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Effect of *Qurs-e-Rewand* (A Hepatoprotective Unani Formulation) on Pentobarbitone induced Sleeping in Mice

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Abstract

Background: *The present study was planned to investigate the effect of Qurse Rewand commonly used compound hepatoprotective drug on sleep duration in sodium pentobarbital (PB)-induced sleeping in mice.*

Methodology: *The animals were divided into five groups of 6 animals each and they were fasted overnight. The animals of group I and II were left untreated, whereas the animals kept in group III, IV and V were administered, Silymarin (100 mg/kg b. w.) Extract of Qurse Rewand (50mg/kg b. w.) and (100 mg/kg b. w.) respectively as single and double dose orally for 7 days. On the 8th day the animals in group II, III, IV and V were given CCl_4 in dose of 2 ml/kg b. w. intraperitonial route. Two hours later the animals in all the five groups were administered 30 mg/ kg b. w. of sodium pentobarbitone I.P. The sleeping time was recorded by observing the CNS activity as pinna, sound and righting reflex in each animal at every five minutes.*

Results: *The findings obtained, indicated the significant reduction ($P \leq 0.05$-0.001) in the onset and duration of pentobarbitone-induced sleep in mice treated with the test drug of both the doses and it was found significant against CCl_4+Pentobarbitone induced animals. The results suggest that Qurse Rewand shortened the pentobarbital hypnosis without major toxic effect and the result exhibited by the double dose of QR was more potent than single dose and shortening of duration is almost equal to the standard drug.*

Conclusion: *On the basis of reduction in sleeping time it can be concluded that the test drug is quite safe and has wide therapeutic index and therefore can be safely used in high doses also.*

Key words: *Hepatoprotective drug, Sodium pentobarbitone, sleeping time, CCl_4, Silymarin*

1. Introduction

Qurs-e-Rewand is one of the reputed compound formulations of Unani system of Medicine, extensively used in various liver disorders such as Chronic fever, Hardness and Inflammatory conditions of Liver and Spleen[1,2,3,4,5], Haemoptysis[6,7,8], Ascitis[9], Obstruction in Liver[10], Bloody Diarrhoea[11], Jaundice, Headache, Liver and Spleenic pain, Dysuria, Indigestion, Anti-dote of poisons[12]. Majority of the drugs are metabolized in the liver and the liver is responsible for the selective uptake, concentration, and excretion of the drugs and toxins that are introduced into the body. While some parent drugs can directly cause hepatotoxicity, it is generally the metabolites of these compounds that lead to drug-induced liver injury[13]. Since the liver is most important place for metabolism and excretion therefore, the normal functioning as well as structure of the liver may be affected resulting in prolongation of sleep duration. Herbal medicines are often mistakenly regarded as safe because they are natural. Nevertheless, those products contain bioactive principles with potential to cause adverse effects. It is therefore, important that all herbal medicines are subjected to efficacy and safety tests by the same methods used for new synthetic drugs[14]. Secondly, the sleeping duration test aims at establishing the therapeutic index. The greater the index, the safer the compound[15]. A number of studies have reported the toxic effects of herbal medicines[16,17,18]. Many drugs, both prescription and over the counter, herbal products, or toxins can cause hepatotoxicity through a variety of mechanisms[19]. The Unani system of medicine has a major role in the treatment of liver ailments, as it possesses several hepatoprotective single and compound drugs that are highly effective, safe and have been subjected to scientific study, mostly with good results. However, a number of drugs particularly compound formulations have still not been scientifically evaluated for their effects on CNS to rule out the integrity and level of injury of liver by the onset and duration of sleep. Presently about eighty percent of the world's population relies on traditional medicine for health care delivery [20]. This interest is channeled into the discovery of new biologically-active molecules by the pharmaceutical industry and into the adoption of crude and extracts of plants for self-medication by the general public. Studies of medicinal plants using scientific approaches showed that various biological components of medicinal plants exhibit a variety of properties and can be used to treat various ailments[21,22]. The different composition of *Qurse Rewand* have been mentioned in various pharmacopoeias however, the composition mentioned in "*Ilaj-ul-Amraz*" got tremendous popularity for its efficacy in liver ailments[7], described to be effective in liver diseases and prescribed commonly by the physicians has not been investigated for its hypnotic effect. *Qurs-e-Rewand* is used in various diseases but it has not been evaluated scientifically for its effect on sleep and toxicity. In present study the sleeping time was evaluated by observing the pentobarbitone narcosis as pentobarbitone sodium metabolized by liver and damaged liver does not metabolize or detoxify drug, so that the effect of the drug

