In vivo study of Fagonia Cretica Linn toxicity in Sudan

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Abstract

Purpose: The study targeted to determine the toxicological effects of ethanolic extracts of Fagonia cretica linn plant in Khartoum, Omdurman, and Shendi towns in Sudan during (January – February) 2013.

Objectives: To determine the LD50 of the plant and to study the effects of the plant on the body weight, on vital organ weights, and anesthesia induction time effects.

Methods: Different methods were adopted to achieve the objectives of this study, including Harborne methods for extraction. A total of (30), young adult Wistar rats, weighing (42.6 – 72.2) grams, fed standard rat food and water ad libitum, were maintained at standard laboratory conditions. All rats were grouped into (3) groups, survived through the experiment by using the recommended progression doses of Fagonia cretica as (175, 550, 1500, 5000, & 5000) mg/kg/body weight screen shot of AOT425 statistical program of main test. Toxicological studies in vivo (LD50 determination) were done in all (3) groups of animals survive till the experiment was over after the administration of a selected high dose (5000) mg/kg/body weight. Ethical approval had been obtained from Omdurman Islamic University, Faculty of Pharmacy, and Department of Pharmacology under the number-(01/0/2013).

Results: The LD50 is greater than 5000 mg/kg, indicates the high safety of the plant. The ethanolic extract of Fagonia cretica in doses of (100 & 300 mg/kg/body weight) has no effects on the rats relative vital organ weight (heart, liver, kidneys, lung and spleen) during the study period of (14) days compared to the control group whilst the dose of (600) mg/kg/body weight change the liver somewhat from the control. The anesthesia induction time on rats, shows that the ethanolic extracts of the plant has neurological effect on rats.

Conclusion: The toxicological determination of LD50 was found to be greater than (5000) mg/Kg, estimated by the special statistical software with three stopping criteria based on long term outcomes. This is indicating wide safety margin of Fagonia ethanol extract which encourages its use by humans for the treatment of many diseases...

Keywords: Fagonia Cretica Linn, Umm Showaika, Ethanolic Extraction, Toxicity.

1. Introduction

Life and diseases go together: Where there is life, diseases are bound to exist. Dependency and sustainability of man and animal life has been revolving around plants through uses as foods, fibers and shelter, but also plants have been used to control and ease diseases, therefore the use of the plants as medicines is an ancient and reliable practice (Sarfaraz Khan Marwat et al 2010). The plant is a small spiny under shrub, mostly found in dry calcareous rocks throughout Pakistan. (Chopra, R.M.et al 1982, p.507) It is reputed to be a medicinal plant in scientific and folkloric literature and its medicinal values are well documented (Saied MA ET al.1969.p.41-43) Vern names: (Ar) Umm Showaika, Shoolib, UmmShok. Family : Zygophyllaceae. Habitat: Sandy hills (Quos), low land plains. In Sudan: ElMazroub, also widespread throughout Northern and central Sudan. (Gamal, E.B.ELGhazaliit et al 1994. p.52-53) it is present abundantly in Shendi Region. Universally: It is found in India, Pakistan, China, Bangladesh and Egypt. Medicinal properties of the plant are attributed to its variety of active phytochemical constituents. In the last fifteen years, this plant and related species have been investigated mainly for the presence of flavonol and terpenoid glycosides. Most of the flavonol glycosides have been isolated from various Egyptian Fagonia species and their phylogenetic affinities have also been investigated. (Crack P.J.et al 2003.p.19-22) Several saponin glycosides have been separated and characterized. (Taylor CP. Et al 1993.p.555-563) Other constituents, such as docosyl docosanoate from hexane extract and water soluble proteins from aqueous extract of air-dried F. cretica plants have been isolated. Furthermore nahagenin (hederagnin, ursolic acid and pinitol from other Fagonia species have also been separated and characterized. (Paglia DE et al.1969.p.194-195) Antimicrobial activity of its flavonoid compounds has been explored previously. While the nutritive values of it and of other species growing wild in the Rajasthan region of India, have also been evaluated. (Paglia DE et al.1967.p.158-169).

