**IJPSR (2020), Volume 11, Issue 11** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 November 2019; received in revised form, 01 February 2020; accepted, 13 March 2020; published 01 November 2020

# ACUTE AND SUB ACUTE TOXICITY STUDY OF HYDROALCOHOLIC EXTRACT OF UNANI FORMULATION: (MAJOON HAJRUL YAHOOD) USED IN UROLITHIASIS

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**Keywords:**

Majoon hajrul yahood, Unani medicine, Toxicity, Formulation

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**ABSTRACT: Background:** Majoon hajrul yahood is a polyherbomineral formulation commonly used in the Unani system of medicine for the management of urolithiasis. In this study safety of a hydroalcoholic extract of Majoon hajrul yahood was evaluate in Wistar rats. **Materials and Methods:** The hydroalcoholic extract of the formulation were tested for acute and sub-acute toxicity study. The single-dose (300, 2000, and 5000 mg/kg) of the extract was administered orally to female rats. In the subacute study, the extract was given at doses of 1/5, 1/10, and 1/15 mg/kg (1000, 500, and 333.33 mg/kg) of maximum dose (5000 mg/kg) of acute toxicity study during 28 days orally. Mortality, changes in body weight and food consumption, organ weights, biochemical, hematological, and histopathology of the organs were performed. **Results:** In the acute study, the extract was classified as safe, according to the OECD guide in category 5. In the present study, results revealed that subacute treatment with test extract, there is no changes in biochemical, hematological, and histological changes in the tissues. **Conclusion:** The no-observed-adverse-effect level (NOAEL) was found 5000 mg/kg/day in this study for both the sexes. The hydroalcoholic extract of the Majoon hajrul yahood did not show any signs of toxicity or changes in hematological, biochemical, and histological parameters in rats when different doses were administered for 28 days.

**INTRODUCTION:** Plant-derived medicines have been known as unique sources for delivering many bioactive lead compounds for the development of new medicines and also for the treatment and prevention of various ailments of the human body and are easily accessed, an affordable, efficacious and culturally acceptable form of drug [1]. Unani system of medicine advocates therapeutic uses of herbal, mineral, and metallic preparations in many diseases since century in clinical practice. Plants are popular remedies for diseases that used most of the world's population.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(11).5694-02

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(11).5694-02

Herbal preparations currently serve the health needs, and there is clear evidence of the therapeutic benefits they have attained widespread acceptability as therapeutic agents [2, 3]. It is a widely held belief that herbal preparations are safe. However, despite the belief and claim of being natural and safe, herbal remedies have been associated with lethal effects, which have been attributed to several factors. These factors include hepatic toxicity of the main constituents and contamination of preparations by heavy metals or microorganisms [3].

The Unani system of medicine is gaining acceptance globally due to the potential of its formulations for the treatment of several chronic conditions. Although it is a traditional system of medicine being practiced since centuries, there is negligible documented evidence regarding their safety and effectiveness. However, the need of the

time is to validate these medicines as per the current regulatory requirements [4]. The major hypothetical advantage of botanicals over conventional drugs is the presence of multiple active compounds that together can provide a potentiating effect that may not be achievable by any single compound. Plant-based formulations have several phytoconstituents which may exert synergistic, potentiation, agonistic antagonistic actions. These pharmacological principles work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects [5].

Majoon (confection) is a semisolid dosage form of Unani medicine. Majoon hajrul yahood is one of the extensively used preparations in clinical practice, prescribed for the management of urolithiasis [6, 7]. Almost all Unani physicians recommended *Lapis judaicus* for urolithiasis as a single drug or in combination with other drugs in various dosage forms. Moreover, this traditional drug is still used in Iraq, Afghanistan, Jordan, India and Pakistan [8]. Majoon hajrul yahood is composed of habbe kaknaj (*Physalis alkekengi)*, hajrul yahood (*Lapis judaicus*), maghz tukhm kharbuza (*Cucumis melo*), maghz tukhm khayar (*Cucumis sativus*) and maghz kaddu (*Legenaria vulgaris*) [6, 9]. Several studies have been carried out to explore the medicinal properties of individual ingredients of tested formulation; a clinical trial on lapis judaicus showed that its efficacy in dissolving calcium renal stone [8].

