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Toxicity Studies on Devil's Claw Herbal Medicine

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Abstract: Toxicity studies on Devil's claw capsules were conducted using mice by investigating the possible biochemical, hematological and histopathological changes. Acute, subacute and chronic toxicity studies were undertaken by treating mice with a single dose of (81 mg kg^{-1}) body weight for acute (27 mg kg^{-1}) body weight administered orally each other day for 7 days for subacute and an oral dose of (5.4 mg/kg/day) body weight of the drug daily for 90 days for chronic toxicity studies. Devil's claw treated animals did not show any sign of toxicity and none of the animals died during the observation period. Hematological and biochemical studies showed non significant differences among the animals in the treatment groups and control groups.

Key words: Devil's claw, acute, subacute, chronic, toxicity, Saudi Arabia

INTRODUCTION

Devil's claw (*Harpagophytum procumbent* D.C.) is a plant with bright pinkish red flowers native of South and Southwestern Africa, Kalahari deserts, Namibia and Island of Madagascar. The name of Devil's claw is derived from the herb's unusual fruits which seem to be covered with numerous small hooks.

The secondary storage roots or tuber of the plant is employed in herbal supplements (Tyler, 1993). Devil's claw is used in rheumatoid arthritis, indigestion and heartburn. The tuber is mostly used as remedy (Mabey, 1988), for gall bladder, liver and kidney problems but Western herbalists use them mostly in the treatment of rheumatic pain and joint problems, mainly arthritis and lumbago (Mabey, 1988; Ody, 1993; Gagnier *et al*., 2004).

It has a cooling energy with bitter and astringent taste and is considered as an alternative, anti-inflammatory and analgesic herb (Frawley and Lad, 1986; Mohamed and Ojewole, 2004; Catelan *et al*., 2006). The analgesic properties can be compared in strength to cortisone or phenylbutazone. It is prescribed for the treatment of rheumatoid and osteoarthritis with serious side effects (Mabey, 1988; Ody, 1993). Devil's claw capsules registered and marketed in Saudi Arabia, manufactured by Bioron, France. The therapeutic dose is one capsule twice daily or as suggested by the physician. The active ingredient per capsule in Devil's claw is 189 mg.

The excipients added in the formulation are magnesium Stearate 5 mg $capsule^{-1}$, precipitated silica (5 μm) 8 mg $capsule^{-1}$ and precipitated silica (3-4 μm) 2 mg $capsule^{-1}$. The total weight of capsule is 204 mg. The capsule contains Devil's claw with main active compound harpagoside. No toxicity data were available in literature and official files. Therefore, the present study was designed to investigate acute, subacute and chronic toxicity effects of Devil's claw capsules.

Experimental animals: Acute, sub acute and chronic toxicity studies were carried out using male and female mice. Swiss albino mice (SWR) aged 6-7 weeks and weighing 20-25 g (home breed) were used. The animals were maintained under standard conditions of humidity, temperature and light (12 h dark/12 h light). The animals were fed with Purina chow diet with free access to water.

Acute toxicity evaluation: For evaluation of acute toxicity a dose of (81 mg kg^{-1}) was chosen which is 15 times the therapeutic dose. The drug in each case was suspended in 1% Carboxymethyl Cellulose solution (CMC) and administered orally (0.5 mL per mouse) in a single acute dose.

The control group received equal amount of vehicle. The animals were observed for signs of toxicity over a period of 7 days following the dose administration.

Subacute toxicity evaluation: A dose of 27 mg kg^{-1}) was given orally each other day for seven days. The control group received vehicle in the same dose (0.5 mL). The parameters included in this study were based on standard toxicological screening program (Robin *et al*., 1982; Chan *et al*., 1982) and included screening on general toxicity systems, mortality, body and organ weight, hematology, biochemistry, genotoxicity and histopathology.

