Research Article

Acute and Sub-acute Toxicity Study of *Kushta Hajrul yahood* (Calx of *Lapis judaicus*): A Unique Herbomineral Unani Formulation

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Received: 04-11-2019; **Revised:** 18-12-2019; **Accepted:** 26-12-2019.

ABSTRACT

Present study was carried out to evaluate the acute and sub-acute toxicity of *Kushta Hajrul yahood* in Wistar rats. study was conducted as per the OECD guidelines. The formulation was administered orally in a single dose (300, 2000 and 5000mg/kg b.w.) in female Wistar rats in acute toxicity study. In sub-acute toxicity, drug was given at doses of 1000, 500 and 333.33 mg/kg b.w. for 28 days. During study animals were observed for any toxic signs such as changes in the animal behaviour, body weight, and food and water intake. After completion of study haematological and biochemical parameters as well as histopathological analysis of kidney, liver, spleen and stomach were performed. In acute toxicity no mortality was observed up to 5000 mg/kg, hence test formulation was classified as safe (Category 5) as per OECD guideline 423. Repeated dose administration in three different doses does not show any statistically significant changes in haematological and biochemical parameters. Histopathology of the organs did not show any significant changes in cellular architecture. From above results it is concluded that *Kushta Hajrul yahood* is safe but long-term toxicity study is needed to confirm its safety.

Keywords: Calx; Herbomineral; *Kushta Hajrul yahood*; Safety Profile; Unani formulation.

INTRODUCTION

The use of inorganic materials in medicine subsists since ancient times and continues to use globally in many tribal and folk medicines.[1]Classical Unani literature has mentioned substances of vegetable, animal and mineral origin. Metals and minerals used include mercury, gold, silver, iron, tin, zinc, Jew's stone, alloys, and gems, etc. chemical and physical processing of these substances and their compounds have been elaborated in texts which are generally known as Calcinology.[2-5] Calcinology deals with the mineral origin drugs, their varieties, characteristics, processing techniques, properties, possibilities of adverse effects and their management etc. in a comprehensive way.[6] *Kushta* (Calx) is a Persian word '*kushtan*' which means 'to kill'.[7,8] Its preparation is time consuming and complex process which involves the purification of mineral or metal compounds and grinding together with a number of herbs, juices required for the making of *Kushta*.[9] The toxic effect of the mineral or metallic constituents is believed to be removed by repeated heat and cold cycles in herbal decoctions.[10]Thousands of formulations and remedies are used in Unani and other traditional systems of medicine which have helped humans since ancient times. These remedies are natural and researchers are showing interest in them to discover new compounds of medicinal importance.[11]

Herbal medicines and formulations which are used in the treatment of certain diseases may pose a toxic hazard and there is a need to evaluate for their safety, efficacy and therapeutic significances [12]as well, there is an emerging increase in the intake of plant based formulation as an alternative or adjuvant drug for the strong faith that these products are safe and devoid of side effects. Meanwhile, their acceptability, effectiveness, affordability, safety and low cost give more attention to use them as alternative medicine.[13] According to WHO, in spite of the wide use of medicinal plants, their efficacy and toxicity have rarely been tested, and it is therefore necessary to evaluate and standardize various herbal formulations used in the management of a numerous diseases.[14]

Kushta is the finest powder form of the medicinal preparations obtained by calcination of metal, minerals and rarely animal origin drugs. These herbo-mineral or herbo-metallic preparations are used since centuries and are claimed to be very effective and potent dosage form. *Kushta* is easily absorbed in human body and highly efficacious and potent inaction in comparison to other dosage form of the same drug.[15,4,8,16] Some of the commonly used *Kushta's* in Unani system of medicine are *Kushta Hajrul yahood* (calx of *Lapis judaicus*), *Kushta Tutia* (calx of Copper sulphate), *Kushta Khabasul hadeed* (calx ofIron oxide), *Kushta Sadaf* (calx of Oyster shell), *Kushta Sammul far* (calx of Arsenic oxide), *Kushta Jast* (calx of Zinc oxide), *Kushta Qalai* (calx of Tin)etc. for the management of various ailments of the body. Efficacy of these preparations in curing various diseases *viz.*, skin diseases, diabetic ulcers, warts, and vitiligo are well established.[17,8,4,8] In reviewing classical literature of Unani medicine, we found that almost all Unani physicians recommended *Lapis judaicus* for urolithiasis as a single drug or in combination with other drugs in various dosage

forms.[18]Moreover this traditional drug is still used in Iraq, Afghanistan, Jordan, India and Pakistan.[19]

Kushta Hajrul yahood is an important Unani pharmacopoeial formulation commonly used for the treatment of urolithiasis.[17,2,20]Its ingredients have been evaluated for various pharmacological activities *viz.,* a clinical trial on *Lapis judaicus* showed that its efficacy in dissolving calcium renal stone.[19] Sub chronic oral toxicity of *Kushta Hajrul yahood* showed it is safe up to 1000mg/kg b.w.in Wistar rats.[21] There are various factors such as environment, preparation of *kushta*, place and geographical conditions affect the metabolism and response to a particular drug. Keeping these points in mind the present study was designed to evaluate the *Kushta Hajrul yahood* for acute and sub-acute oral toxicity studies in Wistar rats.