prolonged and the sleeping time observed with it is a measure of liver damage. This functional parameter was used to determine the metabolic activity of the liver. Therefore, the present study was planned to evaluate the effect of hydro-alcoholic extract of *Qurse Rewand* (HAE) in different doses on pentobarbitone induced sleep in mice.

2. Materials and Methods

Ingredients of *Qurs-e- Rewand*

Botanical Name	Common Name	Unani Name	Quantity (in gm)
Rheum emodi Wall	Indian Rhubarb	Rewand Chini	17.5 gm
Rubia cordifolia Linn	Indian Madder	Majith	10.5 gm
Creteria lacca	Lakh	Luk-e-Maghsool	10.5 gm
Apium graveolens	Celery Seed	Karafs	3.5 gm
Feoniculum vulgare Mill	Fennel	Badiyan	3.5 gm
Agrimonia eupatoria	Agrimony	Gul-e-Ghafis	3.5 gm

2.1 Collection of raw materials and determination of dose

The crude drugs were procured from the local market (Bara Dwari, Aligarh). After the confirmation of purity and identity of the ingredients by the pharmacognosy section of the Department of Ilmul Advia, A. K. Tibbiya College, AMU, Aligarh, all the ingredients of *Qurse-Rewand* were made coarse powdered and the hydro-alcoholic extract of all the ingredients was prepared using soxhlet apparatus, in which they were continuously extracted for 6 hours. The extract was filtered by Whatman No.1 filter paper and evaporated on water bath at 40 - 60^{0}C until it dried completely.

The doses of the extract for albino mice were calculated by multiplying its clinical doses described in Unani literature with conversion factor 7 [23].

2.2 Animals

Thirty albino mice of either sex with weight range of 25-30 gm were used for experiment. The rats were randomly selected and were divided into five groups with six animals in each. They were housed in clean polypropylene cages and the room temperature was maintained at 25 ± 1^0 C with 12 hour light and dark cycle. All the animals received standard diet (Amruta Labs, Pune) and water *ad libitum*. The animals were deprived of food for 12 hours before the treatment. The experimental protocol was approved by the Institutional Ethics Committee.

2.3 Experimental design

The animals were divide in five groups. The animals in group I and II were left untreated. The animals in group III, IV and V were administered Silymarin i.e. standard drug (100 mg/kg b. w.), *Qurse Rewand* single dose (50 mg/kg b. w.) and *Qurse Rewand* double dose (100 mg/kg. b. w.)

orally respectively for seven days. On the 8th day the animals in group II, III, IV and V were given CCl_4 in dose of 2 ml/kg b. w. by intraperitonial route. Two hours later the animals in all the five groups were administered 30 mg/ kg b. w. of sodium pentobarbitone I.P. The sleeping time was recorded by observing the righting reflex in each animal at every five minutes[24]. Pentobarbitone induced sleeping time was observed in untreated rats, rats treated with CCl_4 and those with CCl_4 as well as the drugs. The animals were placed on table after loss of righting reflex. The time interval between loss and regain of righting reflex was measured as Pentobarbitone Sleeping Time (PST).[25,26].

2.4 Drugs and Chemicals

CCl_4, (Thomas Baker Pvt. Limtd. Mumbai), Silymarin (Sigma-Aldrich, Germany), and Pentobarbitone sodium (Sigma U.S.A).