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Chronic toxicity evaluation: A dose of (5.4 mg kg^{-1}) was given orally for 90 days to test chronic toxicity in accordance with WHO (1966) in order to predict long term exposure of a particular drug (Mossberg and Hayes, 1989). The purpose of this investigation was to evaluate the effect of prolonged treatment on the target organs and the physiological and metabolic tolerance of the drug product at low doses. The parameters included in the study were based on the standard toxicological screening program (Robin *et al*., 1982; Chan *et al*., 1982; Mossberg and Hayes, 1989).

MATERIALS AND METHODS

Material: Ether, Haematoxylin, Eosin, Methanol, Formalin, May-Grunewald solution and Giemsa stain all purchased from (Sigma-Aldrich). Test combination reagents (Boehringer MannheimGmbH, Diagnostica-Germany).

Instruments: Coulter counter, Spectrophotometer, Introspect II (LKB). American optical Rotary Microtome. Optical microscope. centrifuge (Beckman coulter).

Metabolic measurements: The animals were anesthetized with ether and blood was taken from the heart by direct puncture.

Biochemistry: The blood was collected, serum samples were separated, stored at -20°C and analyzed for alanine aminotrasnferase (ALT/GPT), aspartate aminostrasnferase (AST/GOT), enzyme MB of Creatine Kinase (CK-MB), glucose, urea and creatinine. The parameters were analyzed by an enzymatic colorimetric method using test combination reagents. The measurements were carried out in a spectrophotometer.

Hematology: The blood was analyzed on a coulter counter for the quantification of different hematological indices such as WBC, RBC, hemoglobin, haematocrit, platelets and MCV.

Histopathological procedures: Tissue samples of liver, heart and kidney were preserved in 10% buffered formalin and processed for routine paraffin block preparation. Using an American Optical Rotary Microtome, sections of thickness of about 5 μm were cut and stained with haematoxylin and eosin. The preparations were analyzed using an optical microscope compared to control animal preparation.

Genotoxic studies: The adhering soft tissue and epiphyses of both the tibiae were removed. The marrow was aspirated from each femur in fetal calf serum and transferred to centrifuge tubes. After centrifugation at 1000 rpm for 5 min. the supernatant was discarded and the residual cells were spread on slides and air dried. The slides were fixed in methanol, stained in May-Grunewald solution followed by Giemsa stain. The coded slides were screened for the presence of micronuclei in polychromatic erythrocytes which Indicated non-disjunction, chromosomal breaks and structural or numerical changes in the chromosomes. The bone marrow depression (mitotic index) was evaluated on the basis of the ratio of Polychromatic to Normochromatic Erythrocytes (PCE/NCE ratio) (Al-Harbi *et al*., 1994).

Statistical analysis: Student's t-test and Chi-square test were used to assess the significance of the values obtained in the treated groups as compared to controls for the evaluation of results obtained during acute, sub-acute and chronic toxicity studies.

RESULTS

Acute toxicity: The body weight changes in both the male and female treated groups were found comparable to the changes observed in the respective control groups. At the end of the treatment the weight of the vital organs and condition of the viscera were normal and comparable to the control. The results of histopathological studies also revealed all organs to be normal and comparable to the control. The results of the hematological studies revealed no significant differences between the treated groups and the control groups (Table 1 and 2). All biochemical parameters remained normal and comparable to the control (Table 3 and 4). Devil's claw acute treatment showed no cytotoxic or clastogenic effect in the femoral cells of mice. All these results clearly indicate that Devil's claw acute treatment is devoid of any toxicity. However, sub-acute and chronic toxicity experiments were considered essential to evaluate any toxic signs in treated groups. During chronic toxicity studies, no toxic symptoms were noticed in the treated groups over the period of 3 months. The increase in water in-take during the period of chronic toxicity studies was comparable to the water consumption in the control groups. All animals in the control and treatment groups were found healthy and active. There was a significant and similar weight gain in all the animals of the control and treated groups (Table 5). At the end of the treatment the visceral conditions of all animals in the treated groups were normal and comparable to the control. It is worth mentioning that during histopathological studies (Fig. 1) all the vital organs of the treated groups were found to be normal and comparable

