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Acute Toxicity Study on Crude and Detoxified Leaves of Mazaryun (*Daphne oleoieds* Schreb.) on Wistar Female Rats



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Sayeedur Rahman^{a*}, Najeeb Jahan^b

^a Dept. of Ilmul Advia (Pharmacology), Hayat Unani Medical College and Research Centre, Lucknow, India.

^b Dept. of Ilmul Advia (Pharmacology), National Institute of Unani Medicine, Bangalore, India

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ABSTRACT

Mazaryun (*Daphne oleoieds* Schreb.) is an Unani drug used as a Purgative, anti-inflammatory, antipyretic, anti-helminthic, diuretic, corrosive, detergent and the skin protective, etc. which is mentioned in Unani classical literature. According to the Unani physicians, the *Mazaryun* is a poisonous drug and has 4th degree of temperament. For this reason, detoxification and rectification of *Mazaryun* is very necessary according to the Unani process of detoxification/ rectification called Tadbeer-e-Advia. Acute toxicity study of crude and detoxified leaves of *Mazaryun* has been done before any other pharmacological evaluation.



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INTRODUCTION

Most of the drugs used in USM are believed to be safe but few drugs are toxic and even some of them are deadly poisonous and produce undesirable or unwanted effects^{1,2,3}. If these drugs are used without rectification as per the method mentioned, then such drugs may produce adverse effects. Therefore, these drugs are detoxified to minimize their toxicity.

The Unani physicians have categorized all dietary and medicinal substances into six groups viz; absolute food, medicinal foods, drugs, nutritional drugs, poisonous drug, and absolute poison [1,2,4,5,6,7].

According to the Unani concept, everything has its respective temperaments, so drugs also have their *Mizaj* (temperaments), and based on this concept of temperament again the drugs are explicitly divided into four degrees viz; First degree drugs, these drugs do not produce any perceptible effect in the body after administration and are considered as the safest drugs. They have medicinal value along with nutritional value. Second-degree drugs are the drugs that produce a strong and perceptible effect in the body but do not cause any harm to physiological functions. Third-degree drugs are not only perceptible but may be harmful to the body. These types of drugs can be used with suitable corrective drugs which protect the normal physiological function. Fourth-degree drugs, the effect these drugs are so strong that the physiological function of the body becomes disturbed and the person is likely to die, such drugs are also considered to be poisonous drugs and always recommended for use after subjecting it to some *Tadbeer* (detoxification processes) [1,2,6].

Mazaryun (*Daphne oleoids* Schreb.) is one of the plant origin drugs used in the Unani System of Medicine. Its leaves [8,9], bark [5] and root [10,11] have therapeutic values. It has some important properties like: Purgative [5,8,9,12,13,14,15], anti-inflammatory [8,9,13,14,15], anti-pyretic [16], anti-helminthic [5,13,15], diuretic [5,13,15], corrosive [8,9], detergent [5] and the skin protective [5,8,9,13,15]. *Mazaryun* is of two types according to the colour and size of the leaves [8,13,15] one which has white colour big leaves and another one has small-sized black colour leaves, the second type is said to be toxic and causes adverse effects. [5,8,9,13,15]. Therefore, black leaves are not used in USM. One more the third type of *Mazaryun* with yellow colour thick leaves is also mentioned in some Unani books [5,9]. But Unani physicians have preferred *Mazaryun* with white colour leaves for medicinal use

[5,8,9,13,15]. Unani physicians have kept this drug in the Fourth degree as it has some toxic effects [5,8,9,14].

Since *Mazaryun* is the fourth-degree drug and may produce toxic effects when taken without detoxification or correction (Islah) or in over dose. Hence, detoxification of this drug is necessary [5,8,9,14] otherwise it may cause loose motions and vomiting [8,9] even patient may die due to its toxic effects [9].

In the present study, the first variety-white colour *Mazaryun* (*Daphne oleoides*) was detoxified as per the method mentioned in Unani literature. The fresh and clean 50 gm leaves of *Mazaryun* were dipped in 800 ml of vinegar (acetic acid) for three consecutive days and nights in an earthen pot, and vinegar was changed daily. Then leaves of *Mazaryun* separated from vinegar and washed with water and dried in an oven at 45 °C, and then crushed and finally roasted with Almond oil [5,8,9,13,15].

In the present study, the safety profile of *Mazaryun* was documented by acute toxicity study of both the samples of test drug according to the OECD guidelines 423, a single dose for the 14 days in female Wistar rats [17].



MATERIALS AND METHODS

1. Animals

The study was carried out on healthy Wistar Female rats weighing 150-200 g. The animals were procured from a registered breeder and allowed to acclimatize for one week. They were housed in clean polypropylene cages at room temperature (25 ± 2 °C), humidity 45e55% with 12 h light/12 h dark cycle throughout the experimental period and were provided with standard diet and water *ad libitum* unless stated otherwise. The animal care procedures and experimental protocol were following the guidelines of the Committee for Control and Supervision of Experiment on Animals (CPCSEA). The study was conducted after obtaining the ethical clearance by the Institutional Animal Ethics Committee (IAEC) of the National Institute of Unani Medicine (NIUM), vide Reg. no. IAEC/06/17/IA/03.

