Toxicological Evaluation of a Unani Herbal Formulation

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ABSTRACT

Qurs-e-mubarak, a Unani herbal antipyretic formulation was studied for its toxicity in albino rats. It has been found to be quite safe since its LD50 in male albino rats is about 7g / kg body weight. The aqueous extract of the drug did not induce significant changes in acute toxicity studies. On prolonged administration for 90 days the aqueous extract did not produce any significant change on metabolic profile. The histopathological studies of liver and brain, on chronic treatment, revealed that these organs were unaffected. The studies suggest that the formulation is safe for use and devoid of toxic effects.

Keywords: Acetylcholinesterase, blood glucose, glycogen, phosphatases, qurs-e-mubarak, serum proteins, transaminases, unani herbal formulation.

INTRODUCTION

Qurs-e-mubarak is a compound of Unani formulation, which has been widely and effectively used to treat pyrexia (fever) in general (Anon., 1981). It has also been found effective against pyrexia of unknown etiology. Its composition includes Zeera Safaid (*Cuminum cyminum*) 1 part, Filfil Daraz (*Piper longum*) 1 part, Maghz-e-karanjawa (*Caesalpinia bonducella* seeds) 2 parts and Barg-e-babool (*Acacia arabica* leaves) 1 part, all w/w. The drug is fairly soluble in water and contains alkaloids, phytosterol, terpenoids and other basic organic compounds, though in low concentrations. Resins and tannins are also present in small amounts and calcium is

also present in appreciable amount (about 30 mg/g drug ash) (Anon.,1991) In the present study the drug was evaluated for its toxicity in albino rats.

MATERIAL AND METHODS

Materials

Adult male albino rats(150 ± 10 g) were used. The animals were maintained on a 12-hour light/dark cycle and had food and water adlibitum. They were kept separate from the females. 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 three groups. Animals of group one served as controls and were given normal saline; those of group two were administered the drug (50 mg/kg body weight,orally) daily for 90 days. Group three animals were divided into two sub-groups: first sub-group of animals were given 500 mg drug/kg body weight orally, while the second sub-group received 1g drug / kg body weight orally. The animals were sacrificed 14 days after the treatment.

Biochemical Estimation and Enzyme Assays

The animals were sacrificed by draining the blood from the jugular vein. The blood was collected in clean centrifuge tubes and kept for serum separation. Brain was removed immediately after the sacrifice and acetycholinesterase (E. C. 3. 1. 1.7) activity was measured according to the method of Ellman *et al.* (1961), using acetylthiocholine as the substrate. The brain glycogen was extracted as per the method given by LeBaron (1955) and estimated colorimetrically as described by Montgomery (1957). Blood glucose was determined by the method of Nelson (1944). Acid and alkaline phosphatases (E. C. 3. 1. 3. 2 and 3. 1. 3. 1 respectively) were assayed according to Wooten *et al.* (1964) using disodium phenyl phosphate as the substrate at pH4 and 10 respectively. SGOT (E. C. 2. 6. 1. 1) and SGPT (E. C. 2. 6. 1. 2) activities were determined by the method proposed by Wooten (1964). LD_{50} was determined by the method of Weil (1952). Total serum proteins were estimated using biuret method given by Gamall *et al.* (1949).

RESULTS

Data on the effect of Qurs-e-mubarak (50 mg/kg body weight given orally for 90 days) on the level of blood glucose, total serum proteins and albumin, hepatic and cerebral cortex glycogen; RBC, cerebral cortex and striatal acetylcholinesterase; liver, brain and kidney alkaline and acid phosphatases, and GOT and GPT (Table 1and 2) indicate that the values in drug treated animals were not significantly different from the control values. Similar results were obtained with the drug (500 or 1000 mg/kg body weight) when administered orally for 14 days (Table 3, 4 and 5).The LD50 in male albino rats was 7 g ± SE.

Table 1. Effect of Qurs-e-mubarak (50 mg/kg body weight, orally, daily) on the level of blood glucose, hepatic and cerebral glycogen, total serum protein and albumin and acetylcholinesterase activity in albino rats. Treatment continued for 90 days. Each group consisted of 6 animals and the values are mean ± SE of six replicates.