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2. Materials and methods

This study was conducted in Omdurman Islamic University laboratories, Khartoum state, Sudan, during (January – February) 2013.

2.1. Ethical-consideration

An ethical approval had been obtained from Omdurman Islamic University, Faculty of Pharmacy, Department of Pharmacology, under the number (01/0/2013). The in vivo toxicological procedures involve the experimental animals and their care was conducted in compliance with the Guidelines for Care and Use of Laboratory Animals in Biomedical Research as promulgated by the Canadian Council of Animal Care (1984) and United States National Institutes of Health (1985). The rats were fed a standard rat food (a mixture of flour, oil, meat and some vegetables) and water ad libitum and were maintained at standard laboratory conditions (12/12 hr dark/light cycle, 23 ± 1 °C temperature, and 55 ± 3 % humidity) The Faonia cretica plants were collected from uncultivated and waste areas of Shendi town from near the Faculty of Medicine and Surgery, University of Shendi, Shendi town, River Nile state, Sudan in (January/February) 2011. These were authenticated by the Herbarium staff, Department of Botany, the Sudanese national centre for research, Khartoum, Sudan.

2.2. Experiment-setup

A voucher specimen was deposited in the Herbarium Department of Botany, the Sudanese national centre for research, Khartoum, Sudan for further reference. Extraction was carried out according to the method described by (Harborne J B, 1984.p.4-7). 2000 g of plant sample was extracted successively with chloroform and 80 % ethanol using shaker apparatus; this was for about (72) hours for chloroform, and (5) days for ethanol. At last extracts were allowed to air till they were dried. A total of thirty, young adult Wistar rats, weighting (42.6 ± 7.2) grams were obtained from the Sudanese National Centre for Research, Khartoum, Sudan. Before the oral doses administration experiment was beginning, rats were fasted overnight for (14 – 16) hours. Group C, which was the control group, received (10) ml/kg distilled water, orally, throughout the study period while Groups (1,2,3) were orally administered single, daily doses, (100, 300 and 600) mg/kg of body weight, respectively of the Fagonia cretica ethanolic extract dissolved in distilled water (1gm/10ml ) for (14) days using acute oral toxicity (425) protocol. Acute oral toxicity study was conducted using the limit dose test of Up-and-Down Procedure according to Organization for Economic Cooperation and Development (OECD) at a limit dose of (5000) mg/kg body weight per oral route. Behavioral manifestations of acute oral toxicity such as lethargy, anxiety, sleep, hyperactivity, changes in eyes, diarrheal etc, were also noted. All observations were systematically recorded and individual records being maintained for each rat. Single, daily doses, (100, 300 and 600) mg/kg of body weight, respectively of the Fagonia cretica ethanolic extract dissolved in distilled water (1gm/10ml) for (14) days using acute oral toxicity (425) protocol. Body weights of all rats were monitored using a sensitive balance at day (0, 7 & 14) this can be shown in Fig.( 1)

2.3. Experiment-results

Table 1: Sequence and Results of Limit Dose Test of Fagonia Cretica in Rats Using AOT425statpgm (Version: 1.0) (Acute Oral Toxicity (OECD Test Guideline 425))

<table>
<thead>
<tr>
<th>Test term</th>
<th>Long-term</th>
<th>Animal ID</th>
<th>Dose (mg/kg)</th>
<th>Short-term</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>O</td>
<td>1</td>
<td>5000</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>O</td>
<td>2</td>
<td>5000</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>O</td>
<td>3</td>
<td>5000</td>
<td></td>
</tr>
</tbody>
</table>

(X = Death, O = Survival)

The LD50 is greater than 5000 mg/kg.

Main test: All rats were survival through the experiment by using the recommended progression doses of Fagonia cretica as (175, 550, 1750, 5000, 5000, 5000) mg/kg/boby weight screen shot of AOT425 statistical program of main test.

i) Limit test such as lethargy, sleeping, hyperactivity, anxiety and sometimes inactivity.

