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RESEARCH ARTICLE

Comparative Physicochemical Evaluation and Acute Toxicity Study of Crude and Processed (Detoxified) Samples of *Saqmunia* (*Convolvulus scammonia* L.) in Albino Wistar Rats

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Abstract

Background and Objectives: *Tadbīr va Islāh-i-Adwiya* (detoxification/rectification of drugs) is a distinctive concept of *Unani* medicine in which drugs that are toxic by nature are detoxified before being administered to the human body. In *Unani* medicine, the resin called *Saqmunia* which is a derivative of the rhizome of *Convolvulus scammonia*, is used for therapeutic purposes. So far, limited research studies have been carried out to appraise the age-old concept of detoxification of *Unani* drugs. Therefore, the present study was designed to explore the physicochemical characteristics and acute toxicity of crude and detoxified samples of *Saqmunia* in albino Wistar rats. **Methods:** *Saqmunia* was detoxified through the *tashwiya* (roasting) method using apple and cow dung cakes. The physicochemical standardization includes moisture content, different ash values, extractive values; pH, fluorescence analysis, and TLC were performed. The acute toxicity study of both samples of *Saqmunia* was carried out at 300 and 2000 mg/kg dose levels in female albino Wistar rats. The data were analyzed using One-way ANOVA followed by Dunnett's comparison test. **Results:** The findings of the physicochemical standardization of crude and detoxified *Saqmunia* were found to be within an accepted range. The acute toxicity study has shown that the non-detoxified form of the test drug produced maximum toxicities, even causing death in rats. The LD_{50} of crude and detoxified *Saqmunia* was found to be 1000 mg and > 2000 mg/kg, respectively, in rats. **Conclusions:** The present study has suggested that *Saqmunia* used in this study was of good quality, and the physicochemical constants found in this study may be taken as a reference for future studies. Moreover, the acute toxicity study validates the *Unani* concept of detoxification of drugs, as is evident from the minimal toxic effects found in the detoxified sample of *Saqmunia* as compared to the crude sample in rats.

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Keywords: Acute Toxicity, *Convolvulus scammonia*, Detoxification, Physicochemical Standardization, *Saqmunia*

Abbreviations: *Ayush: Ayurveda*, *Yoga* and *Naturopathy*, *Unani*, *Siddha* and *Homeopathy*, b. w. Body weight, CBT: Centre for Biodiversity and Taxonomy, CCRUM: Central Council for Research in *Unani* Medicine, CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals, CS: Crude Saqmunia, DMSO: Dimethyl Sulphoxide, DS: Detoxified Saqmunia, IAEC: Institutional Animal Ethics Committee, IP: intraperitoneally, OECD: Organization for Economic Cooperation and Development, RRIUM: Regional Research Institute of *Unani* Medicine, SEM: Standard Error of Mean, SKUAST: Sher-i-Kashmir University of Agricultural Sciences and Technology, TLC: Thin layer chromatography, WHO: World Health Organization

1. Introduction

Unani drugs are obtained from three natural sources, viz., plant, mineral, and animal. All drugs used in the *Unani* system of medicine are classified based on their temperamental qualities and divided into five classes, such as *mo'tadil* (moderate), first, second, third, and fourth degrees[1]. The temperament (*mizāj*) is a unique concept in *Unani* medicine. The drugs obtained from natural sources are contained with organic and inorganic compounds which possess diverse properties and functions[2]. According to the *Unani* concept, the drugs belonging to the first and second degrees of temperament are considered to be safe, whereas the drugs belonging to the third and fourth degrees of temperament may produce serious adverse effects[1]. *Tadbīr va Islāh-i-Adwiya* (detoxification/rectification of drugs) is a unique concept of *Unani* medicine in which drugs that are toxic by nature are detoxified by applying various physical processes[3] like *ihrāq* (burning/charring), *irghā* (removal of froths), *itfā* (cooling), *tahmīs* (roasting), *tadhīn* (oiling), *tashwiya* (parching), *tasfiya* (cleansing), *tasvīl* (decantation), *taghsīl* (washing), *taqliya* (frying), *taklīs* (calcination), etc. Though, these processes are carried out by *Unani* physicians and pharmacists in traditional manner, limited scientific information is available in the public domain pertaining to the physicochemical and phytochemical changes that occur in the processed sample of toxic botanical drugs after conversion into less toxic drugs[1].

Convolvulus scammonia is a perennial herb that belongs to the Convolvulaceae family and is commonly grown in tropical and temperate regions throughout the world. The resin, namely *Saqmunia*, which is collected from the rhizome of *Convolvulus scammonia* after making a transverse or oblique incision, is used medicinally in *Unani* medicine. According to *Unani* pharmacologists, the temperament of this drug is hot and dry in 3rd degree, which may produce adverse effects such as palpitation, restlessness, loss of appetite, nausea and vomiting, depression, and other noxious effects related to the stomach, intestine and heart, if non-detoxified or crude form is used. Hence, it is advised that it should be used after detoxification or purification. The processed form of *Saqmunia* is prescribed for the treatment of several ailments like constipation, joint pain, chronic headache, ascites, bilious fever, pitryasis, vitiligo, jaundice, melasma, scabies, etc. It is also included in some *Unani* compound preparations, which are prescribed in the treatment of several diseases[4]. In other traditional and folk medicines, this drug of botanical origin is used as a purgative, antipyretic, abortifacient, uterine tonic, etc[5].