MATERIALS AND METHODS

Raw materials

The ingredients of *Kushta Hajrul yahood viz., Lapis judaicus*, *Raphanus sativus* L. and Potassium carbonate were purchased from an authentic herb supplier in the local market of Bengaluru, India. They were identified at Trans Disciplinary University (TDU), Bengaluru by Dr. S. Noorunnisa Begum, Associate Professor, (FRLHT Acc. No. 5042, 5043, 5044 and 5045). *Lapis judaicus* was identified and characterized by Regional Ore Dressing Laboratory, Bengaluru, the Indian Bureau of Mines (IBM), Ministry of Mines Government of India, report investigation (No. K-23011/4/Chem/2018-19/Analys/Bng/OD). A voucher specimen (Ref. no. 58/IA/Res/2019) was deposited in the department of Ilmul Advia (pharmacology), drug museum, NIUM, Bengaluru for future reference. *Kushta Hajrul yahood* was prepared as per the procedure mentioned in classical literature of Unani medicine.[2,17,21]

Chemicals and reagents

All the chemicals used in this experiment were of analytical grade. Glucose (GOD-POD method), Cholesterol (CHOD-POD method), Urea (Urease/GLDH method), Creatinine (Modified Jaffe's method), AST (Modifies IFCC method), ALT (Modified IFCC method), Albumin (BCG method), Total Protein (Biuret method) reagents were procured form Aspen Laboratories. Alkaline Phosphatase mono (Kinetic UV test-optimized IFCC method), Bilrubin total, Sodium and potassium reagents were purchased from Lab care diagnostic (India) Pvt. Ltd.

Animals

In this study Wistar rats of both the sex weighing 150 ± 200 g were used. The rats were procured from registered breeder and allowed to get acclimatized for 15 days. They were housed in clean polypropylene cages at room temperature (25±2 °C), humidity 45–55% with 12 h light–12 h dark cycle throughout the experimental period and were provided with standard diet and water *ad libitum*. The animal care procedures and experimental protocol were in accord with the guidelines of the CPCSEA. IAEC ethical clearance was taken from the NIUM, Bengaluru, India, before starting the experiment (Ref. no. IAEC/614/IA/02).

Acute toxicity study

Acute toxicity study was carried out according to OECD Guideline 423 (OECD, 2001). *Kushta Hajrul yahood* was dissolved in distilled water and a single dose of 300, 2000 and 5000 mg/kg b.w. was administered by gavage to rats which were kept on fasting for overnight. The animals were observed individually for signs of acute toxicity and behavioral changes (Table 1) for 3 h post dosing, and at least once daily for 14 days.

Sub acute toxicity study

This test was performed as per the OECD Guidance 407. 50 rats (5 males and 5 females) were randomly divided into five groups. Three treated groups received different doses of aqueous suspension of *Kushta Hajrul yahood* i.e. 1000, 500 and 333.33 mg/kg respectively and the satellite group was treated with 1000 mg/kg. At the same time distilled water was given to control group. The test drug was given once daily by oral gavage for 28 days. The rats were observed for physical and behavioural changes or any abnormal signs. At the end of the study the rats were fasted overnight only water given *ad libitum*. Then they were anesthetized (thiopentone sodium 50 mg/kg, IP) and the blood was collected by cardiac puncture. The liver, kidney spleen and stomach of all the animals were excised and trimmed off any adherent tissue, and their wet weight was taken.

Body weight, food, and water consumption

Body weight of all the animals was taken on '0', 7th, 14th, 21st and on 28th day. The amount of food (g/rat/day) and water (ml/rat/day) consumption was measured daily.

Hematological and biochemical analysis

Blood was collected by cardiac puncture in EDTA-coated tubes (Qyantum Biomedicals, India) for the hematological analyses and for biochemical (without anticoagulant) parameters after 28 days. Hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), platelets count, hematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were evaluated by automated analyzer (ABX Micros ESV60 – Horiba). For the biochemical analysis blood was centrifuged at 3000 rpm for 10 min to obtain the serum. A auto analyzer (Star 20, Rapid Diagnostic Pvt Ltd) and commercial biochemical kits were used to determine biochemical parameters.[22]

Histological analysis

After anaesthesia (thiopentone sodium 50 mg/kg, IP) liver, kidney, spleen and stomach were collected and fixed in 10% formalin solution for histolpathological study, and stained in hematoxylin and eosin.[23]

Statistical analysis

The results were analyzed using one-way analysis of variance (ANOVA), followed by Tukey Kramer was used as statistic tests. Data are expressed as mean ± SEM and the differences between groups were considered to be statistically significant when $p < 0.05$.

