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RESEARCH ARTICLE

ACUTE TOXICITY EVALUATION OF GRANULES OF MAJOON AARAD KHURMA PREPARED WITH *STEVIA REBAUDIANA* AS SWEETENER

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ABSTRACT

Majoon Aarad Khurma (MAK) is a famous traditional Unani medicine formula. It has been used in the treatment of diseases including Jeryan (Spermatorrhea), Muqawwiea Bah (Aphrodisiac) and Mughallize Mani. In present study an important Unani compound formulation Majoon Aarad Khurma has been modified into granular form using natural sweetening agent *Stevia rebaudiana it is new* dosage form. According to development of new dosage form there is need of safety profile. A new drug is always studied for its toxic effects so that its safety profile and therapeutic dose range can be determined. Not only new molecules/drugs but new combinations and new dosage forms are also screened for toxicity.

Aim of the study: To provide information on the potential toxicity of GMAK, evaluated the acute toxicity in Swiss Mice.

Materials and methods: In acute study, GMAK was administered orally at different dosage of 0.39 mg/kg b.wt, 5.73 mg/kg b.wt, 9.73 mg/kg b.wt, and 16.69 mg/kg b.wt respectively, the dose was calculated by Miller's formula. In 24 hours observed behavioural and mortality., clinical signs, body weight changes, food and water consumption, were monitored during the study period.

Results: The acute toxicity study was done on Swiss mice of either sex. The Hydro-Alcoholic extract of GMAK was administered orally at different dosage. In 24 hours observed behavioural and mortality, there was no mortality found.

Conclusions: Hence the acute toxicity study shows that the drug GMAK did not possess any toxicity after inclusion of *Stevia rebaudiana* plant. Hence Stevia can be used as a safe sweetening agent in Unani formulations.

INTRODUCTION

Medicinal herbs are used for the prevention and treatment of diseases, and have a long history. In the long struggle to achieve mastery over the powerful sources of nature, man has always turned to plants for food, shelter, clothing, weapons, healing and even for relief from the hardship of life. Currently approximately 25% of all prescription drugs are derived from herbs, shrubs and trees. The WHO estimates that 4 billion people i.e., 80% of the world's population presently use herbal medicines for some aspects of their primary health care. The practice of using herbal drugs for treating various diseases is well documented in the Unani classics. Sexual activity is also an intimate expression of love and a mode of procreation. Sexual function is an important component of quality of life and subjective well being of humans. Human sexuality is a multidimensional phenomenon having biological, psychological, behavioral, clinical, moral and cultural aspects (Ahmed *et al.,* 2005). A new drug is always studied for its toxic effects so that its safety profile and therapeutic dose range can be determined. Not only new molecules/drugs but new combinations and new dosage forms are also screened for toxicity. Toxicity study may be acute, sub-acute, chronic, and of special type. In an acute toxicity study a drug is tested in different groups of laboratory animals, the 50% of animals dying within 24 hours are used for the purpose of establishing $LD_{50.}$ This study determines the toxicity of a substance after single administration. The aims of acute toxicity study are to: (I) evaluate the degree of toxicity (II) get information about acute mechanism of toxicity (III) obtains a dose level to be used in further studies. In present study an important Unani compound formulation Majoon Aarad Khurma has been modified into granular form using natural sweetening agent *Stevia rebaudiana* which has sweetening property as well as hypoglycemic activity, *Stevia* is a genus of about 240 species of herbs and shrubs in the sunflower family (Asteraceae). The species *Stevia rebaudiana*, is widely grown

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for its sweet leaves. As a sweetener and sugar substitute, *Stevia's* taste has a slower onset and longer duration than that of sugar, With its steviol glycoside extracts having up to 300 times the sweetness of sugar, *Stevia* has garnered attention with the rise in demand for low-carbohydrate, low-sugar food alternatives Stevia is zero calorie natural sweeteners. It is useful in obesity, diabetes, gingivitis, acne, digestive problems, cuts, wounds, mouth sores, heartburn, seborrhoea, eczema, dermatitis, inflammations, and high blood pressure. It is used in mouthwash, toothpaste and used to kill mouth bacteria, sweetener in confectionery, beverages and food industry. It is also used in cosmetic industries in skin shinning and anti-wrinkle creams. It helps the body sustain a feeling of vitality and well-being and used externally for blemishes. Stimulate alertness and counter fatigue, facilitate digestion and gastrointestinal functions (Anonym, 2011). The granules of Majoon Aarad Khurma become palatable and will not cause any harm to diabetic patients who are suffering from sexual dysfunction.

