Acute & Sub-Acute Toxicity Study of Qurse-Nazla in Experimental Animals

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Abstract

The present study was carried out during 2011-2012 to investigate the safety of Qurse-nazla by conducting acute and sub-acute toxicity in Swiss albino mice & rats respectively. Acute toxicity was determined by administering aqueous extract of Qurse-nazla orally to two groups of mice of six each at a dose 1gm/kg and 2gm/kg body weight. The animals were observed for gross behaviour and mortality for 24 hours after drug administration. The formulation was well tolerated by the animals and no abnormality was observed in the general behaviour (salivation, lacrymation, lethargy, sleep and coma) of the animals and no overnight mortality was recorded. Similarly, sub-acute toxicity was determined in albino rats by orally administration of aqueous extract to three groups of seven animals each at a dose ranges from 1gm/kg and 3gm/kg body weight for 28 days. The results of haematology and biochemistry profile done on 29th day were found to be normal and no changes were observed in organ to body wt ratio of liver, heart, kidney and spleen.

Keywords: Acute toxicity, Sub-acute toxicity, Qurse-Nazla, *Ocimum sanctum* Linn., *Cinnamomum zeylanicum* Blume., *Zingiber officinale* Rose., *Tinospora cordifolia* Wild.

Introduction

The World Health Organization is fully aware of the importance of herbal medicines to the health of many people throughout the world, as stated in a number of resolutions adopted by the World Health Assembly and the Regional Committee for the Western Pacific. Thus herbal medicines have been recognized as a valuable and readily available resource for primary health care, and WHO has endorsed their safe and effective use. A comprehensive programme for the identification, cultivation, preparation, evaluation, utilization and conservation of herbal medicines has been developed. Meanwhile, it has been realized that medicinal plants are a valuable resource for new pharmaceutical products and thus a potential source of new drugs as well as for economic development (Anonymous, 1993).

Determination of efficacy and safety of herbal remedies is necessary because many people using these agents as self medication. Since there is limited data available about the safety of the commonly used herbal remedies, therefore, effort to elucidate health benefits and risk of herbal medicine should be intensified. Toxicity testing in animal is typically the initial steps to determine the effect profile of test substance and potential hazards which occurs due to short term exposure of test substance. A toxicity study provides information on the

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hazardous properties and allows the substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity (OECD, 2000).

Formulation

Qurse-nazla is a poly herbal formulation containing main constituents in equal parts:

S.No.	Common Name	Botanical Name
1.	Barg-e-Tulsi	*Ocimum sanctum* Linn.
2.	Darchini	*Cinnamomum zeylanicum* Blume.
3.	Zanjabeel	*Zingiber officinale* Rose.
4.	Satte Gilo	*Tinospora cordifolia* Wild.
5.	Satte Ajwain	*Ptychotica jowar* DC

Methodology

This study was carried out in Pharmacology Research Unit, of Regional Research Institute of Unani Medicine, Aligarh and conducted in accordance with the protocol approved by Institutional Ethics Committee (RRIUM), Aligarh.

Procurement of Drug

The Qurse nazla formulation was procured in the form of Tablet from CRIUM (Central research Institute of Unani Medicine) A.G colony Road Near ESI Hospital, Eragadda Road, Hyderabad.

Animals

The study was carried out in Swiss albino mice (20-25 g) and rats (100-150g) of either sex, for acute & sub-acute toxicity determination respectively. The animals were procured from Mr. Rahat Hussain Enterprises Biological Suppliers Babri Mandi Aligarh. They were acclimatized to the conditions for one week before experimental study. The animals were maintained in a standard environmental condition at a room temperature of (25±2 degree Celsius) with 12 Hrs light/Dark cycles, humidity (50-55%), and had free access to food pellets. The study was conducted after approval of protocol from Institutional ethics committee of RRIUM, Aligarh.

Preparation of Drug Extract

The tablets of the drugs were crushed into fine powder and a weighed quantity was steeped in acidulated distilled water. The water soaked mass of the drug

was warmed over water bath and kept for 24 hours at room temperature. During this period it was occasionally stirred. After 24 hours it was filtered through a filter paper and filtrate was dried over water bath. The aqueous extracts of the drug thus obtained was used in different doses selected according to OECD guidelines for safety evaluations.