In 2002, the World Health Organization (WHO) stated that around 80% of the population of the world uses herbs or herbal products to treat their health care problems[6-8]. Currently, the demand for herbal drugs has increased throughout the world, and consequently, various types of research on botanical products are carried out on a large scale by pharmaceutical industries and government research organizations. In ancient times, the identification and authentication of plants and other herbal materials were based on the available standard literature[8]. At present, adulteration in herbal drugs has become a major concern due to their non-availability, overexploitation, and high cost[7]. In spite of the high demand for herbal medicine, the scientific community and government organizations of developing countries are facing challenges in finding standardized herbal products. The WHO has mentioned standard guidelines and scientific parameters for proper authentication and development of herbal drugs that are helpful in providing safe and effective medical care. There is a notion among the public that drugs obtained from natural sources are safe due to their long history of consumption. In this scientific era, several phytoconstituents isolated from plant materials have been reported to produce various potential adverse reactions[9]. The present study was carried out to develop the physicochemical constants of crude and processed (detoxified) *Saqmunia* samples using standard physicochemical and quality control parameters, as no previous study had been conducted in this regard. Concurrently, the acute toxicity study of both samples was

also carried out in albino Wistar rats to evaluate their safety and LD_{50} values and to validate the old *Unani* concept of detoxification of drugs.

2. Methodology

2.1 Source of Data Collection

The data were obtained from the Regional Research Institute of *Unani* Medicine, Srinagar, Jammu and Kashmir (NABH Accredited), Central Council for Research in *Unani* Medicine, Ministry of Ayush, Government of India, and Sher-i-Kashmir University of Agricultural Sciences and Technology (SKUAST), Srinagar, Jammu and Kashmir, India.

2.2 Collection and Authentication of the Plant Materials

The resin of *Convolvulus scammonia* was procured from an authentic drug supplier in the local market of Srinagar in the month of December, 2020, and the same was authenticated by Dr. Akhtar H. Malik, Centre for Biodiversity and Taxonomy (CBT), Dept. of Botany, University of Kashmir, Jammu and Kashmir, vide voucher specimen number 3161-(KASH) for future reference.

2.3 Procurement of Chemicals and Reagents

Eosin was procured from Merk Life Science Pvt. Ltd. Godrej one, 8th floor, Mumbai - 400079 , and haematoxylin was procured from HiMedia Laboratories Pvt. Ltd. 23, Vadhani Ind. Est., LBS Marg, Mumbai - 400086. DMSO was procured from Sisco Research Laboratories Pvt. Ltd., Plant site 1: D-88/2, MIDC, Turbhe, New Mumbai - 400705. The manufacturing date of DMSO was September 2020. Formaldehyde, DPX mountant, Acetone and Benzene were procured from Merck Life science Private Limited, Godrej one, 8th floor, Pirojshanagar, Eastern express highway, Vikhroli (East), Mumbai - 400079.

2.4 Method of Detoxification of *Saqmunia* (*Convolvulus scammonia*)

Saqmunia (*Convolvulus scammonia*) was detoxified by the method of *tashwiya* (roasting). In this method, apples and cow dung cakes were taken and hollow spaces were made in the apple. The drug was kept in the hollow space of the apple, followed by a covering made of a piece of the same apple and flour paste. Thereafter, the cow dung cakes were burnt at a suitable place, and apples containing *Saqmunia* were kept in the ash of the burnt cow dung cakes till the colour of the flour got brownish. After cooling down, the drug was removed from the hollow space of the apple and was considered detoxified *Saqmunia* (Figure 1)[10-15]. The detoxified sample was then stored in a tightly sealed glass container for further study.

2.5 Physicochemical Standardization

The crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) were subjected to evaluation including foreign matter detection, loss on drying, total ash value, acid insoluble ash value, water soluble ash value, extractive values, pH[16,17], Fluorescence analysis[18], Thin layer chromatography[19], and preliminary qualitative phytochemical analysis[20], by following standard methods and procedures.

2.6 Acute Toxicity Study

The acute toxicity study of crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) was carried out according to the Organization for Economic Cooperation and Development (OECD) 423 guidelines[21]. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of RRIUM, Srinagar, Jammu and Kashmir, vide number 927/GO/Re/S/2006/CPCSEA. The date of IAEC approval of the protocol is May 27, 2019.

2.6.1 Dosage and Mode of Administration of the Test Drug

Two dose levels, i.e., 300 mg and 2000 mg of crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) were administered orally to the rats.

2.6.2 Experimental Design

A total of 15 female albino Wistar rats were randomly divided into five groups of three each. The experimental design was as follows:

Group I (Plain Control): This group received a single dose of Dimethyl Sulphoxide (DMSO) at 1 ml/kg b. w.

Group II: A single dose of crude (non-detoxified) *Saqmunia* (*Convolvulus scammonia*) at 300 mg/kg b. w., dissolved in 1 ml of DMSO was administered to this group.

Group III: This group received a single dose of detoxified *Saqmunia* (*Convolvulus scammonia*) at 300 mg/kg b. w., dissolved in 1 ml of DMSO.

Figure 1. (Different steps of detoxification of *Saqmunia*) Step 1. Hollow space made into an apple. Step 2. *Saqmunia* placed in the hollow space of an apple. Step 3. Apple containing *Saqmunia* packed with paste of wheat flour. Step 4. Apple along with *Saqmunia* placed in the ash of cow dung cakes. Step 5. Apple along with *Saqmunia* after removing from the ash. Step 6: Detoxified *Saqmunia*.

Group IV: When no signs of toxicity or mortality were found in Group II for 48 h after administration of the drug, a single dose of crude (non-detoxified) *Saqmunia* (*Convolvulus scammonia*), at 2000 mg/kg b. w., dissolved in 1 ml of DMSO was administered to this group.

Group V: When no signs of toxicity or mortality were seen in Group III for 48 h after administration of detoxified *Saqmunia* (*Convolvulus scammonia*), a single dose of the same drug at a dose level of 2000 mg/kg b. w., dissolved in 1 ml of DMSO was administered to the rats.