Table 1: Hematological studies on male mice after acute treatment (81 mg kg^{-1}) with Devil's claw

Treatment groups	Hematological indices (Mean±SE)					
	WBC x 10^9 L^{-1}	RBC x 10^{12} L^{-1}	Hemoglobin g dL^{-1}	Platelets 10^9 L^{-1}	MCV fL	HCT ratio (%)
Control	5.71±0.50	6.10±0.60	12.9±0.35	484±28.0	51.5±0.9	38.7±0.80
Devil's claw	5.90±0.36	6.12±0.20	12.9±0.13	491±23.0	51.8±2.0	38.4±0.63

p>0.05 (Student's t-test); 5 male mice were used in each group; treatment group was statistically compared with the control group

Table 2: Hematological studies on female mice after acute treatment (81 mg kg^{-1}) with Devil's claw

Treatment groups	Hematological indices (Mean±SE)					
	WBC x 10^9 L^{-1}	RBC x 10^{12} L^{-1}	Hemoglobin g dL^{-1}	Platelets 10^9 L^{-1}	MCV fL	HCT ratio (%)
Control	5.14±0.40	6.21±0.63	12.5±0.32	464±20.0	51.06±0.45	38.4±0.63
Devil's claw	5.30±0.31	6.02±0.44	12.8±0.50	483±18.0	51.84±2.34	38.3±0.77

p>0.05 (Student's t-test); 5 female mice were used in each group; treatment group was statistically compared with the control group

Table 3: Biochemical studies on male mice after acute treatment (81 mg kg^{-1}) with Devil's claw

Treatment groups	Biochemical indices (Mean±SE)					
	AST (µ L^{-1})	ALT (µ L^{-1})	CK-MB (µ L^{-1})	Creat. (µ mole L^{-1})	Urea (m mole L^{-1})	Glucose (m mole L^{-1})
Control	16.0±0.58	11.15±1.50	145.8±15.61	132.86±2.21	5.84±0.35	5.42±0.09
Devil's claw	17.9±0.63	12.09±1.16	144.3±12.91	134.01±3.69	5.82±0.37	5.09±0.06

p>0.05 (Student's t-test); 5 male mice were used in each group; treatment group was statistically compared with the control group

Table 4: Biochemical studies on female mice after acute treatment (81 mg kg^{-1}) with Devil's claw

Treatment groups	Biochemical indices (Mean±SE)					
	AST (µ L^{-1})	ALT (µ L^{-1})	CK-MB (µ L^{-1})	Creat. (µ mole L^{-1})	Urea (m mole L^{-1})	Glucose (m mole L^{-1})
Control	15.31±0.52	11.12±0.70	140.5±14.60	99.57±3.61	5.53±0.29	5.45±1.02
Devil's claw	16.38±0.70	13.61±1.55	145.7±14.91	96.39±4.92	4.78±0.31	5.44±1.51

p>0.05 (Student's t-test); 5 female mice were used in each group; treatment group was statistically compared with the control group

Table 5: Quantitative data on body weight changes in mice after chronic treatment (5.4 mg kg^{-1}) with Devil's claw

Treatment groups	Male (g)		Female (g)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Control	20.4±1.30	34.7±2.60*	21.5±1.00	32.01±0.70*
Devil's claw	22.3±2.17	34.9±3.10*	22.1±2.63	31.80±0.58*

*p<0.001 (Student's t-test); all results are expressed as: average body weight±SE; pre-treatment body weight was compared with post-treatment body weight; 10 male and 10 female mice were used in each group