Acute Toxicity Study

The current study was carried out in accordance to Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals. Swiss Albino mice of either sex weighing 20-25 g were randomly selected and divide into two groups of six mice each. Mice were kept fasted overnight (12hrs) with free access to water prior to administration of dose ranging 1gm/kg body weight and 2g/kg body weight as per limit test of OECD guideline. The aqueous extract of the drugs was administered orally. The animals were kept in polypropylene cages after drug administration and were observed for Gross behaviour (salivation, lacrymation, lethargy, sleep and coma) & mortality at 1 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour, 12 hour, 24 hour and thereafter once every day up to 14 days after drug administration.

Sub-Acute Toxicity Study

Swiss Albino rats of either sex weighing between 100-150 g were randomly selected and divided into three groups of seven animals each. Rats were kept fasted overnight (12hrs) with free access to water prior to administration of dose ranging 1gm/kg and 3gm/kg body weight for 28 days as per limit test of OECD guideline. Group I was kept as normal control which received distilled water for 28 days, while in the IInd and IIIrd groups aqueous extract of the drug was administered orally at a dose of 1gm/kg and 3gm/kg body weight for 28 days. The animals were observed for Gross behavior (salivation, lacrymation, lethargy, sleep and coma) & mortality at 1 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour, 12 hour, 24 hour and thereafter once every day up to 28 days after drug administration. On 29th day, blood was collected of all the three groups of rats through retro-orbital plexus for estimation of SGOT,SGPT and Serum alkaline Phosphatase, Serum urea and Serum Creatinine, Serum cholesterol, Serum triglyceride and Serum HDL, Percentage hemoglobin, ESR, Total leukocyte count and Differential leukocyte count. After collection of blood the animals in all the three groups were sacrificed and liver, heart, kidney and spleen were excised out for determination of organ to body weight ratio. SGOT, SGPT were estimated by Reitman's and Frankel, method (Reitman *et al.,* 1957). Alkaline Phosphatase was estimated by Bessey and Brock, 1946 method (Bessay *et al.,* 1946). Serum urea was estimated by GLDH, Ureas method

given by (Tiffany *et al.,* 1972), while Serum Creatinine was estimated by Jaffe's method given by (Bower's *et al.,* 1980). Serum HDL was estimated by Phosphotungstic Acid method given by (Burstein *et al.,* 1970), while Serum cholesterol and Triglyceride were estimated by CHOD-PAP method given by (Roeschlau *et al.,* 1974) and GPO-Trindev method given by (Mcgowan *et al.,* 1983). ESR and DLC were estimated by Westergreen and Leishman stain methods given in Medical Laboratory Technology (Mukherjee, 1990). TLC was estimated by Hemoaltometry method (Plum, 1936). Percent hemoglobin was estimated by Sahli's Acid Haematin method given by (Newcomer, 1919).

Statistical Analysis

Statistical analysis was performed by using unpaired t test calculating p value at 5% level. All values are expressed as Mean±SEM (standard error of mean). p value less than 0.05 found to be considered statistically significant.

Results and Discussion

Acute Toxicity Study

The effect of oral administration of single dose of aqueous extract of Qurse nazla in Swiss albino mice shows that the formulation was well tolerated by the animals and no abnormality was observed in the general behaviour of the animals and no overnight mortality was recorded. Herbs and supplements can be toxic when used for inappropriate indication, or prepared inappropriately, or used in large excessive dosages or for a prolonged duration of time. Since it is polyherbal formulation other ingredients present in formulation helps in reducing the toxic effect of active component.

Sub-Acute Toxicity Study

The values of all the parameters including liver functions, renal functions hematology and organ body weight ratio found to be normal as compared to control group. The effects of the studied drug on organ body weight ratio in control and treated animals are presented in Table 4. There were no significant changes observed in organ body weight ratio of the control and the animals treated with various doses. Table 1 and 2 is a summary of the results of the effects of the drug on the biochemical parameters. There were no significant changes in AST and ALT levels in all the treated animals compared with the control. Similarly lipid profile of treated animal was found to be normal as compared to control group. Table 3 reflects the values for hematological parameters of treated group as compared to control showed no significant changes in Hb, TLC, ESR, and % lymphocyte except % polymorph count in