2.6.3 Physical Observations

After administration of the test drug, all the rats were observed at least once during the first 30 mins, periodically during the first 24 hrs, with special attention given for the first 4 hrs and daily thereafter for a period of 14 days. The observations include mortality, changes in the skin, fur, eyes, mucous membrane, behavior pattern, tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. The body weight of each rat was recorded shortly before administration of the test drug and weekly thereafter. The food intake of each group was also measured daily. The relative organ-body weight was recorded at the end of the experiment[21].

2.6.4 Histopathological Examination of Liver, Kidney and Heart

At the end of the study, all the rats were sacrificed by administering Thiopentone sodium (50 mg/kg/b. w.) intraperitoneally[22] after an overnight fast. The liver, kidney, and heart of each rat were collected for gross and microscopic histopathological examinations.

2.7 Statistical Analysis

The recorded data were compiled and entered in a spread sheet, then exported to the data editor GraphPad Instat and GraphPad prism software versions 8.4.2. The continuous variables are expressed as Mean ± Standard Error of Mean (SEM). One-way ANOVA was employed for the analysis of various parameters among different groups. A repeated measure of ANOVA was also employed in case the observations of parameters were recorded more than twice. All these parametric tests were employed subject to the condition that the continuous data under consideration passed the normality test. In order to analyze the possible pair-wise significance between the groups, Dunnett's comparison test was applied. The p value <0.5 was considered to be statistically significant.

3. Results

3.1 Physicochemical Standardization of *Saqmunia* (*Convolvulus scammonia*)

3.1.1 Macroscopic/Organoleptic Evaluation

The colour, odour, taste, and texture of the crude sample of *Saqmunia* (*Convolvulus scammonia*) were found to

be brownish-black, unpleasant, slightly pungent, and irregularly rough, respectively.

3.1.2 Foreign Matter

The whole sample of the crude *Saqmunia* (*Convolvulus scammonia*) was free from any contamination including insects, moulds, animal excreta, soil, and other adulterants. In addition, no abnormal odor, discoloration, sign of deterioration, or innocuous matter were found in the sample. After analysis, the amount of foreign matter in the sample was calculated as 0.0016% (Table 1).

3.1.3 Loss of Weight on Drying at 105°C

The moisture content in crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) was found to be 4.5% and 5.5%, respectively (Table 1).

3.1.4 Ash Values

The total ash values in crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) were recorded as 2.5% and 2%, respectively. The acid-insoluble ash value was found to be 1% and 1%, respectively. The water-soluble ash value in both samples was recorded at 1% and 1.5%, respectively (Table 1).

3.1.5 DMSO and Alcohol Soluble Matter of Crude and Detoxified Saqmunia (Convolvulus scammonia)

The DMSO-soluble matter of crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) tested through the hot-extraction method was found to be 95.6% and 91.6%, respectively. The DMSO-soluble matter of crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) tested through the cold-extraction method was found to be 50% and 34%, respectively. The alcohol-soluble matter of crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) tested through the hot-extraction method was found to be 97.6% and 93%, respectively. The alcohol-soluble matter of both samples tested through the cold-extraction method was found to be 52% and 51%, respectively (Table 1).

3.1.6 pH

The pH of the crude sample of *Saqmunia* (*Convolvulus scammonia*) in 1% and 10% aqueous solution was determined as 7.8 and 6.7, respectively, whereas the pH of the detoxified sample in 1% and 10% aqueous solution was determined as 3.15 and 3.2, respectively (Table 1).

3.1.7 Fluorescence Analysis

The fluorescence analysis of crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) showed different colors under daytime and ultraviolet lights. (Tables 2 and 3).

3.1.8 Thin Layer Chromatography

The R_f values of the ethanolic extract of the crude sample of *Saqmunia* (*Convolvulus scammonia*) in the solvent system, viz., Toluene: Ethyl acetate: Formic acid (7:3:0.05) were calculated as 0.42, 0.64, 0.73, 0.80 and 0.9, respectively. The same in the detoxified sample was found to be 0.4, 0.61, 0.68, 0.77 and 0.85, respectively (Table 4).

Table 1. Physicochemical constants of crude and detoxified *Saqmunia* (*Convolvulus scammonia*)

Particulars	Crude *Saqmunia* (*Convolvulus scammonia*)	Detoxified *Saqmunia* (*Convolvulus scammonia*)
Foreign matter (%)	0.016	
Moisture content (%)	4.5	5.5
Total ash value (%)	2.5	2
Acid insoluble ash (%)	1	1
Water soluble ash (%)	1	1.5
Hot extractive value (DMSO) (%)	95.6	91.6
Hot extractive value (Ethanol) (%)	97.6	93
Cold extractive value (DMSO) (%)	50	34
Cold extractive value (Ethanol) (%)	52	51
pH (1%)	7.8	3.15
pH (10%)	6.7	3.2

Table 2. Fluorescence analysis of crude *Saqmunia* (*Convolvulus scammonia*)

S. No.	Treatment	Day Light	UV (254nm)	UV(366nm)
1	Powder as such	Beige	Sand colour	White sand colour
2	Powder treated with chloroform	Brownish	Greenish	Dark green
3	Powder treated with ethyl acetate	Yellow	Green	Olive
4	Powder treated with methanol	Light brown	Green	White green
5	Powder treated with 10% NaOH	Brown	Greenish	Dark green
6	Powder treated with NH_3	Brown	Yellow green	White green
7	Powder treated with conc. H_2SO_4	Win red	Black green	Mud colour
8	Powder treated with conc. HCl	Brown	Green	Black
9	Powder treated with HNO_3	Yellow brown	Yellow green	Black red
10	Powder treated with picric acid	Yellow	Yellow green	Black
11	Powder treated with Pet. ether	White	White green	White
12	Powder treated with GAA	Brown	Green	White green

Table 3. Fluorescence analysis of detoxified *Saqmunia* (*Convolvulus scammonia*)

