Evaluation of Acute Toxicity of *Solanum dubium* seeds Aqueous Extract in Rats

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ABSTRACT

The current study evaluated the (lethal dose LD 50) of the aqueous extract of *S. dubium* seeds; as a potent anti asthmatic in terms of biochemical, hematological and liver and kidney functions in Wistar albino rats. Five groups of rats (6 rats each) were administered orally with the aqueous extract of *S. dubium* seeds at doses of 2ml, 10ml. 20ml, 40ml/kg/day, respectively for seven consecutive days. No mortality was recorded in any of the groups. There was no hematological neither biochemical changes in all the treated groups.

It is concluded that the crude aqueous extract of *S. dubium* seeds was not toxic in Wister albino rats (LD50>2000 mg/kg/oral) and could be used safely for therapeutic purposes within this dose range.

Key words: LD50, hematological and biochemical parameters, mortality, toxicity, *Solanum dubium* seeds, Sudan, efficacy and safety

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INTRODUCTION

There has been a steady increase in the use of medicinal plants in the last two decades in both developed and developing countries for prevention, management and treatment of diseases. This increase has been due to reasons such as ease of access, better cultural acceptability and compatibility, cost effectiveness and also the bid to “go natural”. The lack of rigorous research to prove the effectiveness and safety of many medicinal plants is of great concern to the health care system.

Herbal medicines have been extensively used in developed countries hence they are natural and relatively safe (Gurib-Fakim, 2006). They contain plant materials as their pharmacologically active components (Pribitkin, 2005). Plants and derivatives of plant played a key role in world health and have long been known to possess biological activity. Thirty percent of all modern drugs are derived from plants (Burns, 2000). According to the World Health Organization about 80% of the world's population living in developing countries relies essentially on plants for primary health care (McKay *et al*., 2007).

World Health Organization (WHO**)** defines traditional medicine as:"the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being" (WHO, 2008).

Solanum dubium L; family Solanaceae, locally known as Gubbain; is a medicinal plant used for cheese coagulating in Sudan (Habbani, 1992; Osman, 1996; Mohamed and Habbani, 1996; Yousif et al., 1996; Osman, 2001). It is a well known wild plant that grows widely in Khartoum State (Sudan) during the rainy season. It also grows in the west and east shores of the White Nile, South of the Blue Nile, Gezira, Kordfan and Darfur regions (Salih, 1979).

This plant is indigenous in northern and central Sudan, is a woody herb with solid erect stem and green in colour. Unripened fruits are green and almost enclosed in spiny calyx, while the ripened ones are yellow. The seeds are dark brown in colour. (Salih, 1979 and Yousif *et al*., 1996). *Solanum dubium* L is used traditionally for the treatment of asthma among natives in Sudan. Therefore, this study addresses not just the efficacy, but also the safety of the aqueous extract of this useful plant.

MATERIALS AND METHODS

Plant Collection and Identification:

The samples of *S. dubium* seeds were collected from the fields of the area Al-Halfaya, Khartoum state- Sudan. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan.

Plant Extraction:

The cleaned and shade-dried plant seeds were fine powdered using a grinding machine, each ground sample was weighed and then stored in a dry container at ambient temperature. 500g of the powdered seeds were extracted in distilled water (1g to 250mls) for 24 hours, filtered and then evaporated to dryness in an incubator. The yield was 2.1 % W/W. All desired concentrations were freshly prepared in normal saline prior to use.

Animals:

Thirty Wister albino rats weighing (60-90g) clinically healthy of either sex were kept within the premises of the Medicinal and Aromatic Plants Research Institute, National Centre for Research, Sudan. The animals were allowed free access to feed and water *ad libitum* throughout the experimental period. The rats were allowed one week acclimatization period before the experiments. The animals were housed in clean plastic cages containing wood shavings for bedding. Each cage contained six rats.

Experimental Design:

The rats were allotted into five groups, six rats each. Group 1 served as a control group and received distilled water. Group 2-5 were given the aqueous extract of the *S. dubium* seeds at doses of 2ml, 10ml. 20ml, 40ml/kg/day, respectively, via the oral route for seven consecutive days.