RESULTS

Acute toxicity study

In acute toxicity study rats were treated with single dose of at 300, 2000 and 5000 mg/kg b.w. and observed for 24 h. Their further for 14 days, no signs of morbidity and any mortality was observed during the 14 days. General and behavioural observations were mentioned in Table 1.

Sub-acute toxicity

No death or any signs of toxicity was observed in animals treated with different dosage of test formulation. A gradual increase in the body weight of animals was seen in throughout the study but statistically not significant when compared with plain control (Fig. 1, tab 2). Haematology, clinical biochemistry, and histopathological observation of organs were recorded after 28 days.

Effect of *Kushta Hajrul yahood* on haematological and biochemical parameters

The hematological parameters were summarized in Table 3. Administration of *Kushta Hajrul yahood* did not show any significant changes in blood parameters and biochemical parameters such as creatinine, urea, total cholesterol, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase and total bilirubin. The results were statistically not significant when test groups were compared to control groups (Table 4).

Histopathology

Histopathological sections of liver, kidney, spleen and stomach of all treated groups revealed the normal architecture with functional integrity of hepatocytes, glomerulus, epithelial lining and parenchyma, all three layers of stomach were found to intact with moderate congestion on comparison with control rats (Fig. 2).

Table 1: Behavioural responses and general appearance of rats treated with single dose of *Kushta Hajrul yahood* in acute toxicity study.

Observation	Control 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
Diarrhoea	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

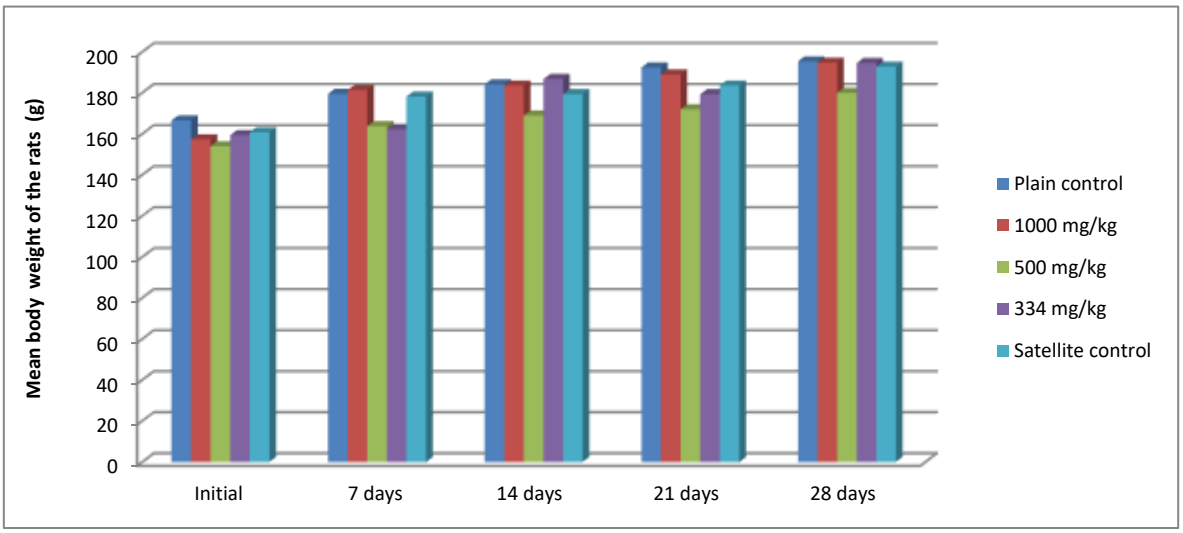

Figure 1: Effect of aqueous extract of *Kushta Hajrul yahood* on body weight of animals

Table 2: Body weight, food and water consumption of rats treated orally with *Kushta Hajrul yahood* for 28 days

Groups	Food	water
Plain control	13.696±1.825	26.88±8.086
1000 mg /kg	13.3446±1.791	25.675±11.272
500 mg/kg	13.067±2.350	24.54±9.217
333.33 mg/kg	13.025±2.119	25.31±13.003
Satellite group	13.7607±1.937	26.27±11.70