S. No.	Treatment	Day Light	UV (254nm)	UV(366nm)
1	Powder as such	Beige	Sand colour	White
2	Powder treated with chloroform	Yellow	Green	White green
3	Powder treated with ethyl acetate	Yellow	Yellow green	White green
4	Powder treated with methanol	Yellow	Olive green	White green
5	Powder treated with 10% NaOH	Brown	Black	Green
6	Powder treated with NH_3	Yellow	Green	White green
7	Powder treated with conc. H_2SO_4	Win red	Black	Green
8	Powder treated with conc. HCl	Brown	Green	Dark green
9	Powder treated with HNO_3	Yellow brown	Green	Black
10	Powder treated with picric acid	Yellow	Yellow green	Red
11	Powder treated with Pet. ether	White	Colourless	White green
12	Powder treated with GAA	Yellow	Green	White green

Table 4. TLC of crude and detoxified *Saqmunia* (*Convolvulus scammonia*)

Extract	Solvent system	No. of spots	R_f Value
Ethanol (Crude)	Toluene : Ethyl acetate : Formic acid (7:3:0.05)	5	0.42, 0.64, 0.73, 0.80, 0.91
Ethanol (Detoxified)	Toluene : Ethyl acetate : Formic acid (7:3:0.05)	5	0.4, 0.61, 0.68, 0.77, 0.85

The TLC plates of both samples showed various spots (Figure 2).

3.1.9 Preliminary Qualitative Phytoconstituents

Various phytochemical constituents were found in the ethanolic extract of *Saqmunia* (*Convolvulus scammonia*) (Table 5).

3.2 Acute Toxicity Study

3.2.1 Physical Observations

The rats were treated with the crude *Saqmunia* (*Convolvulus scammonia*) at the dose level of 300 mg/kg b. w. showed diarrhoea, piloerection, and vocalization, whereas the rats receiving the same dose of the detoxified *Saqmunia* (*Convolvulus scammonia*) showed diarrhoea

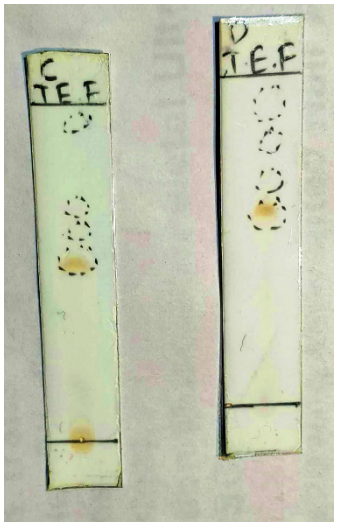

Figure 2. TLC plates showing various spots of ethanolic extract of crude and detoxified *Saqmunia* (*Convolvulus scammonia*).

and vocalization six hours after administration of the drug. Two rats out of three were treated with crude *Saqmunia* at a dose level of 2000 mg/kg b. w., died within 48 h after administration of the drug. Some abnormal signs such as redness in the eye, diarrhoea, piloerection, disturbance in respiration, sluggish reflexes, lethargy, abnormal gait, tremors, and vocalization were also noted in this group. The rats that received the detoxified *Saqmunia*at 2000 mg/kg b. w. did not show any mortality except for some abnormal signs, including diarrhoea, piloerection, and vocalization.

3.2.2 LD_{50}

Since no mortality was observed in the rats belonging to group V (2000 DS) during the whole study period, this suggests that the detoxified sample of the test drug is classified under category 5, which means the $LD_{50,}$ is greater than 2000 mg/kg b. w. for rats. Two rats belonging to group IV (2000 CS) died within 48 h after administration of the drug that falls under category 4 as per OECD 423 guidelines, which referred that the LD_{50} is 1000 mg/kg b. w. Therefore, it is concluded that the LD_{50} of crude *Saqmunia* (*Convolvulus scammonia*) is 1000 mg/kg b. w. for rats.

3.2.3 Body Weight

Before administering the dose, the mean body weight of the rats in Groups I, II, III, IV and V was recorded as 181±19.86, 157.67±3.72, 160.34±6.07, 155±1.53, and 170.67±7.69, respectively. On the 7th day, the mean body weight of the rats belonging to groups I, II, III, IV, and V was found to be 212.34±1.13, 169±4.94, 176.67±1.85, 159.34±7.89, and 197.34±14.82, respectively. On the 14th day, the mean body weight of the rats in groups I, II, III, IV, and V was found to be 229±18.15, 179.67±6.36, 182.67±3.29, 160±4.24, and 210±11.55, respectively (Table 6).

3.2.4 Food Intake

The mean value of the food intake for each rat per day belonging to groups I, II, III, IV, and V was found to be 30.51±0.22, 27.44±0.21, 28.46±0.15, 13.34±0.08 and 32.72±0.41, respectively (Table 7).

3.2.5 Relative Organ-body Weight

The relative organ-body weight of the liver of rats belonging to Groups I, II, III, IV, and V was found to be 3.17±0.21, 4.04±0.18, 3.91±0.24, 4.87±0.33, and 3.25±0.18, respectively. The relative organ-body weight of kidneys of rats belonging to Groups I, II, III, IV, and V was recorded as 0.77±0.09, 0.96±0.01, 0.96±0.03, 1.01±0.02, and 0.81±0.07, respectively. The relative organ-body weight of the heart of rats belonging to Groups I, II, III, IV, and V was found to be 0.35±0.02, 0.38±0.02, 0.36±0.01, 0.43±0.02, and 0.38±0.02, respectively (Table 8).

3.2.6 Histopathological Examination

3.2.6.1 Gross Examination of the Liver

The morphology of the liver of rats belonging to Groups I, II, III, and V showed normal four distinct lobes with a reddish-brown color, whereas the liver of rats belonging to Group IV exhibited congestions and a dark color (Figure 3).

