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Toxicity studies on a Unani herbal formulation "Capsule Hudar" in albino rats

Seema Akbar[1*], A. Masood[2], Shamshad A. Khan[3] and Showkat Ali[1]

[1]Regional Research Institute of Unani Medicine and [2]Department of Biochemistry, the University of Kashmir Campus, Srinagar 190 006 JK

[3]Central Council for Research in Unani Medicine, New Delhi

Abstract

Capsule Hudar, a very effective compound formulation of Unani System of Medicine, contains Azraqi (*Strychnos nux vomica*) a deadly poison as one of its constituents. However, it (*Strychnos nux vomica*) is detoxified, using a classical crude method, before its use to prepare the drug. Results on the evaluation of the drug at dose rate of 5 mg/kg body weight, orally as aqueous extract for 60 days in either sex for its toxicity in Wistar albino rats (150 ± 10g body weight) did not show any significant change in the blood glucose, blood urea and blood urea nitrogen and serum albumin and proteins levels. The activities of GOT/GPT and ACP/ALP levels in serum, brain, kidney, liver and spleen were also not significantly different when compared to controls.

Keywords: capsule hudar, detoxification, strychnine, *strychnos nux vomica*, Unani herbal formulation,

Introduction

Some drugs of the plant, animal and mineral origin used in Unani system of medicine have components that are naturally toxic in their properties. Such drugs, are therefore, detoxified in order to reduce/ overcome their toxicity. In Oriental medicine *Strychnos nux-vomica L.* (Loganiaceae) seeds have been used in activating the channels, alleviating pain and reducing swelling (Bensky and Gamble, 1989). The pharmacological effects of this plant have also been known to increase spinal reflexes and stimulate respiratory and sensory centers of the cerebral cortex (Chung and Shin 1989). In Unani system of medicine the *Strychnos nux-vomica* seeds(Azraqi) in combination with dried ginger ((*Zingiber officinalis)* powder (1:1 w/w) have been used very effectively to elevate blood pressure but after their detoxification in the form of capsule Hudar. The capsule is also used to treat peripheral neuritis, nocturnal enuresis, body aches due to nervine weakness and arthalgia in adults (Anonymous, 1981a). The drug is fairly soluble in water (28.9% w/v) and contains alkaloids as major constituents. Proteins, starch, and resins are also present in small amounts (unpublished results). Azaraqi is a deadly poison and is detoxified before its use for the preparation of drug. Its main constituent is alkaloid strychnine with human LD50 as 0.7-2.1 mg/kg (Jackson and Marsh, 1997). In the present study the drug has been evaluated for its toxicity in albino rats of either sex.

Corresponding author:
E-mail: akbar_masood@hotmail.com
Mob.: +911942424697, +919906966281

Materials and Methods

Adult male and female Wistar albino rats (150 ± 10g body weight) were used. The animals were maintained on a 12 hr light / dark cycle and had food and water *ad libitum*. The two sexes were kept separate from each other throughout the study. The animals (both experimental and controls) were fasted for 18 hours before their sacrifice, since this gave more uniform and consistent results. They were divided into four groups *viz.* I, II, III and IV. Animals of group I and III served as male and female controls respectively while group II and IV animals were male and female experimental. The institute is registered with CPCSE and the study has the clearance of institutional animal ethics committee.

Enzyme assays and biochemical estimation

The animals were sacrificed by draining the blood from the jugular vein. The blood was

collected in clean centrifuged tubes and kept for serum separation. Blood glucose was determined by the method of (GOD/POD) Trinder (1975) ;Acid phosphatase (E.C. 3.1.3.2) was assayed according to King and Jagatheesan (1959); Alkaline phosphatase (E.C. 3.1.3.1) according to Kind and King (1954). SGOT (E.C. 2.6.1.1) and SGPT (E.C. 2.6.1.2) activities were determined by the method of Reitman and Frankel (1957). Total proteins were estimated using biuret method given by Gornell et.al (1949). Serum albumin was estimated following Bromocresol green method proposed by Doumas et.al (1975) and blood urea was estimated by DAM method. All the estimations were done using diagnostic reagent kits manufactured by Span Diagnostic India Ltd and the statistical analysis was done using student's t test.