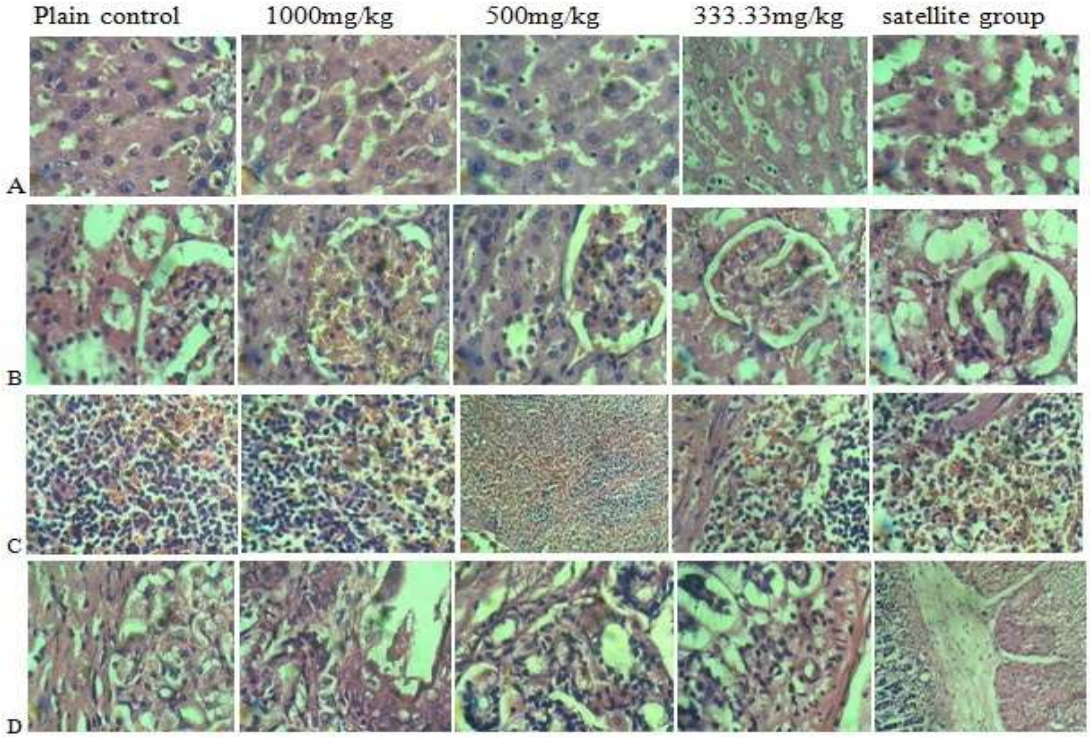

Figure 2: Histopathology (H&E stained 40x) of rat tissues of treated groups rats after 28 days. (A) Liver, (B) Kidney,(C) Spleen and (D) Stomach.

Table 3: Effects of *Kushta Hajrul yahood* on hematological parameters and platelets levels of rats - repeated dose 28-day oral toxicity study

Hematological parameters	Control	1000 mg /kg	500 mg/kg	333.33 mg/kg	Satellite
Male					
Hemoglobin (%)	13.34±0.82	13.08±1.58	13.54±0.51	13.02±1.16	13.96±0.46
Total RBC (10^6/mm^3)	6.69±0.72	6.95±0.48	6.73±0.29	6.73±0.34	6.67±0.18
WBC (10^3/mm^3)	9.42±0.27	9.22±0.21	9.62±0.45	9.44±1.40	9.16±0.84
Platelets (10^3/mm^3)	492.4±59.30	408.4±28.88	464.6±39.04	460.8±55.64	463.8±29.58
HCT (%)	40.12±2.01	41.76±3.01	40.32±2.11	39.64±2.09	39.52±1.01
MCV (µm^3)	54.4±1.33	54.2±0.86	53.2±0.049	54.2±0.66	54.8±0.74
MCH (pg)	20.92±0.45	20.46±0.69	19.56±0.36	20.38±0.54	20.86±0.34
MCHC (g/dl)	32.68±0.45	31.258±1.95	31.2±2.30	31.78±2.91	30.92±1.05
Female					
Hemoglobin (%)	13.2±0.4438	12.52±0.6476	12.4±0.6588	12.74±0.6063	12.2±0.6993
Total RBC (10^6/mm^3)	6.758±0.400	6.612±0.1965	6.51±0.2106	6.384±0.3637	6.428±0.3434
WBC (10^3/mm^3)	7.98±0.5508	7.504±0.3771	6.982±0.5344	7.528±1.043	7.92±0.5687
Platelets (10^3/mm^3)	479.6±59.095	475.6±46.73	420.4±38.413	417.6±55.280	439.4±47.781
HCT (%)	41.06±1.15	40.38±1.186	4.648±2.068	40.9±3.932	40.24±4.194
MCV (µm^3)	56.2±0.37	55.2±0.86	54.8±0.66	56.2±1.77	56.4±5.09
MCH (pg)	23.2±0.85	22.48±0.39	21.86±0.88	22.08±0.55	22.18±0.12
MCHC (g/dl)	39.36±2.36	38.86±0.81	38.22±1.52	39.92±1.07	39.32±0.45

All data are reported as the mean±SEM, analysed by ANOVA one way followed by Tukeys multiple comparison.