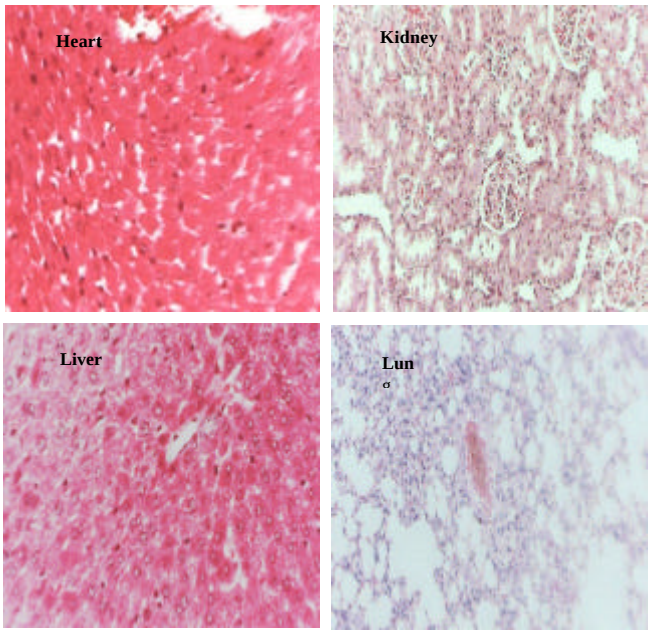

Fig. 1: A section through the heart, kidney, liver and lung of mice after control chronic treatment showing normal appearance (Hematoxyline and eosin X 400)

Table 6: Hematological studies on male mice after chronic treatment (5.4 mg kg^{-1}) with Devil's claw

Treatment groups	Hematological indices (Mean±SE)					
	WBC x 10^9 L^{-1}	RBC x 10^{12} L^{-1}	Hemoglobin g dL^{-1}	Platelets 10^9 L^{-1}	MCV fL	HCT ratio (%)
Control	5.70±0.45	6.30±0.60	13.00±0.58	517±25.0	52.2±1.80	38.40±0.83
Devil's claw	5.65±0.78	6.70±1.10	13.30±0.90	584±86.5	52.3±1.20	38.50±2.61

p>0.05 (Student's t-test); 5 male mice were used in each group; treatment group was statistically compared with the control group

Table 7: Hematological studies on female mice after chronic treatment (5.4 mg kg^{-1}) with Devil's claw

Treatment groups	Hematological indices (Mean±SE)					
	WBC x 10^9 L^{-1}	RBC x 10^{12} L^{-1}	Hemoglobin g dL^{-1}	Platelets 10^9 L^{-1}	MCV fL	HCT ratio (%)
Control	5.41±0.40	6.9±0.4	13.0±0.20	535±50.0	51.6±0.7	38.8±2.10
Devil's claw	5.20±0.70	7.0±0.2	13.2±0.65	585±87.0	52.3±2.4	38.5±1.35

p>0.05 (Student's t-test); 5 female mice were used in each group; treatment group was statistically compared with the control group

Table 8: Biochemical studies on male mice after chronic treatment (5.4 mg kg^{-1}) with Devil's claw

Treatment groups	Biochemical indices (Mean±SE)					
	AST (μ L^{-1})	ALT (μ L^{-1})	CK-MB (μ L^{-1})	Creat. (μ mole L^{-1})	Urea (m mole L^{-1})	Glucose (mmole L^{-1})
Control	16.40±2.40	12.80±1.90	96.00±22.00	83.00±13.05	4.55±0.50	5.11±0.30
Devil's claw	18.50±2.91	13.09±1.86	101.13±24.00	84.23±13.69	4.69±0.63	5.15±0.35

p>0.05 (Student's t-test); 5 male mice were used in each group; treatment group was statistically compared with the control group

Table 9: Biochemical studies on female mice after chronic treatment (5.4 mg kg^{-1}) with Devil's claw