MATERIAL AND METHODS

Granules of Majoon Aarad Khurma were prepared in the laboratory of Dept. of Ilmul Saidla, NIUM. And Maximum tolerable dose of the test drug was also evaluated in mice. Before starting the animal experiment the research protocol was submitted to the IAEC of NIUM, Bangalore for ethical clearance. The protocol was approved vide Reg. No 953/C/06 CPCSEA.

Procurement of raw drugs

All the required ingredients of Majoon Aarad Khurma and Granules of Majoone Aarad Khurma were procured from the raw drug dealers under the supervision of the Guide, and all the raw drugs were identified and authenticated by the expert Dept. of Ilmul Advia, NIUM Bangalore, (Karnataka).

Preparation of Granules of Majoon Aarad Khurma

The granule of Majoon Aarad Khurma was prepared as per the formulation mentioned in the National Formulary of Unani Medicine, Part-1, Govt. of India, the composition of granules of Majoon Aarad Khurma is as given below: All the dried ingredients were powdered and sieved in (sieve number 80). All the Maghaziyat (kernels) were powdered separately and sieved in (sieve number 40), and dates were separately dried in a hot air oven at 100 °C for 4 hours and then powdered and passed through sieve number 60. *Stevia* plant extract was prepared with 120 ml water at low temperature for 15 minutes, and sieved through muslin cloth, the total quantity of this extract obtained was 80 ml. All the dried drugs were mixed one by one in Stevia extract, and subjected into the granulator (sieve number 20) for formation of granules and then stored in container at room temperature for further study (Razzak M A, 2007).

Acute toxicity study

Materials

Acute oral toxicity study was performed as per OECD-423 guidelines.

Experimental Animal

Swiss mice of both sexes, weighing 25-35 gm were used. The animals were procured from the, Sri Raghvendra Enterprises, Vijayanagar, Bangalore, Karnataka (India). Prior to the experiment the animals were allowed to acclimatize for at least one week. They were maintained under standard laboratory conditions throughout the experimental period and were provided with standard diet and water *ad libitum* unless stated otherwise. They were housed in clean polypropylene cages at room temperature 25±2°C, humidity at 45-55% with 12 hours light : 12 hours dark cycle. The animal care procedures and experimental protocol were in according with the guidelines of CPCSEA.

Extractive values

For the determination of extractive values in non-successive of GMAK was carried out in Soxhlet apparatus, with hydro-alcoholic solvents i.e. 50% distilled water and 50% ethanol (1:1) ratio. The extract carried out for 6 hours in that solvent on a heating mantel at temp 80°C. After cooling, the extract was filtered (Whatman filter no. 41) and filtrates were dried by evaporating the solvent in previously weighed Petri-dish on water- bath. Later on after evaporating the solvent the Petri-dish kept in hot air oven for 5 hours at temp 105°C and it was followed by taking accurate weight of Petri-dish, the weight of extracts were calculated by subtracting the weight of Petri-dish. The percentage was calculated with reference to the air dried drug. This procedure was repeated three times and the mean value and standard deviation was calculated. (Turner RA 1965)

Dosage and Administration

The dose of test drug for swiss mice was calculated by multiplying ths human dose with conversion factor of 12 by method of Freirich *et al*., The dose of Granules of Majoon Aarad Khurma is 4.25 gm was calculated excluding the weight of sugar from original formula. Sugar was replaced by natural sweetening agent *Stevia reabudeana* leaves extract and found to be 0.85 gm/ kg body wt. Since, the drug was used in extract form, the dose of extract corresponding to 0.85 gm/kg body wt. Of granules was calculated on the basis of yield percentage of extract and was found to be 0.398 gm / kg body wt was selected for acute toxicity study. Further higher doses were calculated by Miller's formula and the dose previously used was multiplied by $1/\sqrt{2}$ to calculate the next higher doses. The same formula was used to calculate each succeeding doses (Shamsi 2008).

Methodology for Acute Toxicity study

Acute toxicity test was performed according to the World Health Organization (WHO) guideline (WHO 2000) and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals 420 (OECD 2001). Swiss mice of either sex weighing 25-35 gram were randomly assigned to four groups (I, II, III, & IV,) of 7 mice each. Mice were fasted overnight (12 hrs) with free access to water prior to administration of single doses (0.398, 5.73, 9.73, & 16.69 g/kg b.wt.). The extract dissolved in distilled water and administered orally once a day.