Table 1: Effect of Aqueous Extract of Qurse- Nazla on Liver Function & Renal Function Test in Albino Rats

Groups	Liver Function Test			Renal Function Test	
	SGOT Unit/L	SGPT Unit/L	SALP KAU	Serum Urea Mg/dl	Serum Creatinine Mg/dl
Group I Normal Control (n = 7)	178.07±15.39	47.45±3.83	164.74±20.08	33.70 ± 4.03	1.29 ± 0.07
Group II Drug treated 1g /kg(n = 7)	177.09±14.52 p = 0.9638	45.40±2.43 p = 0.694	166.85±19.30 p = 0.9409	33.56± 2.41 p= 0.9767	1.42 ± 0.06 p = 0.1839
Group III Drug treated 3g /kg(n = 7)	176.02±13.50 p = 0.9219	47.86±4.85 p = 0.9482	169.42±17.42 p = 0.8632	34.83± 4.90 p = 0.8616	1.38±0.05 p = 0.3161

Values are expressed as Mean±SEM
n = number of rats in a group.
P* < 0.05, P** < 0.01, P*** < 0.001

Table 2: Effect of Aqueous Extract of Qurse- Nazla on Lipid Profiles in Albino Rats

Groups	Serum Cholesterol Mg/dl	Serum HDL Mg/dl	Serum Triglyceride Mg/dl
Group I Normal Control (n = 7)	47.12± 2.95	10.82±1 .30	43.60± 3.34
Group II Drug treated 1g /kg (n = 7)	48.76±4.01 p = 0.7475	11.32±0.68 p = 1.00	41.20±4.06 p = 0.6562
Group III Drug treated 3g /kg (n = 7)	49.31±3.96 p = 0.6653	12.17±1.55 p = 1.00	43.56±5.87 p = 0.9554

Values are expressed as Mean±SEM
n = number of rats in a group.
P* < 0.05, P** < 0.01, P*** < 0.001

group III animals where p value = 0.0319. This is not quite significant to effect safety of the drugs. It can be concluded on the basis of above observation that drug is quite safe.

Table 3: Effect of the Aqueous Extract of Qurse- Nazla on Haematological Parameters in Albino Rats

Groups	Haemoglobin gm %	TLC / Cumm	ESR mm/hr	DLC	
				% Polymorph count	% Lymphocyte count
Group I Normal Control (n = 7)	14.08±0.20	4264.28±147.88	1.71±0.28	30.85±3.23	65.14±2.46
Group II Drug treated 1g kg (n = 7)	13.88±0.19 p = 0.4824	4350.00±186.56 p = 0.7520	1.57±0.29 p = 0.7344	36.57±2.91 p = 0.2129	58.00±3.70 p = 0.1340
Group III Drug treated 3g /kg (n = 7)	14.65±0.33 p = 0.1654	4157.14±4157.14 p = 0.9719	1.42±0.29 p = 0.4857	40.85±2.56 p* = 0.0319	57.71±3.88 p = 0.1318

Values are expressed as Mean±SEM
n = number of rats in a group.
P* < 0.05, P** < 0.01, P*** < 0.001

Table 4: Effect of the Aqueous Extract of Qurse-Nazla on Organ to the Body Weight Ratio on Albino Rats

Groups	Organ weight in g/100 g body weight			
	Liver	Heart	Kidney	Spleen
Group I Normal Control (n = 7)	4.50± 0.10	0.50± 0.01	0.99 ± 0.03	0.51± 0.07
Group I Drug treated 1 g /kg (n = 7)	4.88±0.22 p = 0.1418	0.51± 0.01 p = 0.4930	1.15± 0.08 p = 0.0857	0.57 ± 0.03 p = 0.4461
Group I Drug treated 3g /kg (n = 7)	4.80± 0.23 p = 0.2547	0.50± 0.01 p = 1.000	1.11 ± 0.05 p = 0.0620	0.52± 0.01 p = 0.889

Values are expressed as Mean±SEM
n = number of rats in a group.
P* < 0.05, P** < 0.01, P*** < 0.001

Conclusion

The formulation was well tolerated by the animals and no abnormality was observed in the general behavior of the animals and no overnight mortality was recorded. There were no finding of any organ toxicity and hematological changes as laboratory findings were normal. It can be concluded on the basis of above observation that drug is quite safe in animals.

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