Mortality and clinical symptoms of toxicity in animals were monitored seven days. After seven days, all the animals were fasted overnight, body weights were recorded and then, they were scarified. Blood samples were collected from the cervical blood vessels and hematological and biochemical parameters were evaluated (Ramirez, *et al*. 2007). Haematological parameters assessed were: Haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and differential WBC counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) by using an automated Hematology Analyzer (Sysmex kx-21, Japan, 1999). Serum samples were analyzed

for the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and for concentrations of total protein, albumin, globulin, bilirubin, cholesterol, urea, sodium and potassium by using Roche Diagnostic Hitachi902 Analyzer (Germany, 1996).

Statistical analysis

Data were analyzed by using SPSS version 20 by using Duncan's multiple range test after T. test for two ways classified data (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The effect of different doses of *S. dubium* seeds aqueous extract on the body weight and weight gain of rats is presented in Table (1). It was found that groups 3and 5 had the lowest ($p<0.05$) body weight gains and the treated group (2, 4) showed no significant change after one week compared to the control rats.

Table (1): The effect of aqueous extract of *S. dubium* seeds on weight gains of rats

Treatment groups	Body weight (g) 0 week	Body weight (g) one week
Control distilled water	40±0.0	40±2.23
S. dubuim seed (2ml/kg)	50+0.0	51.67±2.10
S. dubium seed (10ml/kg)	60±0.0	54.16±1.53*
S. dubuim seed (20ml/kg)	55±0.0	52.5±2.14
S. dubuim seed (40ml/kg)	50±0.0	45±1.82*

Values are expressed as Mean ±SE; *Significant at P<0.05

Haematological changes for rats given daily oral doses of *S. dubium* seeds aqueous extract at 2ml/kg (group2), 10ml/kg (group3), 20ml/kg (group4), 40ml/kg (group5), for one week is presented in table (2). MCH levels in (group 2, 3, 4, 5), MCHC in (group 5) were higher

($p<0.05$) than in the control group (group1) and lymphocytes levels in (group3) was lower than the control rats (group1).

Table (2): The effect of aqueous extract of *S. dubium* seeds on hematological parameters of rats

Parameters	Control (distilled water)	*S. dubuim* seed (2ml/kg/)	*S. dubuim* seed (10ml/kg/)	*S. dubium* seed (20ml/kg/)	*S. dubuim* seed (40ml/kg/)
Hb (g/dl)	12.68±0.28	13.06±0.25	12.70±0.45	12.71±0.39	13.16±0.37
RBC ($X10^6mm^3$)	9.04±0.16	8.80±0.23	7.62±0.73	7.94±0.55	7.47±1.07
PCV (%)	52.36±1.18	52.26±0.93	45.7±4.46	46±2.68	42.63±6.12
MCV (m^3)	58.40±0.84	58±0.81	59.83±0.30	58.16±1.22	57.33±0.55
MCH (pg)	14.05±0.18	14.71±0.23*	15.25±0.22*	15.26±0.19*	15.01±0.142*
MCHC (%)	24.55±0.056	24.98±0.19	25.3±0.45	26.2±0.31	29.4±1.73*
WBC ($X10^3mm^3$)	9.36±1.78	8.14±0.88	6.29±1.27	6.52±1.30	8.44±1.59
Lymphocytes (%)	44.74±4.84	49.08±4.03	31.58±3.0*	34.31±5.47	41.58±5.47
Monocytes (%)	1.66±0.74	0.833±0.095	2.28±1.44	3.26±1.18	3.32±1.50
Granulocytes (%)	53.58±5.32	50.08±4.11	66±2.84	62.43±3.73	55.35±4.94

Values are expressed as Mean ±SE; *Significant at $P<0.05$.