Detoxification of Azaraqi seeds

Azaraqi detoxified as per the method given in National Formulary of Unani Medicine (Anonymous, 1981b). 70 grams of Azraqi seeds are buried in Peeli Matti (yellow clay) for 10 days. The clay is kept moist throughout. The seeds are then removed and washed. The seed coat is peeled off with the help of a sharp knife and the cotyledons are separated after removing the embryo. The healthy cotyledons are then washed with hot water. The cotyledons in a cloth bag are boiled in 2 lit of milk till it evaporates (the cloth bag should not touch the bottom of the container). The water washed cotyledons are then used subsequently.

Results and Discussion

Animal studies were conducted on either sex because the active principal of the main ingredient of the drug, strychnine is reported to have LD 50 different in male and female rats (Stuart, 2002) the results indicate that aqueous extract of capsule Hudar does not alter the values of glucose, blood urea and blood urea nitrogen.

GOT/GPT and ACP/ ALP are important in determining the adverse effects on the vital organs of the body. Our results indicate that these enzymes were not significantly changed in serum, brain, liver, kidney and spleen even after prolonged administration of the drug for 60 days. Furthermore, the histopathological findings in rat organs are in agreement with the results of biochemical studies in animal tissues (Unpublished results). In the process of detoxification of *Strychnos nux vomica* seeds the amount of strychnine present in the drug plays key role. If the same dose of the drug containing the seed of strychnine without detoxification was given to the rats for the same period, animals showed convulsions and increase sensitivity towards light,

Table 1: Effect of Capsule Hudar (5mg / kg b.w. orally daily) on the level of blood glucose, Total serum protein, albumin, Blood urea and BUN activity in female and male albino rats. Treatment continued for 60 days. Each group consisted of 6 animals and the values are mean±SE of six replicates.

	Blood[a] Glucose	Serum[a] protein	Serum[a] Albumin	Blood[a] urea	Blood urea[a] nitrogen
Male control	88.17 ± 5.8	7.30 ± 0.37	3.47 ±0.13	27.19 ±2.9	12.70 ±1.4
Male treated (5mg / kg b.w. orally, Cap Hudar)	90.99 ± 7.6	7.37** ±0.54	3.24** ±0.16	34.6*` ±4.6	16.17* ±2.2
Female control	69.18 ± 5.6	6.94 ±0.98	3.61 ±0.13	33.20 ±4.7	15.50 ±1.27
Female treated (5mg / kg b.w. orally, Cap Hudar)	73.09 ± 7.79	7.14** ±1.0	3.52** 0.30	35.35** ±3.3	16.15** ±1.5

a = mg/100 ml; * $p<0.5$ but $p>0.1$, difference non significant.; **$p>0.5$, significantly not different from control.

Table 2: Changes in the level of Acid phosphatase in serum, liver, brain, kidney and spleen of capsule Hudar treated male and female albino rats 5mg / kg b.w. of drug was given orally for 60 days. Each figure represents mean ± SE of 10 samples.

Group	Serum[a]	Liver[b]	Brain[b]	kidney[b]	Spleen[a]
Male control	13.30 ± 0.46	2.20 ± 0.29	0.476 ±0.026	3.82 ±0.31	6.50 ±0.31
Male treated (5mg / kg b.w. orally, Cap Hudar)	14.78 ± 0.98	2.50^{+} ±0.25	0.483 ±0.31	4.01 ±0.29	6.42 ±0.28
Female control	15.18 ± 0.12	2.60 ±0.04	0.57 ±0.034	3.30 ±0.29	6.64 ±0.17
Female treated (5mg / kg b.w. orally, Cap Hudar)	18.37* ± 2.39	2.66* ±0.23	0.51* 0.028	2.64** ±0.15	7.18* ±0.42

$^{+}$p = 0.5, significantly not different from control; *p>0.5, significantly not different from control;
** p<0.5 but p>0.1, difference non significant; a=mg phenol librated /100ml serum; b=mg phenol librated /100ml tissue.