Treatment groups	Biochemical indices (Mean±SE)					
	AST (μ L^{-1})	ALT (μ L^{-1})	CK-MB (μ L^{-1})	Creat. (μ mole L^{-1})	Urea (m mole L^{-1})	Glucose (mmole L^{-1})
Control	15.50±6.66	12.30±1.63	98.50±9.61	135.61±2.25	4.26±0.35	5.40±0.14
Devil's claw	16.37±5.93	14.73±2.96	93.14±8.39	130.18±4.39	4.20±0.37	5.43±0.16

p>0.05 (Student's t-test); five female mice were used in each group; treatment group was statistically compared with the control group

Table 10: Effects of chronic treatment (5.4 mg kg^{-1}) with Devil's claw on the femoral cells in mice

Treatment groups	Polychromatic erythrocytes (PCE) screened	Micronucleated polychromatic erythrocytes (%)	Normochromatic erythrocytes (NCE) screened	PCE/NCE ratio
Control	5200	0.23±0.03	5060	1.04±0.07
Adriamycin	5000	5.32±0.33**	7596	0.67±0.04*
Devil's claw	5148	0.26±0.04	5546	0.93±0.07

*p<0.05, **p<0.01 (Student's t-test); five male mice were used in each group; treatment group was statistically compared with the control group

to the control. There was statistically non-significant effect on the bone marrow cells of treated group as compared to the control. The results of the hematological studies revealed no significant differences between the treated groups and control groups (Table 6 and 7). All biochemical parameters remained normal and comparable to the control (Table 8 and 9).

Adriamycin treatment, however, caused significant ($p<0.01$) increase in micronucleated polychromatic erythrocytes and reduction in PCE/NCE ratio confirming its known clastogenic and cytotoxic potential (Table 10).

DISCUSSION

Devil's claw is known to possess Iridoid glycosides, including harpagide, harpagoside and procumbide; flavonoids, mainly kaempferol and luteolin glycosides; phenolic acids; chlorogenic and cinnamic acid; harpagoquinone; triterpenes, sterols, oleanolic and ursolic acid derivatives, esters, bitter principles, gum resin and sugars (Mabey, 1988). The slight increase observed in the liver enzymes in the female mice treatment groups as compared to the control during current study may be attributed to the anti-inflammatory principles of Devil's claw. Histopathology results demonstrated all vital organs of treated mice in different groups to be normal and comparable to the control. During current study hematological indices showed no significant differences in the treated male and female animals as compared to the control. The anti-inflammatory properties of Devil's claw were attributed to harpagoside and β-sitosterol.

However, Devil's claw does not appear to inhibit prostaglandin synthesis like most non-steroidal anti-inflammatory drugs (Moussard *et al*., 1992; Whitehouse, 1983; Andersen *et al*., 2004). Earlier reports on Devil's claw suggested this natural drug to be as potent as cortisone and pheylbutazone with regards to its anti-inflammatory properties (Ody, 1993; Mabey, 1988).

The effects of *H. procumbens* (Devil's Claw) found strong evidence that daily doses standardized to 50 or 100 mg harpagoside were better in short-term improvements in pain and rescue medication

(Gagnier *et al*., 2004). The current literature review revealed a major safety concern about potential herb-drug interactions.

This issue is especially important with respect to drugs with narrow therapeutic indexes such as warfarin. Devil's claw was listed among the herbal drugs which could potentially increase the risk of bleeding or potentiate the effects of warfarin therapy (Heck *et al*., 2000). Patients taking anticoagulants along with Devil's claw are at high risk (Izzo *et al*., 2005). Devil's claw and many other medicinal plants, reinforce warfarin action by heterogeneous mechanisms. Therefore, such herbal drugs should not be used in patients on oral anticoagulant and/or antiplatelet therapy (Argento *et al*., 2000).

CONCLUSION

Histopathological studies revealed all the animals to be normal and comparable to the control. Devil's claw acute treatment showed no cytotoxic or clastogenic effects in the femoral cells of mice. These results clearly demonstrated that Devil's claw treatment is devoid of any toxicity and indicates a wide margin of safety for therapeutic doses of Devil's claw.

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