3.2.6.2 Microscopic Examination of the Liver

The liver specimens of the rats belonging to the plain control group showed normal hepatic parenchyma of hexagonal lobules with a Centered Vein (CV), and Sinusoids (S). The bile duct hyperplasia (B), hepatic artery (Ha) in acinar pattern, and mildly swollen hepatic cells were found in the liver of Group II, whereas the liver specimens of Group III showed binucleate cells in hepatocytes and increased nuclear size in hepatocytes. The liver of Group IV exhibited pyknosis of nuclei, congestion in the blood vessels (C) and necrosis (N), whereas the liver specimens of Group V showed only congestion in the Portal Vein (PV) (Figure 4).

Table 5. Phytochemical screening of crude and detoxified *Saqmunia* (*Convolvulus scammonia*)

TESTS	Inference extract	Crude Sample	Detoxified Sample
Alkaloids			
Mayer's test	No ppt formation	-	-
Dragendroff's reagent	Orange ppt	+	-
Wagner's test	Reddish brown ppt	+	-
Hager's test	Yellow ppt	+	+
Tannins and Phenolics			
5% $FeCl_3$	Green colour	+	+
Lead acetate	White ppt	+	+
Carbohydrates			
Molish's test	Violet ring	+	+
Fehling's test	Brick red ppt	-	-
Benedict's test	Red ppt	-	-
Flavonoids			
Shinoda test	Pink colour	+	+
Glycosides			
Coumarin glycosides	Yellow fluorescence	+	+
Anthraquinone Glycosides			
Borntrager's test	Red Colour layer	+	+
Cardaic Glycosides			
Keller killiani Test	Reddish brown ring at junction	+	+
Legal test	Pink colour	-	-
Steroids			
Salkowski's test	Golden yellow ring at junction	+	+
Proteins			
Million's test	White ppt	-	+

Symbol denoted: (+) Positive, (-) Negative

Table 6. Effects of crude and detoxified *Saqmunia* (*Convolvulus scammonia*) on total body weight of rats

Showing average body weight variation by animals among different groups										**P Value**
Groups	**1st day**			**7th day**			**14th day**			
	Mean	**SD**	**SEM**	**Mean**	**SD**	**SEM**	**Mean**	**SD**	**SEM**	
Group I (Plain control)	181	34.4	19.86	212.34	36.62	21.13	229	31.44	18.15	<0.01**
Group II (300 CS)	157.67	6.43	3.72	169	8.55	4.94	179.67	11.02	6.36	<0.01**
Group III (300 DS)	160.34	10.5	6.07	176.67	3.22	1.85	182.67	5.69	3.29	<0.01**
Group IV (2000 CS)	155	9.75	1.53	159.34	13.65	7.89	177	11.2	4.24	<0.001***
Group V (2000 DS)	170.67	13.32	7.69	197.34	25.65	14.82	210	20	11.55	>0.05
Test applied repeated measures of ANOVA.										

(CS: Crude *Saqmunia*; DS: Detoxified *Saqmunia*)
p>0.05: non-significant; **p <0.01: highly significant; ***p <0.001: extremely significant [comparison between groups was made using one-way ANOVA followed by Dunnett's multiple comparison test; values are expressed as Mean ± SEM (n=3)]

Table 7. Effects of crude and detoxified *Saqmunia* (*Convolvulus scammonia*) on food intake of rats

Groups	Food intake / Day/ Rat (Mean ± SEM)
Group I (Plain control)	30.51 + 0.22
Group II (300 CS)	27.44 + 0.21
Group III (300 DS)	28.46 + 0.15
Group IV (2000 CS)	13.34 + 0.08**
Group V (2000 DS)	32.72 + 0.41

(CS: Crude *Saqmunia*; DS: Detoxified *Saqmunia*)
**p <0.01: highly significant [comparison between groups was made using one-way ANOVA followed by Dunnett's multiple comparison test; values are expressed as Mean ± SEM (n = 3)]

3.2.6.3 Gross Examination of the Kidneys

The kidneys of rats in all groups did not show any abnormal changes.

3.2.6.4 Microscopic Examination of the Kidneys

The kidney specimens of the rats belonging to the plain control group showed normal architecture of cortex and medulla (M) with glomeruli (G) and tubules (T). The kidney specimens of the rats belonging to Group II showed hypocellularity of glomerular cells and congestion in inter-tubular capillaries and glomeruli, whereas the kidneys belonging to Group III did not show any marked changes. The kidney specimens of rats belonging to the Group IV showed necrotic tubules (N), casts within the lumen, and congestion, whereas the kidneys of rats belong to Group V exhibited increased Bowman space (E) and congestion of tubular capillaries (Figure 5).

3.2.6.5 Gross Examination of the Heart

The hearts of rats belonging to all groups did not show any abnormal changes.

3.2.6.6 Microscopic Examination of the Heart

The heart specimens of the rats belonging to groups I and III showed normal appearances of centrally arranged nucleus, connective tissue, and well-arranged cardiac muscle fibers. The heart specimens of the rats belonging to group II showed displacement of nuclei in myocytes, whereas the hearts of rats belonging to group IV exhibited marked hemorrhage (H) and degeneration of myofibers. The heart specimens of the rats belong to the group V showed mild hemorrhage (H) in muscle fibers (Figure 6).

