica* were collected in the month of February from Al-Resifah area of Makkah, Saudi Arabia. Then, they were washed and air dried. The dried *A. indica* leaves and seeds were then grounded to powder form and these were stored in an air tight container placed at room temperature.

Water extraction of plant samples: *A. indica* leaves and seeds powders (10 g) were mixed individually in 100 mL of distilled water. After incubation in water bath (70°C) for 24 h the mixture were then filtered and the clear solution obtained were kept in the dark at 4°C and used within 3 days.

Animal samples: Male rats (*R. norvegicus*) were under age of 31 days weighing 70-80 g. All rats were obtained from King Fahd Medical Research Center in King Abdulaziz University, Jeddah, Saudi Arabia. The rats were housed in cages made from woods and metal screens. Each cage contained 6 rats. Rats' food and sterilized water were supplied *ad libitum* during the period of study. All rats were acclimatized for 7 days at room temperature of 25°C, relative humidity of 50-60% and 12 h dark/light cycle.

Experimental design: Experimental rats were divided into 9 groups (6 rats/ group, n = 54). In order to select optimum dose of *A. indica* leaves and seeds water extracts, different doses of *A. indica* leaves water extract were

selected as follows (0.05, 0.071, 0.084, 0.092 and 0.1 g mL^{-1}) while *A. indica* seeds water extract were (0.1, 0.15, 0.17 and 0.2 g mL^{-1}).

Acute toxicity study was carried out by intramuscular injection of the rats in each group with different doses of *A. indica* leaves and seeds water extracts (0.07 mL g^{-1} of body weight). The percentage of death was calculated after 48 h of injection. The LD_{50} of the extract was calculated using the arithmetic method of Karber (Aliu and Nwude, 1982).

Statistical analyses: All data were expressed as Mean±SME (standard mean error) by using SPSS 14.0.

RESULTS

The death percentage of rats injected with different doses of *A. indica* leaves and seeds water extracts during 48 h were shown in Fig. 1 and 2. The present study found that, highest death percentage (100%) was shown in rats injected with 0.1 and 0.092 g mL^{-1} of *A. indica* leaves water extract (Fig. 1) and 0.2 g mL^{-1} of *A. indica* seeds water extract (Fig. 2). However, all injected rats with different doses of *A. indica* leaves and seeds water extracts showed decreased in death percentage from 100% to 33% which was positively correlated ($R^2 = 0.9$) with the doses decreased. The 50% lethal dose (LD_{50}) values for *A. indica* leaves and seeds water extracts were 6.2, 9.4 mL kg^{-1}, respectively (Table 1).

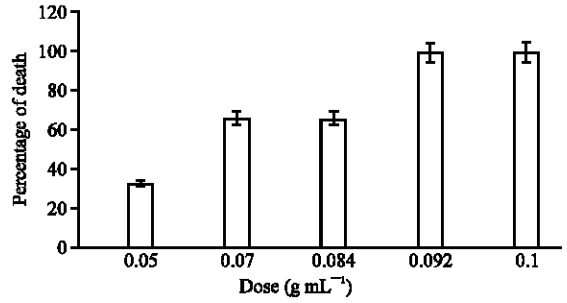

Fig. 1: Death percentage of the rats injected with different doses of water extract of *Azadirachta indica* leaves during 48 h

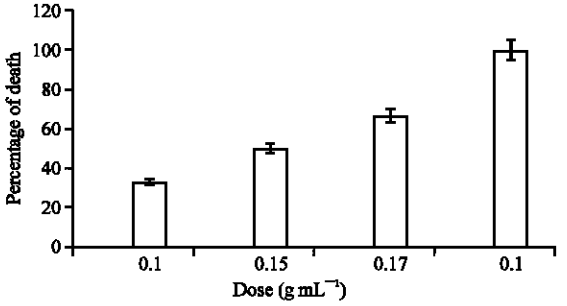

Fig. 2: Death percentage of the rats injected with different doses of water extract of *Azadirachta indica* seeds during 48 h

Table 1: LD_{50} values of *A. indica* water extracts of leaves and seeds during 48 h (n = 54)

A. indica water extracts	LD_{50} mL kg^{-1}
Leaves	6.2
Seeds	9.4

DISCUSSION

The current results is in agreement with Tandan *et al.* (1995) who reported acute toxicity in mice with doses between 1.0-28.2 g kg^{-1} of b.wt. of neem oil during 24 h. Rahman and Siddiqui (2004) showed 10% mortality of rats treated orally for long-term (90 days) with 0.3 g kg^{-1} of Vepacide, a neem-based pesticide (isolated from seed kernels of the neem). Vinod *et al.* (2011) showed 50% mortality in mice after 24 h of injection with 0.1 mL/mouse of 1.4 g kg^{-1} b.wt. dose of neem oil extract by using dimethyl sulphoxide (NDE). Wulari Mbaya *et al.* (2010) reported that rats treated intra-peritoneally with ethanolic extract of *Azadirachta indica* stem bark (1 g mL^{-1}) for 24 h showed 100% of death in the group given 6.4 g kg^{-1} and 70% in the group given 3.2 g kg^{-1} while those given 1.6 g kg^{-1} had 50% mortality. This could explain that water extract of *Azadirachta indica* leaves and seeds had higher toxicity than ethanolic extract of *Azadirachta indica* stem bark even at low concentration of the extract (0.1 g mL^{-1}). The present study suggested that the differences in the solvents and methods used to prepare the extracts could affect the toxicity level. However, LD_{50} values in the present study were lower than LD_{50} value (14.1 mL kg^{-1}) that reported in previous study for acute oral toxicity after rats ingestion of the neem oil (10-80 mL kg^{-1} b.wt.) during 24 h (Gandhi *et al.*, 1988; Boeke *et al.*, 2004). This is indicated that water extract of neem leaves and seeds could have higher toxicity than neem oil. Similar study was conducted by Biu *et al.* (2010) who found that the LD_{50} for chickens treated intraperitoneally with water extract of neem leaves for 24 h was 4.8 g kg^{-1}. Besides, this was reported to be associated with pharmacotoxic symptoms which target lungs and central nervous system (Gandhi *et al.*, 1988).

Nimbolide and nimbic acid are major chemical components of neem seed oil found to be toxic to mice when given intravenously or intraperitoneally (Glinsukon *et al.*, 1986). These components showed less toxic effect to rats and hamster. Besides, nimbolide and nimbic acid at a lethal dose cause death in most animals

by dysfunction of kidney, small intestine and liver, as well as sudden drop of arterial blood pressure (Glinsukon *et al*., 1986). Additionally, oral administration of neem oil in male rats found to increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in blood after 15 h (Sinniah *et al*., 1985). Moreover, aqueous extract of the leaf reduced the elevated serum ALT, AST and gamma-glutamyltransferase (GGT) in rats with paracetamol damaged livers (Bhanwara *et al*., 2000). However, further research such as biochemical and histo-pathological are required.

CONCLUSION

Based on the present results, *A. indica* leaves and seeds water extracts exhibit acute toxicity when injected to rats at all doses under study. The LD_{50} of *A. indica* leaves and seeds doses (mg kg^{-1}) are important. Therefore, the doses of the extracts could be categorized in the WHO classification of acute toxicity ratings of *A. indica*.

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