Serobiochemical changes of rats given daily oral doses of *S. dubium* seeds aqueous extract at 2ml/kg (group2), 10ml/kg (group3), 20ml/kg (group4), 40ml/kg (group5), for one week is presented in Table (3). After one week, the total protein, albumin and Sodium in (group3) and creatinine in (group 4) were lower ($p<0.05$) than the control group (group1). In the observational study, no behavioral changes and death was observed, no any signs of toxicity at the end of the treatment, even at the highest dose of the *S. dubium* seeds is noticed.

In the acute oral toxicity study LD_{50} was considered as more than 2000 mg/kg b.w. The observed changes, plus or minus, in rats weight in different doses, might be due to some other factors, may be environmental, however, no significant change in other hematological and biochemical parameters were noticed. This study is supported by (Bello, *et al.,* 2005) who revealed that the aqueous crude extract of *Solanum melongena* L was found to be not toxic in Swiss albino mice (LD50>3000mg/kg/oral). Also Prashanta, *et al.*, 2014 revealed that the crude methanolic extract

of the dried fruits of *Solanum indicum* Linn. to be safe till a dose of 2000 mg/kg in adult Wister albino rats. Another study strongly indicates the protective effect of *Solanum xanthocarpum* against acute liver injury which may be attributed to its hepatoprotective activity, and thereby scientifically support its traditional use (Ramesh, *et al*., 2011). Marwa, *et al.*, 2013 revealed that the effect of *Solanum nigrum*, dried fruits or its ethanolic extract against lambda cyhalothrin toxicity in rats was able to reverse the pathological parameters and may be useful as a hepatoprotective agent. Osman, 2001 studied the toxicity of *S. dubium* seed by feeding rats an enzyme extracted from the plant as well as white cheese made with enzymes revealing that *S. dubium* seed extract and *Solanum* cheese did not significantly affect the total protein and minerals of serum of all food groups, and no remarkable gross or histopathogical alteration was detected in the liver or kidney of all experimental and control group indicating that *S. dubuim* L. is free of toxins.

Table (3): The effect of aqueous extract of *S. dubium* seeds on biochemical parameters of rats

Parameters	Control distilled water	*S. dubium* seed (2ml/kg/)	*S. dubium* seed (10ml/kg/)	*S. dubium* seed (20ml/kg/)	*S. dubium* seed (40ml/kg/)
AST u/l	21.36±13.14	20.66±26.28	19.82±29.17	24.40±11.69	22.42±6.33
ALT u/l	56.55±3.19	53.73±4.51	59.5±4.44	66.25±6.86	57.71±4.86
ALP u/l	186.33±13.46	160.5±16.39	207.67±3.29	169.33±10.2	173±8.41
Total protein g/dl	6.03±0.149	5.98±0.11	4.88±0.69*	5.98±0.10	5.53±0.46
Albumin g/dl	3.76±0.04	3.73±0.03	2.81±0.34 *	3.6±0.081	3.58±0.083
Globulin g/dl	2.26±0.17	2.25±0.09	1.9±0.30	2.38±0.05	1.95±0.39
Total bilirubin mg/dl	0.56±4.33	0.55±0.04	0.66±0.04	0.48±0.10	0.51±3.43
Direct bilirubin mg/dl	0.36±0.10	0.33±0.05	0.34±0.07	0.425±0.08	0.19±0.09
Urea mg/dl	26.5±5.39	23.08±1.48	20.4±3.8	23.3±1.94	21.08±3.9
Creatinine mg/dl	0.83±0.082	0.81±0.03	0.76±0.03	0.78±0.04	0.68±0.08*
Cholesterol mg/dl	61.8±1.6	57.6±7.7	51.6±5.9	75.5±5.01	68.3±7.5
Potassium mmol/l	5.24±0.06	5.17±0.12	5.44±0.08	5.29±0.18	5.26±0.08
Sodium mmol/l	145.3±0.91	145.3±0.55	142±1.09*	143.6±0.91	146.6±0.76

Values are expressed as Mean ±SE; *Significant at P<0.05.