Statistical analysis

The data of all groups (Gp I-V) were statistically compared for determining significance of difference by one-way ANOVA test, followed by pair-wise comparison of various groups by LSD. The analysis was carried out by using the software of the website, www. Analyse it.com. P< 0.05 or less was considered significant.

3. Result

The sleeping time which is the duration of onset of loss of righting reflex and reappearance. The mean sleeping time in control group (Group I) which was treated only with 30 mg/kg Pentobarbitone Sodium i.p. was found as 90 ± 2.6 minutes, whereas in the CCl_4 treated group (Group II) the Sleeping time was observed significantly prolonged to 155 ± 5.3 minutes ($p<0.001$). The mean sleeping time in standard group (Group III) was recorded as 115.5± 3.5 minutes ($p<0.001$), which has shortened the sleeping time. The pretreated extract of *QR* (Group IV) and (Group V) significantly decreased the duration of sleeping time as 120 ± 2.5 minutes and 118 ± 3.2 minutes respectively as single as well as double doses ($p<0.001$). The results are summarized in Table as well as in Graph.

Effect of the hydroalcoholic extract of *Qurse Rwand* on the duration of Pentobarbital-induced sleeping time (CCl_4-intoxicated mice)

Groups	Sleeping time (ST) (min) (mean ± SE)	
	Onset (min) (mean ± SE)	Duration (min) (mean ± SE)
Pentobarbitone Control (30 mg/kg)	8.5 ± 1.2	90 ± 2.6
CCl_4 Control (2 ml/kg)	4.2 ± 0.5	155 ± 5.3
Silymarin (100 mg/kg)	6 ± 0.8 b^3	115.5± 3.5 b^3
Qurse Rewand I (50 mg / kg)	5.2 ± 0.7 b^3	120 ± 2.5 b^3
Qurse Rewand II (100 mg/kg)	5.6 ± 0.6 b^3 c^1	118 ± 3.2 b^3 c^1

(n=6) 1 = P < 0.05, 2 = P < 0.01, 3 = P < 0.001
a= against PBT control, b= against CCl_4, c=against Silymarin

4. Discussion

We investigated the effect of *Qurse Rewand* (*QR*) in varying doses to observe the changes of

duration in sodium pentobarbitone (PB)-induced sleeping time by CNS activity as pinna, sound and righting reflex [27]. The effect was recorded for disappearance (latency) and reappearance (duration) of the righting reflex, as sleeping time is considered to be the time interval between disappearance and reappearance of the righting reflex. The animals treated with CCl_4 alone showed marked increase in pentobarbitone induced sleeping time as the administration of CCl_4 caused significant potentiation of the effect of pentobarbitone in mice as compared to the untreated animals. However, the potentiation of pentobarbitone was observed in all treated groups. The probable cause of potentiation appears to be a delayed metabolism of pentobarbitone by liver [27]. The administration of Silymarin, hydro alcoholic extract of *Qurse Rewand* along with CCl_4, significantly reduced the usual elevation of sleeping time, but did not bring it down that observed in untreated animals. The fall in sleeping time is more marked with Silymarin and double dose of *QR* as compared to the Single dose of *QR*. To substantiate our findings in context of depletion of toxic constituents in test drug pentobarbitone sleeping study of *Q.R.* was assessed in terms of disappearance (latency) and reappearance (duration) of the righting reflex to ascertain the maximum effect of the drug on CNS. It is obvious from the tabulated results that test drug is more safe. On the basis of reduction in sleeping time it can be concluded that the test drug is quite safe and has wide therapeutic index and therefore can be safely used in high doses also.

Drug metabolizing capacity of liver is severely affected due to the damaging effects of any hepatotoxic agents on liver microsomal enzyme system, thus resulting in prolongation of Pentobarbitone induced sleeping time it indicates physiological parameter which highlights the normal or delayed metabolic activity of liver [26]. *Qurse Rewand* single dose (120 min) and double dose (118 min) reduced elevated levels of pentobarbitone induced sleeping time, indicating protective effect on metabolic functions of liver.

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