Table 4: Effects of *Kushta Hajrul yahood* on biochemical parameters of rats after 28 days of treatment

Biochemical parameters	Control	1000 mg /kg	500 mg/kg	333.33 mg/kg	Satellite
Male					
Glucose (mg/dL)	126.232±4.729	128.016±6.683	129.9675±11.460	115.088±9.960	128.628±14.309
Total cholesterol (mg/dL)	169.506±12.076	136.096±3.414	135.356±9.839	154.356±18.217	160.778±15.979
Urea (mg/dL)	40.1308±1.286	38.588±1.840	39.14±1.676	38.754±2.967	39.436±2.619
Creatinine (mg/dL)	1.032±0.2595	0.97±0.1456	0.908±0.1290	0.82±0.02811	0.74±0.08706
SGOT (U/L)	33.61±2.904	32.51±3.372	30.132±1.342	32.496±3.447	29.726±2.219
SGPT (U/L)	39.106±3.334	36.578±2.683	36.496±1.879	34.162±2.796	33.328±2.027
Albumin (g/dL)	3.352±0.1750	2.37±0.1629	3.452±0.3008	3.792±0.4649	3.238±0.3722
Total protein (g/dL)	6.146±0.5669	5.07±0.6588	5.312±0.4264	6.2±0.9148	5.458±0.8148
ALP (U/L)	144.2±9.074	142.6±2.874	144.8±2.653	142.8±3.200	139.2±10.375
Total bilirubin (mg/dL)	1.076±0.1899	0.698±0.1577	0.812±0.2403	0.674±0.1699	0.712±0.1568
Sodium (mEq/L)	137.684±4.803	133.05±5.057	135.216±5.302	139.49±1.454	137.834±2.241
Potassium (mEq/L)	4.82±0.3367	3.98±0.3308	4.9±0.3114	4.74±0.2522	4.64±0.3415
Female					
Glucose (mg/dL)	133.828±2.8664	129.356±2.031	129.612±5.215	128.652±9.680	123.518±16.544
Total cholesterol (mg/dL)	165.326±14.877	131.576±12.266	133.512±8.999	148.938±13.063	131.73±7.611
Urea (mg/dL)	44.308±3.267	40.05±1.879	41.748±4.246	41.734±2.213	41.906±2.623
Creatinine (mg/dL)	1.094±0.1421	0.964±0.1685	0.922±0.1131	0.996±0.1132	0.962±0.1950
SGOT (U/L)	33.93±1.792	31.104±2.549	32.876±3.826	31.422±3.021	30.696±2.093
SGPT (U/L)	34.588±2.962	32.17±4.981	32.446±2.620	32.072±3.022	30.972±1.915
Albumin (g/dL)	3.62±0.2368	2.818±0.4574	2.856±0.5388	2.918±0.1620	2.62±0.1791
Total protein (g/dL)	5.664±0.3494	4.85±0.2614	4.962±0.3834	4.674±0.1461	5.01±0.4449
ALP (U/L)	138.2±9.541	131.6±8.577	128.8±7.344	133.6±9.298	127.4±4.202
Total bilirubin (mg/dL)	0.974±0.2194	0.664±0.1536	0.928±0.1969	0.88±0.0712	0.74±0.1245
Sodium (mEq/L)	136.15±2.544	129.178±4.379	130.466±6.424	130.774±2.327	129.1±3.404
Potassium (mEq/L)	4.94±0.4771	4.64±0.3829	4.24±0.2786	4.06±0.3530	4.018±0.3709

All data are reported as the mean±SEM, analysed by ANOVA one way followed by Tukeys multiple comparison. ALP; Alkaline phosphatase

Table 5: Relative organ weight (g/100 g of body weight) of rats treated orally with *Kushta Hajrul yahood* for 28 days

Sub-acute toxicity Kushta					
Male					
Liver	5.62±0.22	4.69±0.12	4.66±0.26	4.66±0.23	4.64±0.34
Kidney	1.12±0.03	0.99±0.04	0.916±0.01	0.77±0.11	1.03±0.20
Spleen	0.43±0.04	0.40±0.03	0.46±0.01	0.55±0.08	0.40±0.04
Heart	0.46±0.035	0.38±0.09	0.40±0.018	0.45±0.04	0.37±0.01
Stomach	0.60±0.03	0.61±0.04	0.60±0.02	0.74±0.06	0.72±0.04
Lungs	1.36±0.10	1.14±0.06	1.13±0.07	1.22±0.14	1.08±0.07
Liver	5.62±0.22	4.69±0.12	4.66±0.26	4.66±0.23	4.64±0.34
Female					
Liver	5.39±0.42	4.60±0.15	4.35±0.23	4.36±0.25	4.88±0.17
Kidney	1.02±0.10	0.99±0.04	0.941±0.04	1.23±0.084	1.04±0.042
Spleen	0.60±0.048	0.43±0.04	0.39±0.04	0.56±0.09	0.45±0.034
Heart	0.42±0.05	0.40±0.02	0.43±0.03	0.45±0.042	0.34±0.01
Stomach	0.61±0.02	0.786±0.18	0.83±0.09	0.82±0.07	0.55±0.04
lungs	1.27±0.09	1.26±0.08	1.19±0.14	1.22±0.14	1.26±0.18