Group	Blood[a] Glucose	Glycogen[b]		Serum[c] protein	Serum[c] albumin	Acetylcholinesterase		
		Cerebral cortex	liver			Cerebral[d] Cortex	Corpus[d] striatum	RBC[e]
Control	98	51	322	6.80	3.90	5.00	32	4.04
	±6	±4	±22	±0.49	±0.24	±0.44	±2.59	±0.30
Qurs-e-mubarak	97	52	318	7.00	3.76	4.98	33	4.09
	±8	±3	±27	±0.79	±0.18	±0.30	±2.78	±0.32

a. mg / 100 ml blood b. mg /100 g fresh tissue c. g / 100 ml serum
d. µ moles of acetyl thiocholine hydrolysed / min / g fresh tissue
µ moles of acetyl thiocholine hydrolysed / min / ml RBC.

Table 2. Effect of Qurs-e-mubarak (50 mg/kg body weight, orally, daily) on enzymes activity in liver, brain and kidney of rats. Treatment with Qurs-e-mubarak continued for 90 days. Each group consisted of six animal and the values are mean ± SE of six replicates.

Enzymes	Liver		Brain		Kidney	
	control	treated	control	treated	control	treated
Alkaline phosphatase[a]	4.98 ±0.78	5.68 ±0.95	10.1 ±1.1	11.0 ±1.02	272 ±12	288 ±13
Acid phosphatase[a]	17.17 ±0.7	18.20 ±0.92	13.3 ±1.4	12.9 ±0.75	37.33 ±3.5	36.2 ±2.5
GOT[b]	10.5 ±2.64	11.2 ±0.78	18.6 ±0.41	17.7 ±0.62	29 ±3.0	28 ±1.29
GPT[b]	5.7 ±0.3	5.9 ±0.2	16.1 ±0.41	17.5 ±0.94	40 ±1.76	42 ±1.33

a. nmoles of phenol liberated / min / mg protein

b. nmoles of pyruvate formed / min / mg proteins.

Table 3. **Effect of Qurs-e-mubarak (500 and 1000 mg / kg body weight, orally, daily) on the level of blood glucose, hepatic and cerebral glycogen, total serum protein and albumin and acetyl-cholinesterase activity in albino rats. Treatment continued for 14 days. Each group consisted of 6 animals and the values are mean ± SE of six replicates.**

Group	Glycogen[b]					Acetylcholinesterase		
	Blood[a] glucose	Cerebral cortex	liver	Serum[c] protein	Serum[c] albumin	Cerebral[d] Cortex	Corpus[d] striatum	RBC[e]
Control	96 ± 3	52 ± 3	318 ± 11	6.50 ± 0.62	3.85 ± 0.24	4.99 ± 0.10	31 ± 2.78	4.04 ± 0.19
Qurs-e-mubarak (500 mg/kg body weight, orally)	89 ± 10	54 ± 4	321 ± 31	6.50 ± 0.85	4.00 ± 0.30	5.02 ± 0.19	32 ± 2.63	4.14 ± 0.28
Qurs-e-mubarak (1000 mg/kg body weight, orally)	100 ± 9	54 ± 5	320 ± 28	6.70 ± 0.58	4.11 ± 0.33	4.95 ± 0.46	32 ± 2.87	4.02 ± 0.33

a. mg / 100 ml blood

b. mg / 100 g fresh tissue

c. g / 100 ml serum

d. μ moles of acetyl thiocholine hydrolysed / min / g fresh tissue

e. μ moles of acetyl thiocholine hydrolysed / min / ml RBC

Table 4. Effect of Qurs-e-mubarak (500 mg /kg body weight, orally, daily) on enzymes activity in liver, brain and kidney of rats. Treatment continued for 14 days. Each group consisted of six animals and the values are mean ± SE of six replicates.