Diuretic activity of *Physalis alkekengi* [10] *Cucumis melo* [11] *Legenaria vulgaris* [12, 13]. Anti-inflammatory activity of *Physalis alkekengi* [14] *Cucumis melo* [15, 16] *Legenaria vulgaris* [12, 13]. Antioxidant and analgesic activity of *Cucumis melo* [15, 16] *Legenaria vulgaris* [12, 13, 17]. Antimicrobial, hepatoprotective and activity of *Legenaria vulgaris* [12, 13, 17]. Nephroprotective [18] antiurolithiatic activity of *Cucumis melo* [19] has been reported. However, the studies done were on pharmacological activities but no scientific report is available in support of safety on long term use. The individual constituents' literature is available, but formulation still not evaluated for its toxicity. This study was aimed to examine the possible oral toxicity of repeated dosing of hydroalcoholic extract of the Majoon hajrul yahood for 28 days on liver and kidney function in Wistar rats. The finding from the present study could provide essential information to ensure the safety and quality of the Majoon hajrul yahood for future reference. Apart from the scientific assessment of the safety of the extract, other potential benefits in the treatment of human diseases, associated with long-term administration, may also be detected in this study. It also served as a reference for the researchers to select an appropriate dose for their pharmacological studies.

## MATERIALS AND METHODS:

1. **Raw Materials:** All individual ingredients of the Majoon hajrul yahood were procured from the authentic herb supplier in the local market of Bengaluru, India. They were identified at 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, Ministry of Mines Government of India, (report investigation no. K-23011/4/Chem/2018-19/Analys/Bng/OD). A voucher for the specimen (Ref. no. 58/IA/Res/2019) was deposited in the drug museum of the Department of Ilmul Advia (pharmacology), NIUM, Bengaluru for future reference. Majoon hajrul yahood was prepared as per the procedure mentioned in classical literature of Unani medicine [6, 9, 20, 21].

2. **Preparation of Plant Extract:** The powder of *Physalis alkekengi* L. prepared by grinding in an electrical grinder and passed through 80 mesh sieve to get a coarse powder of desired particle size added to the powder of Lapis judaicus. The remaining drugs ground and sieved through the 40 mesh sieve are added to this powder and mixed thoroughly [9, 20].

The hydroalcoholic extract (Aqueous: ethanol; 30:70 *v/v*) was prepared by maceration for 6 h at room temperature (37 °C) with occasional shaking. The extract was filtered using a muslin cloth, and the filtrate was then concentrated in a carefully regulated water bath maintained at a temperature of 80 ºC [22, 23]. The extract was stored in a refrigerator at 4 °C throughout the study. The yield was found to be 42% w/w.

**Preliminary Phytochemical Screening:** Preliminary phytochemical analysis was performed

for the presence of alkaloids, carbohydrates, saponins, phenols, glycosides, phytosterols, flavonoids, proteins, and amino acids [24, 25]. The animal care procedures and experimental protocol were in accord with the guidelines of the CPCSEA. Animals were procured from Biogen® Laboratory Animal facility (CPCSEA Reg. no: 971/PO/RcBiB/S/2006/CPCSEA). IAEC clearance was taken from the NIUM, Bengaluru, India, before starting the experiment (Ref. no. IAEC/ 614/IA/02).

**TABLE 1: EXPERIMENTAL ANIMALS**

| S. no. | Information about experimental animals | |
|---|---|---|
| 1 | Animals | Wistar rats of both sexes |
| 2 | Weight | 150 ± 200 g |
| 3 | Housing | 6 rats/polypropylene cage 15 days prior to the experiment |
| 4 | Feed | standard rat diet and water *ad libitum* |
| 5 | Room temperature and humidity | room temperature (25 ± 2 °C) humidity 45-55% with 12 h light-12 h dark cycle throughout the experimental period |

**Acute Toxicity Study:** Acute toxicity study was tested 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 was kept on fasting for overnight. The animals were observed individually for signs of acute toxicity and behavioral changes in **Table 1** post-dosing at least once during the first 30 min for the first 4 h and at least once daily for the next 14 days [26, 27].