DISCUSSION

The use of herbal plants as a source of folk medicine in primary health care has become popular globally. Although herbal medicinal products are generally considered to be safe in comparison to conventional drugs, they are not completely free from the toxicity. Therefore, toxicological evaluation of plant derived products is necessary so that they can be used safely.[24] Large number of plant products is available for the prevention and cure of various diseases of the body. *Kushta Hajrul yahood* is used widely in Unani system of medicine for the treatment of urolithiasis. *Kushta* is prepared by grinding the *Lapis judaicus* with *Raphanus sativus* L. Juice. The leaves and roots of *Raphanus sativus* L. are used as vegetables in various parts of the world. It have been reported to possess a wide range of pharmacological activities like gut stimulatory, hepato protective, cardioprotective, antioxidant, anti urolithiatic, antiulcer and diuretic activity[25,26]. The anti urolithiatic property of this formulation makes it imperative to be used for long period of time; hence there is a dire need to establish its safety profile.

The acute and sub acute toxicity study are different from each other on the basis of the amount of test drug duration and observation period. The acute effects are usually observed soon after a single exposure of test substance, while sub-acute effects are generally monitored over an extended period of time during which animals are exposed to repeated dose of test drug.[27] Acute toxicity is usually an initial study performed to serve as the basis for classification and labeling, mode of toxic action of a substance, to decide the dose of a new compound and to help in dose determination in pharmacological studies.

In the present study, at the end of the acute toxicity study, no death or toxic signs were observed. Also, there were no significant changes in water and food consumption treated with a single dose of *Kushta Hajrul yahood* (300, 2000 and 5000 mg/kg). Furthermore, gross examination of the vital organs after 14 days showed no change in colour and volume of any organ. Subacute test showed no mortality or any behavioural changes after oral administration of 1000, 500 and 333.33 mg/kg for 28 days. There were no significant changes in food and water consumption in treated rats throughout the study, which clearly indicates that the test drug did not disturb the metabolic patterns of animals.

Comparison of relative organ weights between treated and control groups of animals have traditionally been used in evaluation of toxic effect of the test substance.[28] Previous study reported that, after exposure to potentially toxic substances, a slight reduction in body weight gain and internal organ weights was observed.[29] Body weights of each animal were carefully recorded on the day of initiation of the experiment and thereafter at weekly intervals. The weight of the organs is used as markers of pathological and physiological status of animals. The toxic effect is most likely to be seen in spleen, heart, liver and kidneys because of their vital functions that they perform in the body.[14,23] No statistically significant difference was observed between relative organ weights, indicating that the multiple dose of the formulation did not affect the wet weight, organ-to-body weight ratio and macroscopic characteristics.

The haematological parameters is an important index of physiological and pathological status and one of the most sensitive targets for toxic compounds both in man and animals.[23] It also provides information about the hematopoietic system and immunological responses.[28] In haematological parameters no statistically significant differences were observed between the groups. This indicates that the repeated dose administration of the *Kushta Hajrul yahood* is safe.[29,30]

The liver is an essential organ for detoxification of drugs, chemicals and other substances. The activities of the liver enzymes and serum proteins are best markers for detection of liver function and hepatocellular damage. Previous studies reported that increased levels indicate inflammation, cellular leakage and damage of cell membrane to cells in the liver. No significant change was observed in these enzymes in any of treated groups. The liver is also involved in the synthesis of proteins. Inflammatory responses are mostly triggered by a decrease in plasma albumin levels.[14] In present study the total protein, albumin and globulin levels of the treated rats did not change significantly (Table 4). The result may suggest that protein metabolism was not affected.

The kidneys are important organs and extremely vulnerable to toxic drugs because high volumes of blood flow. It filters various toxins, which can accumulate in the kidney tubules. Serum urea and creatinine are used as important indicators of renal function test.[30,31]In the present study no significant alteration in serum urea and creatinine was observed in the animals treated with *Kushta Hajrul yahood* in various doses compared with the control group (Table 4). The finding clearly indicates that the test formulation did not have any adverse effect and did not obstruct kidney's ability to excrete the above mentioned metabolites.[14]

Histopathology is considered as a yard stick for determining pathological changes in tissues and organs. Histology of heart, liver, kidney and spleen (Fig. 2) revealed that *Kushta Hajrul yahood* did not adversely affect the morphology of the vital organs; no cellular derangement was observed in any of the organ. Glomerulus was found to be intact with normal renal tubules in treated rats. The results of our study was found to be similar to the study reported by Dar et al., (2016) as no toxic sign was observed at the dose of 1000mg/kg of *Kushta Hajrul yahood*. The results of these two studies further confirmed the safety of *Kushta Hajrulyahood* in rats.