The protective action of *Solanum trilobatum* extract (STE) was evaluated by Shahjahan, *et al*., (2004) in an animal model of hepatotoxicity induced by carbon tetrachloride (CCl_4), the observations suggested that the treatment with *S. trilobatum* extract enhances the recovery from CCl_4 induced hepatic damage due to its antioxidant and hepatoprotective property.

CONCLUSION

From these findings, it is concluded that the aqueous seeds extract of *Solanum dubium* is not toxic and is safe to be used as a herbal remedy.

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REFERENCES

Bello S.O, B.Y. Muhammad, K.S., Gammaniel,. Abdu-Aguye, H. Ahmed, C.H. Njoku, U.H., Pindiga., and A.M. Salka. (2005). Preliminary Evaluation of the Toxicity and Some Pharmacological Properties of the Aqueous Crude Extract of *Solanum Melongena*. Research Journal of Agriculture and Biological Sciences. 1 (1): 1-9.

Burns MM (2000). Alternative medicine: Herbal preparation. *Clin. Ped. Emerg. Med*., 1: 186-190.

Gurib-Fakim A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol. Aspects Med*, 27: 1-93.

Habbani, E. S. (1992)." A study of plant rennet extracted from *Solanum dubium* (Gubbain)". M.Sc. (Agric.) Thesis, Faculty of Agriculture, University of Khartoum, Sudan.

Marwa M Abd Elkawy, Ghada Z A Soliman, Emam Abd-El Mobd'ea and Abd-El Rehim. (2013). Effect of *Solanum nigrum* Linn. against Lambda Cyhalothrin-Induced Toxicity in Rats. *Journal of Pharmacy and Biological Sciences* ,5: 55-62.

Mohamed, B. E. and Habbani, E. S. (1996). Utilization of *Solanum dubium Fresen* for cheese making U.K. J. Agric. Sci., 4 (2): 139-148.

McKay DL and Blumberg JB (2007). A review of the bioactivity of South African herbal teas: Roobos (*Aspalathus linearis*) and Honey comb (Cyclopia intermedia). *Phytother. Res.*, 21: 1-16.

Osman, D. A. A. (2001)." Studies on a rennet substitute from *Solanum dubium* Fresen (Gubbain)". Ph.D. Thesis, University of Khartoum, Sudan.

Osman, M. S. (1996)." Purification and characterization of milk clotting protease from *Solanum dubium*". M.Sc. Thesis, University of Khartoum, Sudan.

Prashanta Kr. Deb, Ranjib Ghosh, Raja Chakraverty, Rajkumar Debnath, Lakshman Das, and Tejendra Bhakta. (2014). Preliminary phytochemical screening and pharmacological evaluation of the dried fruit extract of *Solanum indicum* Linn. *International Journal of Pharmaceutical Sciences Review and Research*, 25 (2): 28-32.

Pribitkin EA (2005). Herbal Medicine and Surgery. *Seminars in Integrative Medicine*, 3: 17-23.

Ramesh K Gupta, Talib Hussain, G Panigrahi, Avik Das, Gireesh Narayan Singh, K Sweety, Md Faiyazuddin, and Chandana Venkateswara Rao.(2011). Hepatoprotective effect of *Solanum xanthocarpum* fruit extract against CCl_4 induced acute liver toxicity in experimental animals. Asian Pacific Journal of Tropical Medicine . 4(12): 964-8.

Salih, H.M. (1979)."Investigation of alkaloidal content of certain *Solanum* species." M.Sc. Thesis, University of Khartoum, Sudan.

Shajahan, M, K.E Sabitha, Mallika Jsinu and C. S. Shyamala Devi. (2004). Effect of *Solanum trilobatum* against carbon tetrachloride induced hepatic damage in albino rats. Indian J. Med. Res. 120: 194-198.

World Health Organization. (2008). "Fact sheet no. 134: Traditional medicine".

Yousif, B.H., Mc Mahon, D.J. and Shammet, K.M. (1996)." Milk clotting enzyme from *Solanum dubium* plant", International Dairy j. 6: 637- 644.