4. Discussion

The present study was carried out to evaluate the physicochemical characteristics, preliminary qualitative phytochemical screening, and acute toxicity of crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia* L.) in rats. The organoleptic properties of the crude sample were found to be identical to those mentioned in *Unani* classical literature[10,11], confirming the purity and quality of the test drug. The moisture content in any herbal product should be between 10-20%[8] to prevent decomposition due to microbial contamination. In our study, the moisture content in crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) was found to be within the permissible limits. The ash value is an important parameter to detect adulterants and establish the quality and purity of the drug. Moreover, it is a useful parameter for detecting low grade products, exhausted drugs, the presence of sand or earthy matters, etc. This test is appropriate for powdered drugs that consist mainly of carbonates, phosphates, silicates, and silica and gives an indication of the degree of mineral interaction in the structure and properties of the polysaccharides. The total ash values of crude and detoxified samples of *Saqmunia*

Table 8. Effects of crude and detoxified *Saqmunia* (*Convolvulus scammonia*) on relative organ-body weight of rats

Groups	Relative organ-body weight in albino Wistar rats (Mean ± SEM)		
	Liver (g/ 100 g)	Kidneys (g/ 100 g)	Heart (g/ 100 g)
Group I (Plain control)	3.17+0.21	0.77+0.09	0.35+0.02
Group II (300 CS)	4.04+0.18	0.96+0.01	0.38 + 0.02
Group III (300 DS)	3.91+0.24	0.96+0.03	0.36 + 0.01
Group IV (2000 CS)	4.87+0.33*	1.01+0.02*	0.43 + 0.02*
Group V (2000 DS)	3.25+0.18	0.81+0.07	0.38 + 0.02

(CS: Crude *Saqmunia*; DS: Detoxified *Saqmunia*)
*p<0.05: significant [comparison between groups was made using one-way ANOVA followed by Dunnett's multiple comparison test; values are expressed as Mean ± SEM (n = 3)]

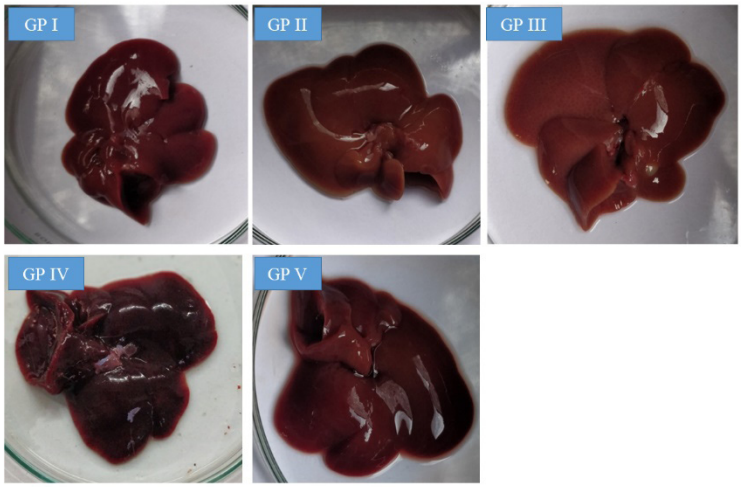

Figure 3. Morphology of liver showed normal four distinct lobes of reddish brown colour in all Groups (I, II, III, V) except Group IV, which showed congestion and dark colour.

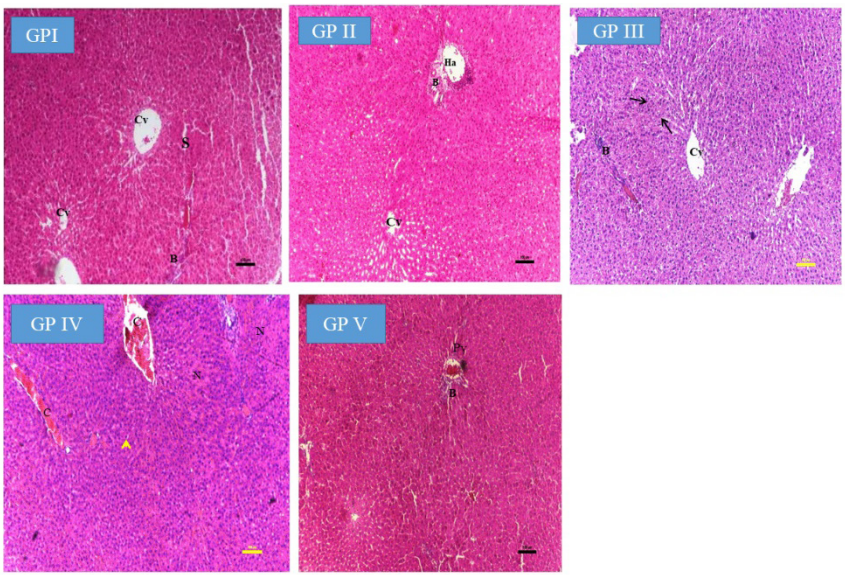

Figure 4. Liver. GP I. Normal hepatic parenchyma of hexagonal lobules with centered vein (Cv) and sinusoids (S), GP II. Bile duct hyperplasia (B) Hepatic artery (Ha) in array pattern of hepatocytes and mild swollen hepatic cells, GP III. Binucleate cell (black arrow) in hepatocytes and increased nuclear size of hepatocytes, GP IV. Pyknosis of nuclei (yellow arrow), Necrosis (N) and congestion (C) of blood vessel, GP V. congestion of Portal Vein (PV). (H and E100X).

(*Convolvulus scammonia*) were determined as 2.5% and 2%, respectively. Similarly, the acid-insoluble ash is the residue that is obtained after boiling the total ash in the diluted hydrochloric acid. It is ignited in the muffle furnace, and the remaining insoluble matter is calculated[23]. The acid-insoluble ash value of both samples was recorded as 1%. The water-soluble ash is used to identify the existence of material exhausted by water. In the present study, the water-soluble ash of crude and detoxified samples was calculated as 1% and 1.5%, respectively. The results of the different ash values of both samples of *Saqmunia* indicate that the drug was free from any adulterants. The extractive value is an amount of the extract that a drug yields in a particular solvent[23]. The extractive values of a particular drug depend on the solubility of the chemical constituents in the respective solvents. The water-soluble extractive indicated that the drug possesses water-soluble constituents like glycosides, tannins, mucilage, etc. The alcohol-soluble extractive referred to the fact that the drug contains tannins, glycosides, resins, etc., whereas