2. Plant materials and preparation of powder

The leaves of *Mazaryun* (*D. oleoids* Schreb.) were purchased from Dr. Mohd. Afsahul Kalaam, Research Officer Unani RRIUM, Habak, Nasewmbagh Campus, Kashmir

University, Srinagar, 190006, India. Acetic acid and Roghan-e-Badam (almond oil) were purchased from an authentic herbal supplier from a local market in Bangalore, India. The leaves of Mazaryun were identified by Dr. S. Noorunnisa Begum, Associate Professor, Centre for Repository of Medicinal Resources (C-RPR) at Trans-Disciplinary University (TDU) 74/2, Bangalore-64, vide authentication number (FRLHT Acc. No. 5355). A voucher specimen (Ref. no. 77/IA/Res/2020) was deposited in the Department of Ilmul Advia (pharmacology), drug museum, NIUM, Bangalore, for future reference. The leaves of Mazaryun were divided into two equal parts - one part was kept crude and another part was detoxified. The leaves selected for detoxification were kept in an earthen pot, and soaked in acetic acid for three consecutive days and nights (72 h) and the acetic acid was changed daily as mentioned in Unani classical literature [13,18]. After completion of 72 h, the Mazaryun was taken out from acetic acid and washed with freshwater, and then dried in an oven at 45 °C. Then, the dried leaves were powdered and charb (anointed) with almond oil [11,18,19]. The half crude undetoxified leaves of Mazaryun were simply ground into fine powder at the laboratory of the Department of Ilmul Advia, NIUM.

3. Dosage of the drug

The human therapeutic dose of Mazaryun mentioned in Unani classical literature is 3e5 g [13,15,20]. The dose for rats in Acute Toxicity Study of Crude and Detoxified *Mazaryun* at 300, 2000 & 5000 mg /kg BW on female Wistar rats according to OECD. The dose of the test drug powder for each rat was dissolved in 1 ml of freshly prepared 1% CMC, daily before each administration.

RESULTS

The results of Acute Toxicity Study of Crude and Detoxified *Mazaryun* at 300, 2000 & 5000 mg /kg BW on female Wistar rats according to OECD revealed no observable side effects, on behavior and other subjective parameters as general physique, food intake, and temperature found to be normal at these different doses; there was no effect on the skin, eye colour, and sedation; and no animals were found with diarrhea, drowsiness, tremor, breathing difficulty and all the animals were alive. (Table 1 & 2).

Table No. 1. Behavioral responses and general appearance of rats treated with a single dose of Crude *Mazaryun* in the acute toxicity study

Observation	Crude Drug group	300 mg/kg	2000 mg/kg	5000 mg/kg
Temperature	Normal	Normal	Normal	Normal
Change in skin	No effect	No effect	No effect	No effect
Eye color change	No effect	No effect	No effect	No effect
Food intake	Normal	Normal	Normal	Normal
General physique	Normal	Normal	Normal	Normal
Diarrhea	Not present	Not present	Not present	Not present
Coma	Not present	Not present	Not present	Not present
Drowsiness	Not present	Not present	Not present	Not present
Breathing difficulty	Not present	Not present	Not present	Not present
Sedation	No effect	No effect	No effect	No effect
Tremor	Not present	Not present	Not present	Not present
Death	Alive	Alive	Alive	Alive

Table No. 2. Behavioral responses and general appearance of rats treated with a single dose of detoxified *Mazaryun* in the acute toxicity study

Observation	Detoxified Drug group	300 mg/kg	2000 mg/kg	5000 mg/kg
Temperature	Normal	Normal	Normal	Normal
Change in skin	No effect	No effect	No effect	No effect
Eye colour change	No effect	No effect	No effect	No effect
Food intake	Normal	Normal	Normal	Normal
General physique	Normal	Normal	Normal	Normal
Diarrhea	Not present	Not present	Not present	Not present
Coma	Not present	Not present	Not present	Not present
Drowsiness	Not present	Not present	Not present	Not present
Breathing difficulty	Not present	Not present	Not present	Not present
Sedation	No effect	No effect	No effect	No effect
Tremor	Not present	Not present	Not present	Not present
Death	Alive	Alive	Alive	Alive

DISCUSSION

The ancient Unani physicians were well aware of the toxicity of the drugs, their correctives, and the process of purification and detoxification, to remove their toxins and minimize their harmful effects and have mentioned the details of all these concepts in their treatises. According to the Unani concept, everything has its respective temperaments, so drugs also have their *Mizaj* (temperaments), and based on this concept of temperament the Unani physicians have divided the drugs into 4 degrees: 1⁰, 2⁰, 3⁰ and 4⁰ [1,2,6].

Fourth-degree drugs are also considered to be poisonous and always recommended for use only after subjecting them to a certain *Tadbeer* (*detoxification*) process. According to the Unani concept, the drugs belonging to the *Mizaj* of 3rd and 4th degree have greater potency in treating diseases with minimal doses, but at the same time, they may have some harmful effects as well. After the purification process, the drugs become physically and chemically pure, therapeutically more effective, and less toxic.