	Liver		Brain		Kidney	
Enzymes	**control**	**treated**	**control**	**treated**	**control**	**treated**
Alkaline phosphatase[a]	4.93 ±0.44	5.05 ±0.48	9.8 ±1.1	10.1 ±0.96	272 ±23	276 ±18
Acid Phosphatase[b]	16.97 ±1.5	17.18 ±1.22	13.1 ±1.2	12.8 ±0.89	36.73 ±3.3	36.40 ±3.5
GOT[b]	10.3 ±1.63	10.0 ±0.86	17.8 ±1.61	17.7 ±1.44	27 ±2.8	26 ±2.23
GPT[b]	5.9 ±0.4	5.6 ±0.3	16.8 ±1.43	17.2 ±1.96	37 ±3.71	39 ±3.33

a. nmoles of phenol liberated / min / mg protein
b. nmoles of pyruvate formed / min / mg proteins.

Fig. 1. Section of brain from Qurs-e-mubarak treated albino rats. Treatment continued for 90 days.

Table 5. Effect of Qurs-e-mubarak (1000 mg /kg body weight, orally, daily) on enzymes activity in liver, brain and kidney of rats. Treatment continued for 14 days. Each group consisted of six animals and the values are mean ± SE of six replicates.

	Liver		Brain		Kidney	
Enzymes	**control**	**treated**	**control**	**treated**	**control**	**treated**
Alkaline phosphatase[a]	5.01± 0.38	5.03± 0.51	10.0± 0.9	9.96± 0.92	281± 27	279±31
Acid Phosphatase[a]	16.66± 1.3	16.31± 1.66	12.88± 1.3	12.7± 1.86	34.88± 3.8	35.01± 3.
GOT[b]	10.6	10.2	16.9	17.1	29	27
	± 1.08	± 0.96	± 1.42	± 1.63	± 2.5	± 2.63
GPT[b]	6.1	5.9	17.0	16.8	36	37
	± 0.51	± 0.46	± 1.63	± 1.66	± 3.53	± 3.66

a. nmoles of phenol liberated / min / mg protein

b. nmoles of pyruvate formed / min / mg proteins

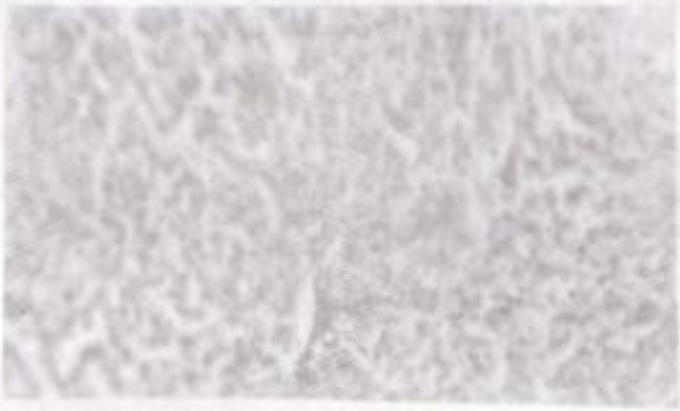

Fig. 2. **Section of liver from Qurs-e-mubarak treated albino rats. Treatment continued for 90 days.**

DISCUSSION

The results indicate that aqueous extract of Qurs-e-mubarak does not alter the values of glucose and cerebral or peripheral glycogen. Cerebral acetylcholinesterase activity was in the normal range even after administration of high doses of the drug. GOT/ GPT and ALP/ ACP are important in determining the adverse effects on the vital organs of the body. Our results indicate that these enzymes were not significantly changed even after prolonged administration of the drug. LD50 of the compound which is often relied upon for predicting the safety of the drug was about 7 g/kg body weight, which also suggests that the compound is devoid of toxic effects. Histopathological studies also showed similar results. Section of brain of the treated rats (Fig.1) did not show any dense mass of the nuclei and also was not fragmented. Pykinosis was also not seen. In liver section (Fig.2), cynocytes were not dilated, portal vein was normal and peripheral and central zones were also intact. These studies support the conclusion that Qurs-e-mubarak did not have adverse effects on vital organs like brain and liver.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Mohammad Khalid Siddique, Director, Central Council for Research in Unani Medicine, New Delhi, India, for his interest in this work. Thanks are also due to Prof. A. R. Yousuf, Director, CORD, University of Kashmir, Srinagar, who provided required laboratory facilities.

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