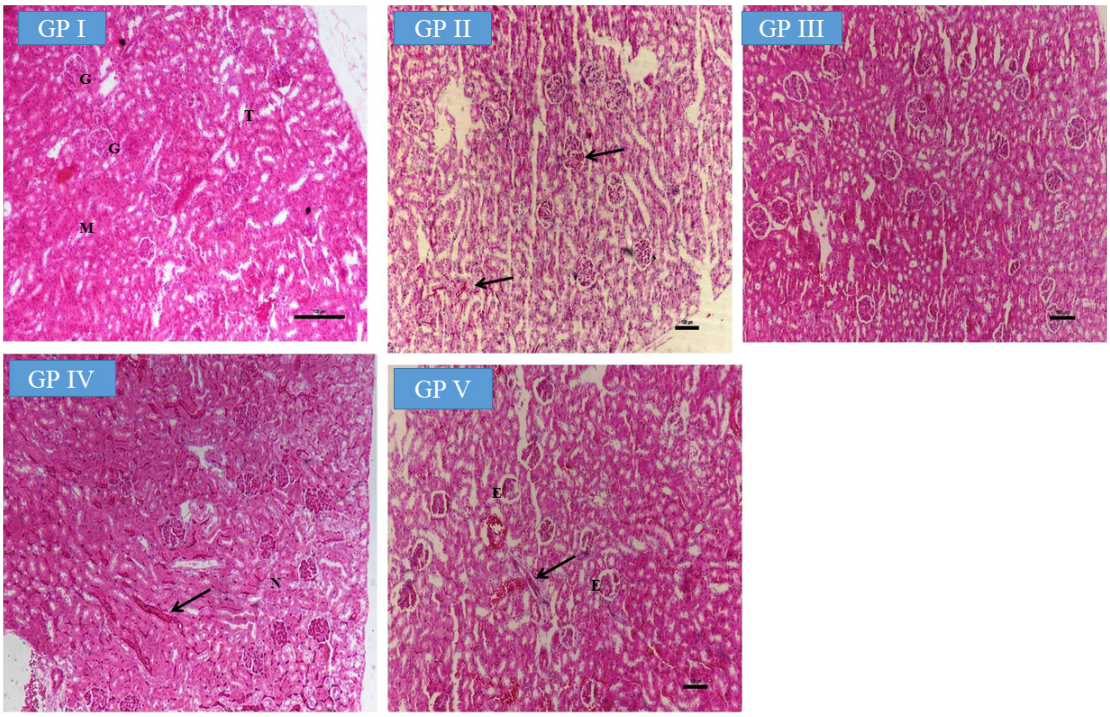

Figure 5. Kidney. GP I: Normal architecture of cortex and medulla (M) with glomerulus (G) and Tubules (T); GP II: Hypocellularity of glomerular cells and congestion (arrow) in inter-tubular capillaries and glomeruli; GP III: No marked alteration; GP IV: Necrotic tubules (N), casts within lumen and congestion (arrow); GP V: Increased bowman space (E) and congestion (arrow) of tubular capillaries. (H and E 100X).

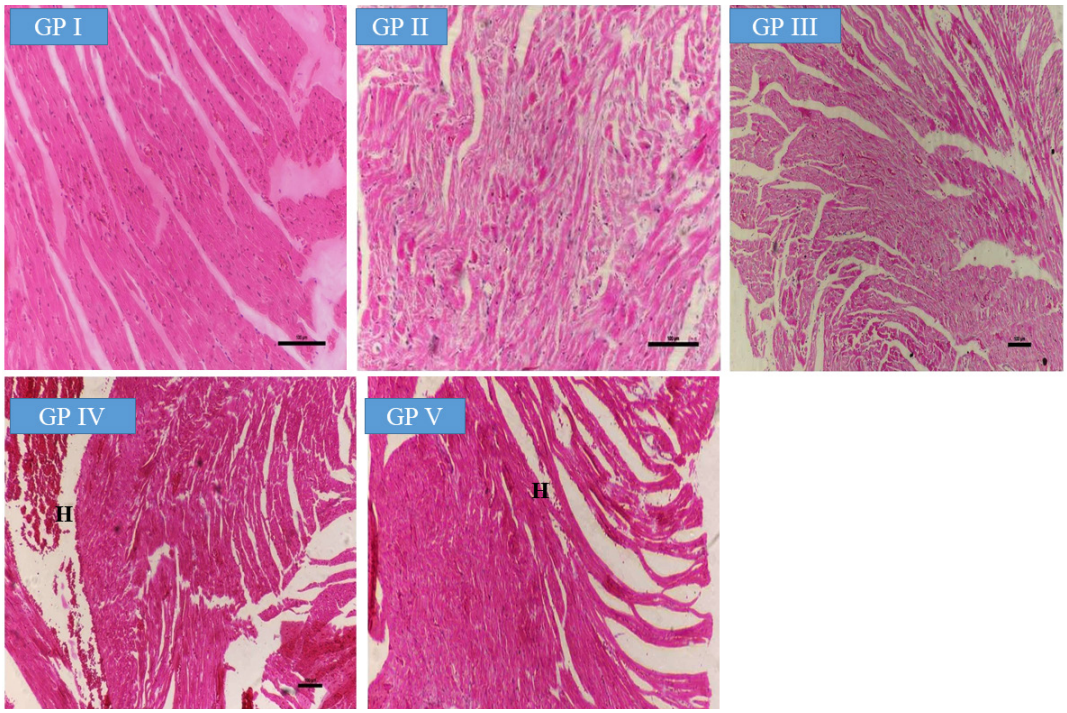

Figure 6. Heart. GP I and III: Normal appearance of centrally arranged nucleus, connective tissue, and cardiac muscle fibers; GP II: Displacement of nuclei in myocytes; GP IV: Marked haemorrhage (H) and degeneration of myofibre; GP V: Mild haemorrhage (H) in muscle fibres. (H and E 100X).