Mazaryun (*Daphne oleoides* Schreb.), is one of the plant origin drugs used in the Unani System of Medicine. Its leaves [8,9], bark [5] and root [10,11] have therapeutic values. It has some important pharmacological properties like: Purgative [5,8,9,12,13,14,15], anti-inflammatory [8,9,13,14,15], antipyretic [16], anti-helminthic [5,13,15], diuretic [5,13,15], corrosive [8,9], detergent [5] and the skin protective. [5,8,9,13,15]. *Mazaryun* is of two types according to the colour and size of the leaves, [8,9,15] the type one *Mazaryun* has white colour big leaves and the second type has small-sized black colour leaves, the second type is said to be toxic and causes adverse effects. [5,8,9,13,15]. Therefore, black leaves are not used in USM. One more the third type of *Mazaryun* with yellow colour thick leaves is also mentioned in some Unani books [5,9]. But Unani physicians have preferred *Mazaryun* with white coloured leaves for medicinal use [5,8,9,13,15]. Unani physicians have kept this drug in the Fourth degree as it has some toxic effects [5,8,9,14]. Many studies have been conducted on “*Mazaryun*” to evaluate its pharmacological activities like Antimicrobial property [21], Anti-oxidant property, wound healing and gut modulatory property, etc.[16].

But, to date, no scientific study has been carried out to evaluate the safety profile of this drug before and after the detoxification process.

Therefore, after a thorough literature review on the concept of *Tadbeer-e-Advia*, a fourth-degree drug, *Mazaryun*, was selected in the present study, to validate the significance of the

Unani concept of *Tadbeer-e-Advia* (detoxification) on scientific parameters, by detoxifying this fourth-degree drug, and to evaluate the changes that occur in its physical and chemical profile after detoxification processes by analytical methods, the acute toxicity was also carried out by OECD guidelines 423 to document the toxicity profile of the drug and finally pharmacological activities like anti-inflammatory and anti-pyretic activities of the drug were evaluated to check whether the efficacy or potency of the drug, increases or decreases or remain same after detoxification process, by using both the crude and detoxified forms of *Mazaryun*.

In the study, the first variety-white colour *Mazaryun* was detoxified as per the method mentioned in Unani literature. The fresh and clean 50 gum leaves of *Mazaryun* were dipped in 800 ml of vinegar (acetic acid) for three consecutive days and nights in an earthen pot, and vinegar was changed daily. Then leaves of *Mazaryun* were separated from vinegar and washed with water and dried in an oven at 45 °C, and then crushed and finally roasted with Almond oil [5,8,9,13,15].

Toxicity testing is paramount in the screening of newly developed drugs before they can be used on humans. The guiding principles of toxicity testing are to check the effect of the test substances on laboratory animals and its direct toxic effect on humans and secondly, the exposure of laboratory animals to high doses to evaluate its possible hazard on the human that is exposed to much lower dose [22]. Toxicity studies are divided into acute toxicity studies, sub-acute toxicity studies and chronic toxicity studies [23]. The acute toxicity study can be evaluated by four methods viz., Graphical method of miller and tainter, Arithmetic method of Reed and Muench, Arithmetic method of Karbar and Lorke's method [23]. But now a day's acute toxicity study is done according to the OECD guidelines. Therefore, in the present study acute toxicity was carried according to OECD guidelines 423 [17].

In the present study, the results of Acute Toxicity Study of Crude and Detoxified *Mazaryun* at 300, 2000 & 5000 mg /kg BW on female rats, revealed no observable side effects, on behavior and other subjective parameters as general physique, food intake, and temperature found to be normal at these different doses; there was no effect on the skin, eye colour, and sedation; and no animals were found with diarrhea, drowsiness, tremor, breathing difficulty and all the animals were alive. After the results of the acute toxicity study, the other two, sub-acute and chronic toxicity studies were not conducted. Thus, the result of the acute toxicity study of *Mazaryun* has provided the documentation of safety measures, and generated the

data about adverse effects of *Mazaryun* in its crude and detoxified forms on animals for academics and regulating purpose [24].



CONCLUSION

The present study was conducted to explore the concept of *Tadbeer-e-Advia* (detoxification), a fourth-degree drug, *Mazaryun*, which has been used in the study, and its acute toxicity was evaluated before and after detoxification processes by OECD guidelines 423. In this study, the safety profile of *Mazaryun* was documented by acute toxicity study of both the samples of test drug according to the OECD guidelines 423, a single dose for the 14 days in female Wistar rats.

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	<p><i>Dr Sayeedur Rahman (corresponding author)</i></p> <p><i>Assistant Professor</i></p> <p><i>Department of Pharmacology</i></p> <p><i>Hayat Unani Medical College and Research Centre, Sikrori, Kakori, Lucknow (U.P.) 226101.</i></p>
	<p><i>Prof. Najeeb Jahan</i></p> <p><i>Professor</i></p> <p><i>Department of pharmacology</i></p> <p><i>National Institute of Unani Medicine, Kottigepalya, Magadi Main Road, Bengaluru, Karnataka.</i></p>

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