The present study verified that *Kushta Hajrul yahood* is safe in rats as NOAEL was observed up to 5000mg/kg b.w.; and no significant changes were observed in body weight, behaviour, haematological and biochemical parameter of the animals. However, it is not necessary that the toxic effects observed in animals may also be found in humans or vice versa which is evident by many studies. The reasons behind these variations may be due to pharmacokinetic differences. Thus, the consequences produced by oral administration of test drug in Wistar rats can be manifested differently in various species.[29] Since *Kushta Hajrul yahood* is an important pharmacopeial herbomineral formulation of Unani system of medicine which is used effectively in the treatment of urolithiasis therefore, its complete safety profile should be established by sub chronic and chronic toxicity studies in different species.

CONCLUSION

On the basis of above results and discussion it may be concluded that *Kushta Hajrul yahood* is well-tolerated by rats and has broad safety margin for therapeutic use. To better understand and establish the safety profile of this herbomineral formulation, further long term and special toxicity studies are essential.

Acknowledgement: Authors are thankful to the Ex director of NIUM, Porf. Mohd. Zulkifle and present director Prof. Abdul Wadud for providing best possible facilities to carry out present research work. Authors also express their gratitude to Prof. Ghulamuddin Sofi for his help in statistical analysis of data.

REFERENCES

1. Lev E. Healing with minerals and inorganic substances: a review of Levantine practice from the Middle Ages to the present. International Geology Review. 52(7-8), 2010, 700–725.
2. Makbul SAA, Jahan N, Ahmad G. Hajrul yahood (Lapis judaicus): An important mineral drug of Unani system of medicine for the management of urolithiasis. Journal of Ethnopharmacology. 222, 2018, 165-170.
3. Makbul SAA, Wadud A, Jahan N, Sofi G, Khan IM. Scientific appraisal of urolithiasisand its remedial measures in Unani medicine. Journal of Herbal Medicine. 8(1), 2017, 1-7.
4. Sudha A, Murty VS, Chandra TS. Standerdization of metal based Hebal Medicines. Americal Journal of Infectious Diseases. 5(3), 2009, 193-199.
5. Kapoor RC. Some observations on the matal based preparations in the Indian system of the Medicine. Indian Journal of Traditional Knowledge. 9(3), 2010 July, 562-575.
6. Singh TR, Gupta LN, Kumar N. Standar manufacturing procedure of teekshna lauha bhasma. Journal of Ayurveda and Integrative Medicine. 7, 2016, 100-108.
7. Tariq M, Chaudhary SS, Imtiyaz S, Rahman K, Zaman R. Priliminary physicochemical evaluation of kushta tutia: a Uani formulation. Journal of Ayurveda and Integrative Medicine. 5(3), 2014, 148-153.
8. Aziz N, Gilani AH, Rindh MA. Kushat(s): unique herbomineral preparations used in South Asian traditional medicine. Medical Hypothesis. 59(4), 2002, 468-472.
9. Sivasankaran S, Sankaranarayanan S, Ramakrishnan S. A Novel Sonochemical Synthesis of Metal Oxides Based Bhasmas. Materials Science Forum. 754, 2013, 89-97.
10. Nagarajan S, Sivaji K, Krishnaswamy S, Pemiah B, Rajan KS, Krishnan UM, et al. Safety and toxicity issues associated with lead based traditional metallic preparations. Journal of Ethnopharmacology. 151, 2014, 1-11.
11. Pour ZS, Hosseinkhani A, Asadi N, Shahraki HR, Vafaei H, Kasraeian M, et al. Double-blind randomized placebo-controlled trial on efficacy and safety of Lactuca sativa L. seeds on pregnancy-related insomnia. Journal of Ethnopharmacology. 227, 2018, 176–180.
12. Onyeka IP, Suleiman MM, Bako SP. Toxicity Effects of Methanolic Extract of Euphorbia hirta-Honey Mixture in Albino Rats. Journal of Pharmacognosy & Natural Products. 4(1), 2018, p. 1-8.
13. EL-Hak HNG, Moustafa ARA, Mansour SR. Toxic effect of Moringa peregrina seeds on histological and biochemical analyses of adult male Albino rats. Toxicology Reports. 5, 2018, 38–45.
14. Unuofin JO, Otunola GA, Afolayan AJ. Evaluation of acute and subacute toxicity of whole-plant aqueous extract of Vernonia mespilifolia Less. in Wistar rats. Journal of Integrative Medicine. 16, 2018, 335–341.
15. Tariq M, Chaudhary SS, Zaman R, Rahman K, Imtiyaz S. Physicochemical Standardization of Kushta Abrak Safaid: A Herbo-Mineral Unani Formulation. International Journal of Pharmaceutical Sciences and Drug Research. 5(3),2013, 129-132.
16. Bhowmick TK, Suresh AK, Kane SG, Joshi AC, Bellare JR. Physicochemical characterization of an Indian traditional medicine, Jasad Bhasama: detection of nanoparticles containing non stoichiometric zinc oxide. J Nanopart Res. 11, 2009, 655-664.
17. Kabeeruddin HM. Bayaze Kabeer Hyderabad Deccan: Hikmat book depot, 1938.
18. Sina I. Al Qanoon Fil Tib New Delhi: Idara KItabul Shifa; 2007.
19. Faridi P, Seradj H, Samani SM, Vossoughi M, Mohagheghzadeh A, Roozbeh J. Randomized and double blinded clinical trial of the safety and calcium kidney stone dissolving efficasy of Lapis judaicus. Journal of Ethnopharmacology. 2014, 82-87.
20. Vohora SB, Athar M. Mineral Drugs (used in Ayurveda and Unani medicine). 1st ed. New Delhi: Narosa Publishing House Pvt. Ltd.New Delhi, 2008.
21. Dar SA, Akbar S, Ghazanfar K, Hamdani M, Nazir T, Mir MS, et al. Sub chronic oral toxicity study of kushta Hajrul Yahood (A Herbo-mineral Unani formulation) in Wistar rats. Journal of Applied Pharmaceutical Science. 6(11), 2016 November, 105-113.
22. Lima Rd, Guex CG, da Silva ARH, Lhamas CL, Moreira KLdS, Casoti R, et al. Acute and subacute toxicity and chemical constituents of the hydroethanolic extract of Verbena litoralis Kunth. Journal of Ethnopharmacology. 224, 2018, 76–84.
23. Traesel GK, de Souza JC, de Barros AL, Souza MA, Schmitz