Table 3: Changes in the level of Alkaline phosphatase in serum, liver, brain, kidney and spleen of capsule Hudar treated male and female albino rats 5mg / kg b.w. of drug was given orally for 60 days. Each figure represents mean ± SE of 10 samples.

Group	Serum[a]	Liver[b]	Brain[b]	kidney[b]
Male control	47.18±0.08	0.054± 3.21	0.230± 0.002	4.82±0.87
Male treated (5mg / kg b.w. orally, Cap Hudar)	51.23± 2.99	0.052*±0.001	0.213**±0.021	4.76**±0.97
Female control	50.44± 4.8	0.0675±0.013	0.231±0.014	7.55±0.42
Female treated (5mg / kg b.w. orally, Cap Hudar)	60.93***± 6.01	0.052***±0.001	0.211***±0.019	6.22**±0.67

* p=0.5, significantly not different from control; **p>0.5, significantly not different from control.
***p<0.5 but p>0.1, difference non significant; a=mg phenol librated /100ml serum; b=mg phenol librated /100ml tissue.

Table 4: Effect of Capsule Hudar (5mg / kg b.w. orally) on the level of Glutamate Pyruvate transaminase (GPT) in serum, liver, brain, kidney and spleen of male and female albino rats. Treatment continued for 60 days. Each figure represents mean ± SE of 10 animals.

Group	Serum[a]	Liver[b]	Brain[b]	kidney[b]	Spleen[a]
Male control	53.87 ± 0.61	10.08 ± 1.12	4.40 ±0.18	4.44 ±0.23	4.52 ±0.33
Male treated (5mg / kg b.w. orally, Cap Hudar)	54.66 ± 3.6	11.23* ±1.06	4.87 ±0.46	4.27** ±0.17	4.25*** ±0.17
Female control	49.79 ± 2.45	8.97 ±0.035	4.23 ±0.11	4.23 ±0.104	4.25 ±0.28
Female treated (5mg / kg b.w. orally, Cap Hudar)	46.86* ±3.89	9.51* ±0.40	4.10 ±0.17	4.06* ±0..24	4.17** ±0.054

* p<0.5 but p>0.1, difference non significant; *p>0.5, difference non significant; *** p = 0.5, difference non significant
[a]μmoles of pyruvate formed / minute / L serum; [b]μmoles of pyruvate formed / minute / g tissue.

Table 5: Effect of Capsule Hudar (5mg / kg b.w. orally) on the level of Glutamate oxaloacetate transaminase (GOT) in serum, liver, brain, kidney and spleen of male and female albino rats. Treatment continued for 60 days. Each figure represents mean ± SE of 10 animals.

Group	Serum[a]	Liver[b]	Brain[b]	kidney[b]	Spleen[a]
Male control	38.31 ±0.83	5.45 ±0.16	5.85 ±0.39	5.05 ±0.11	3.55 ±0.04
Male treated (5mg / kg b.w. orally, Cap Hudar)	41.46* ±4.2	5.51** ±0.94	5.05*** ±0.081	4.81*** ±0.19	3.44 ±0.09
Female control	35.85 ±1.3	5.61 ±0.08	5.63 ±0.18	5.24 ±0.23	3.94 ±0.003
Female treated (5mg / kg b.w. orally, Cap Hudar)	36.70** ±0.63	5.54** ±0.18	5.52** 0.12	5.38** ±0.96	3.70*** ±0.14

* p = 0.5, difference non significant; **p>0.5, difference non significant; *** p<0.5 but p>0.1, difference non significant. [a]μmoles of pyruvate formed / minute / L serum; [b]μmoles of pyruvate formed / minute / g tissue.

smell and sound. Detoxification of Azraqi seeds resulted in a decrease in the strychnine content (Unpublished results). Though larger doses of strychnine are known to be deadly poison (Jakson and Marsh, 1997), but in lower doses it gives subjective feeling of stimulation (Samulesson, 1992).

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