the ether-soluble extractive indicates that the drug yields volatile constituents and fats[24]. In the present study, the extractive values of crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) with respect to DMSO in hot extraction were found to be 95.6% and 91.6%, respectively. The alcohol-soluble extractive values of crude and detoxified samples in hot extraction were found to be 97.6% and 93%, respectively. The DMSO-soluble extractive values in cold extraction were found to be 52% and 51%, respectively. The alcohol-soluble extractive values in cold extraction were found to be 50% and 34%, respectively. Such results suggested that both samples of the test drug yielded maximum constituents in hot extraction. The presence of foreign organic matters in a drug indicates the presence of contaminants like insects, moulds, earthy materials, animal excreta, sand, etc[24,25]. A very low percentage of foreign organic matter found in the crude sample of *Saqmunia* (*Convolvulus scammonia*) indicated that the test drug was free from any contaminants. The pH of any drug plays a pivotal role in its absorption through biological membranes like the oral mucosa, gastro-intestinal membrane, skin, etc. The absorption of an ionized drug through the gastrointestinal mucosa is explained by the pH partition theory[26]. In the present study, the pH of detoxified samples of *Saqmunia* was found to be acidic in nature, which suggested that its absorption through gastrointestinal mucosa might be good. The fluorescence analysis of the powdered crude and detoxified *Saqmunia* showed different colours in daylight and ultraviolet light, which suggested that both samples were free from any type of adulterant and were of good quality. In this study, different types of solvent systems (binary, ternary, etc.) were tried to develop an effective solvent system for the analysis of phytoconstituents present in the crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*). The solvent system of Toluene: Ethyl acetate: Formic acid in the ratio of 7:3: 0.05 gave satisfactory results. The phytochemical screening revealed both samples contain alkaloids, tannins, phenols, coumarins, cardiac and anthraquinone glycosides, carbohydrates, flavonoids, steroids, proteins, etc. Hence, it is concluded that the physicochemical constants of crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) found in our study may be taken as references for future studies since no previous studies have been carried out so far in this regard.

In the acute toxicity study, the LD_{50} value of crude *Saqmunia* (*Convolvulus scammonia*) was found to be1000 mg/kg b. w. Since no mortality was seen in the rats treated with the higher dose level of detoxified *Saqmunia*, it is suggested that its LD_{50} value is more than 2000 mg/kg b. w. for rats. The physical observations revealed that most of the clinical findings, viz., diarrhoea, haemorrhage in the eyes, tremors, lurching gait, lethargy, emaciation, piloerection, sluggish reflexes, etc., were found in the rats that were treated with the higher dose level of the crude *Saqmunia* (*Convolvulus scammonia*). Except for diarrhoea no other abnormal clinical signs were observed in the rats treated with both dose levels of the detoxified sample of the test drug during the whole study period. The *Unani* literature revealed that *Saqmunia* (*Convolvulus scammonia*) is used as a purgative[10-13]. The *Unani* scholars have described that the crude *Saqmunia* may be harmful for the stomach, liver, intestines, and heart and may produce adverse effects like irritability, syncope, retching, diarrhea, etc[10,11,13]. A study has reported that the active constituent of *Convolvulus scammonia* was found to be inert until it went to the gastrointestinal tract, where it came into contact with bile and produced a chemical reaction with taurocholate and glycocholate of sodium and became a strong purgative compound[5]. The body weight and food intake of rats belonging to group IV treated with the higher dose of crude *Saqmunia* were found to be reduced at the end of the treatment as compared to those of other groups, which suggested that the crude sample is toxic in nature. A study revealed that the resin glycosides present in *Convolvulus scammonia* have a strong purgative effect, which might be responsible for anorexia and reduced body weight[27]. The significant abnormal histoarchitecture findings in the liver, kidney, and heart specimens of the rats that received the greater dose level of crude *Saqmunia* suggested that it produces organ-level toxicity.

The overall outcome has suggested that the test drug used in this study was of good quality, and the physicochemical constants found in the present study may be taken as references since no previous study has been carried out so far in this regard. The overall comparative data of the acute toxicity study has suggested that the higher dose of crude *Saqmunia* (*Convolvulus scammonia*) produces significant toxic effects in rats as compared to the detoxified sample of the same drug, which further validates the *Unani* concept of *Tadbīr va Islāh-i-Adwiya* (detoxification/ rectification of drugs). The overall reduced toxic effects observed in the rats treated with detoxified *Saqmunia* (*Convolvulus scammonia*) may be due to either qualitative or quantitative chemical changes

in the sample after its detoxification, which further needs to be explored. Some previous studies have supported the scientific validation of the concept of detoxification/ rectification of herbal drugs. A study has reported that the concentration of toxic constituents such as strychnine and brucine in *Strychnos nux-vomica* was found to be reduced in the detoxified sample of the test drug[28]. Another study demonstrated that the amount of strychnine was found to be less in the detoxified sample of *Strychnos nux-vomica* using water and milk[29]. It has also been observed that the anticancer activity of the detoxified sample of *Semecarpus anacardium* was potentiated due to some chemical changes that occurred in the drug[30].

5. Conclusion

The findings of physicochemical standardization reveal that all the constants of crude and detoxified samples of *Saqmunia* (*Convolvulus scammonia*) were found within normal limits, which suggested that the test drug was of good quality and free from adulterants. In addition, the findings of the acute toxicity study exhibit that the non-detoxified *Saqmunia* produced mortality and serious toxicities in rats, whereas the detoxified *Saqmunia* didn't produce serious adverse effects or mortality up to 2000 mg/kg b. w. Hence, it is concluded that the detoxified form of *Saqmunia* can be safely used with a wide therapeutic window.

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