WO, Muzzi RM, et al. Acute and subacute (28 days) oral toxicity assessment of the oil extracted from Acrocomia aculeata pulp in rats. Food and Chemical Toxicology. 2014, 74, 320-325.

24. Mahon CPCdAN, Colodel EM, Balogun SO, de Oliveira RG, Martins DTdO. Toxicological evaluation of the hydroethanolic extract of Dilodendron bipinnatum Radlk. Journal of Ethnopharmacology. 155, 2014, p. 665–671.
25. Devaraj VC, Krishna BG, Viswanatha GL, Prasad VS, Babu SNV. Protective effect of leaves of Raphinus sativus Linn on experimentally induced gastric ulcers in rats. Saudi Pharmaceutical Journal. 19, 2011, 171–176.
26. Vargas R, Perez G RM, Perez G S, Zavala S MA, Perez G C. Antiurolithiatic activity of Raphanus sati6us aqueous extract on rats. Journal of Ethnopharmacology. 68, 1999, 335–338.
27. Nath P, Yadav AK. Acute and sub-acute oral toxicity assessment of the methanolic extract from leaves of Hibiscus rosa-sinensis L. in mice. Journal of Intercultural Ethnopharmacology. 4(1), 2014, 70-73.
28. Balogun SO, da SilvaJr IF, Colodel EM, de Oliveira RG, Ascêncio SD, de Oliveira Martins DT. Toxicological evaluation of hydroethanolic extract of Helicteres sacarolha A. St.- Hil. et al. Journal of Ethnopharmacology. 157, 2014, 285–291.
29. Almanca CCJ, Saldanha SV, Sousa DR, Trivilin LO, Nunes LC, Porfírio LC, et al. Toxicological evaluation of acute and sub-chronic ingestion of hydroalcoholic extract of Solanum cernuum Vell. in mice. Journal of Ethnopharmacology. 138, 2011, 508– 512.
30. Jin SE, Seo CS, Lee MY, Shin HK, Yang MJ, Ha H. Sub-chronic toxicity of Gyejibokryeong-hwan in Sprague-Dawley rats. Journal of Ethnopharmacology. 224, 2018, 441–450.
31. Lim KT, Lim V, Chin JH. Subacute oral toxicity study of ethanolic leaves extracts of Strobilanthes crispus in rats. Asian Pac J Trop Biomed. 2(12), 2012, 948-952.
32. Rajesh. Study of Hasate Kulya (Renal Calculus) with Therapeutic Evaluation of Unani Formulation (Sufoof Hajrul Yahud) in its Management. International Journal of Science and Research. 5(10), 2016, 268-271.
33. Vargas S R, Perez G RM, Perez G S, Zavala S MA, Perez G C. Antiurolithiatic activity of Raphanus sati6us aqueous extract on rats. Journal of Ethnopharmacology. 68, 1999, 335–338.

Source of Support: Nil, **Conflict of Interest:** None.