Table 2: Effect of Ethanol Leaves Extract of Fagonia Cretica on the Relative Organs Weight and Lethality in Rats

<table>
<thead>
<tr>
<th>Doses (mg/kg)</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Lung</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.35±0.01</td>
<td>3.02±0.1</td>
<td>0.66±0.03</td>
<td>0.63±0.03</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>100</td>
<td>0.35±0.02</td>
<td>3.08±0.2</td>
<td>0.68±0.03</td>
<td>0.63±0.03</td>
<td>0.20±0.01</td>
</tr>
<tr>
<td>300</td>
<td>0.33±0.02</td>
<td>2.52±0.6</td>
<td>0.63±.002</td>
<td>0.59±0.02</td>
<td>0.19±0.01</td>
</tr>
<tr>
<td>600</td>
<td>0.36±0.02</td>
<td>3.50±1.1</td>
<td>0.64±0.02</td>
<td>0.60±0.02</td>
<td>0.20±0.01</td>
</tr>
</tbody>
</table>

iii) Anesthesia induction time effects:

The ethanolic extract of Fagonia cretica in doses of (100 & 300 mg/kg/boby weight) has no effect on the anesthesia induction time on rats after the study period of (14) days compared to the relevant control.
control group whilst the dose of (600) mg/kg/body retard the anesthesia induction time on rats from the relevant control.

![Fig.2: Anesthesia Induction Time for All Groups Using Diethyl Ether at the End of the Study (Day 15) - Sacrifying Date.](image)

## 4. Discussion

This is an in vivo current study of the Sudanese Fagonia cretica plant that attempted to examine the toxicological effects, in order to fill the gap due to lack of such information’s in Sudan. Nowadays, there is an increase and popular utilization of Fagonia for treating various health problems worldwide particularly in Pakistan, India, Far East and some other parts of the world including Sudan; hence there were limited scientific data available regarding its safety. This current case-control (14) days study evaluates the safety of Fagonia ethanolic extract using protocol 425 guidelines. Observations of rats’ behaviors can be summarized as follows; rats were normally behaving during the adaptation period, after (½) hours of dose administration some of them from different study groups develop inactivity, lethargy and aspetic associated with some rats’ hyperactivity. No behavioral changes occurred during the remaining test period. Statistical estimates based on long term outcomes. The LD50 is greater than (5000) mg/kg, which indicates the high safety of the plant, which encourages its use by human. This result is in line with (Matthew Lam 2012) who describes for the first time that an aqueous extract of Fagonia cretica shows potent in vitro cytotoxic activity towards breast cancer epithelial cell lines which was not seen towards normal mammary epithelial cells.

The ethanolic extract of Fagonia showed no effect in rat’s body weight, which means absence of any substance that may cause increase in body weight. No effects were observed on different vital organs using the tested doses, except for the large dose which has significantly increased the liver size, this hepatomegaly was not due to any pathological conditions because the macroscopically investigations showed normal liver texture, besides our liver function tests revealed normal functionality. This was in line with the traditional use in treating liver disorders (Avinash K et al 2004, p. 4-11). Also the literature survey revealed the presence of antioxidant effect that act as Hepato protective (Avanish, K et al. 2004).

Only the dose of (600) mg/kg/body retarded the anesthesia induction time on rats, which means that the ethanolic extracts has a neurological effect, which agrees with (Avinash K et al., 2004).

## 5. Conclusion

The toxicological determination of LD50 was found to be greater than (5000) mg/Kg, estimated by the special statistical software with three stopping criteria based on long term outcomes. Which indicating wide safety margin for Fagonia ethanolic extract.

## 6. Recommendations

1) Further studies targeting the identification of the active phytochemical components of Fagonia cretica and their role of action are recommended.
2) Pharmaceutical formulation of Fagonia cretica as herbal medicine is highly recommended.
3) The LD50 of the plant showed a high margin of safety which encourages its use by human for the treatment of many diseases.
4) Further studies on the Sudanese Fagonia cretica as antioxidant, immune modulating agent, anticancerous, and anti-inflammatory is also recommended.

## References