Table 1. Ingredients of granules of Majoon Aarad Khurma

Sl. No.	Unani name	Botanical name	Part used	Quantity
1	Khurma	*Phoenix dactylifera*	Fruit	200gm
2	Kamagh arbi	*Acacia arabica*	Gum	200gm
3	Singhara khushk	*Trapa bispinosa*	Fruit	200gm
4	Satawar	*Asparagus rasemosus*	Root	50gm
5	Jaiphal	*Myristica fragrans*	Nutmeg	1.25gm
6	Javitri	*Myristica fragrans*	Mace	1.25gm
7	Qaranfal	*Myrtus caryophyllus*	Fruit	2.5gm
8	Maghaze Badam	*Prunus amygdalus*	Fruit	25gm
9	Maghaze Chilghoza	*Pinus gerardiana*	Fruit	25gm
10	Maghaze Fundaq	*Corylus avellana*	Fruit	25gm
11	Maghaze Pambadana	*Gossypium herbaceum*	Fruit	5gm
12	Stevia plant powder	*Stevia rebaudeana*	leaves	3.50gm

Table 2. Acute toxicity of granules of Majoon Aarad Khurma

Gps.	Treatment dose (gm/kg B.Wt.)	Mice Death/Treated Sex D/T		Effects	Mortality latency (h)	Symptoms of toxicity
A.	0.398	M/F	0/7	None	None	None
B.	5.73	M/F	0/7	None	None	None
C.	9.73	M/F	0/7	None	None	None
D.	16.69	M/F	0/7	None	None	None

After the administration of the test drug all the animals were kept in polypropylene cages singly and were observed for Gross behaviour and mortality at 0 min, 30 min, 60 min, 120 min, 240 min and 24 hrs. The Gross behavioural changes such as piloerection, grooming, trembling, wriggling, diarrhoea, breathing difficulty, constant changing position, immobility, asthenia, anorexia, ataxia, urination and syncope were monitored continuously for any above abnormal changes (Mohammad TH 2010, Acute and sub-chronic oral toxicity studies of an aqueous stem bark extract of Pterocarpus soyauxii Taub (Papilionaceae) in rodents, 2011, and Ibn Baitar, 1999).

RESULTS AND DISCUSSION

The acute toxicity study was done on Swiss mice of either sex. The Hydro-Alcoholic extract of GMAK was administered orally at different dosage of 0.39 mg/kg b.wt, 5.73 mg/kg b.wt, 9.73 mg/kg b.wt, and 16.69 mg/kg b.wt respectively, the dose was calculated by Miller's formula. In 24 hours observed behavioural and mortality and there was no mortality found. It was highly tolerable and safe dosage form. The acute toxicity of GMAK was studied, even though the test drug possessed safe and effective raw drugs but as *Stevia rebaudiana* was used in its preparation as it being a new drug hence it was subjected for acute toxicity study to evaluate its safety. The study was conducted in adults with Swiss mice of either sex they were divided into four group each consisting of seven mice. The Hydro-Alcoholic extract of GMAK was administered orally at different dosage of 0.39 mg/kg b.wt, 5.73 mg/kg b.wt, 9.73 mg/kg b.wt, and 16.69 mg/kg b.wt respectively, the dose was calculated by Miller's formula (Shamsi 2008). The animals were observed continuously for 24 hours, for any behavioural changes and mortality, after 24 hours observation, no behavioural changes and mortality was observed in all the treated groups. Hence the acute toxicity study shows that the drug GMAK did not possess any toxicity after inclusion of *Stevia rebaudiana* plant. Hence Stevia can be used as a safe sweetening agent in Unani formulations (Irshad S 2009).

Conclusion

The present study was aimed to modify the Unani Aphrodisiac formulation Majoone Aarad Khurma into the granular form with *Stevia*, a natural sweetening agent instead of sugar as a base in Majoon. *Stevia* also possess the action of lowering the glucose level. Therefore the Granules of MAK which contain *Stevia* will serve as a good aphrodisiac as combination of both these will be beneficial for the diabetics having the sexual dysfunction. Stevia a natural sweetening agent which was used as base for granules was evaluated for its toxicity in animal models and no toxicity was found upto 16.69 mg/kg b.wt, hence Stevia can be used as safe and efficacious sweetening agent in preparation of granules as well as in other Unani formulations.

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