**Sub Acute Toxicity Study:** This test was performed as per the OECD Guidance 407 (OECD, 2008). Fifty rats (5 males and 5 females) were randomly divided into five groups. Three treated groups received different doses of aqueous extract of Majoon 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 the control group.

The test drug was given once daily by oral gavage for 28 days. The rats were observed for physical and behavioral changes or any abnormal signs. At the end of the study, the rats 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 were taken on '0', 7$^{th}$ and 14$^{th}$, 21$^{st}$ and on 28$^{th}$ day. The amount of food (g/rat/day) and water (ml/rat/day) consumption was measured daily [28].

**Hematological and Biochemical Analysis:** All the chemicals used in this experiment were of analytical grade. 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 hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were evaluated by an automated analyzer (ABX Micros ESV60 – Horiba, hematology analyzer). For the biochemical analysis, blood was centrifuged (REMI, R 8 C, Laboratory Centrifuge, REMI Centrifuge, Mumbai) at 3000 rpm for 10 min to obtain the serum. Auto analyzer (Star 20, rapid diagnostic Pvt. Ltd.) and commercial biochemical kits were used to determine the glucose (GOD-POD method), cholesterol (CHOD-POD method), urea (Urease/GLDH method), creatinine (Modified Jaffe's method), aspartate aminotransferase (AST) (Modifies IFCC method), alanine aminotransferase (ALT) (Modified IFCC method), albumin (BCG method), total protein (Biuret method) reagents were procured from 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 [28, 29].

**Histological Analysis:** After anesthesia (thio-pentone sodium 50 mg/kg, IP) liver, kidney, spleen, and stomach were collected and fixed in 10% formalin solution for a histopathological study, and stained in hematoxylin and eosin [29, 30].

**Statistical Analysis:** The results were analyzed using a 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:

**Preliminary Phytochemical Analysis:** Preliminary phytochemical of hydroalcoholic extract of Majoon hajrul yahood revealed the presence of alkaloids, carbohydrates, saponins, phenols, glycosides, phytosterols, flavonoids, proteins, and amino acids.

**Acute Toxicity Study:** In acute toxicity study, rats were treated with a single dose of 300, 2000, and 5000 mg/kg b.w. Majoon hajrul yahood.

All the animals were observed for that appearance of signs of toxicity or death for 14 days. General and behavioral observations were mentioned in **Table 2**.

**TABLE 2: GENERAL APPEARANCE AND BEHAVIOURAL RESPONSES OF RAT TREATED WITH SINGLE DOSE OF HYDROALCOHOLIC EXTRACT OF MAJOON HAJRUL YAHOOD IN ACUTE TOXICITY STUDY**

| Observation | Control group | 300 mg/kg | 2000 mg/kg | 5000 mg/kg |
|---|---|---|---|---|
| Change in skin and fur | No effect | No effect | No effect | No effect |
| Eye and mucous membranes colour change | No effect | No effect | No effect | No effect |
| 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 |

**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 bodyweight of both sexes of animals was seen throughout the study; moreover, food, and water consumption also no statistically significant when compared with plain control **Fig. 1**. Hematological, biochemical, and histopathological analysis of organs was performed after 28 days.

**Effect of Hydroalcoholic Extract of Majoon Hajrul Yahood on Haematological Parameters and Biochemical Parameters:** The hematological parameters were summarized in **Table 3**. Administration of formulation extract did not show any significant changes in blood parameters and biochemical parameters such as creatinine, urea, total cholesterol, total protein, albumin, AST, ALT, alkaline phosphatase, and total bilirubin. The results were statistically not significant when test groups were compared to control groups in **Table 4**.

**Histopathology:** Histopathological sections of liver, kidney, spleen, and stomach of all aqueous extract treated groups revealed the normal architecture with the functional integrity of heap-tocytes, glomerulus, epithelial lining and parenchyma, all three layers of stomach intact with moderate congestion on comparison with control rats **Fig. 2**.

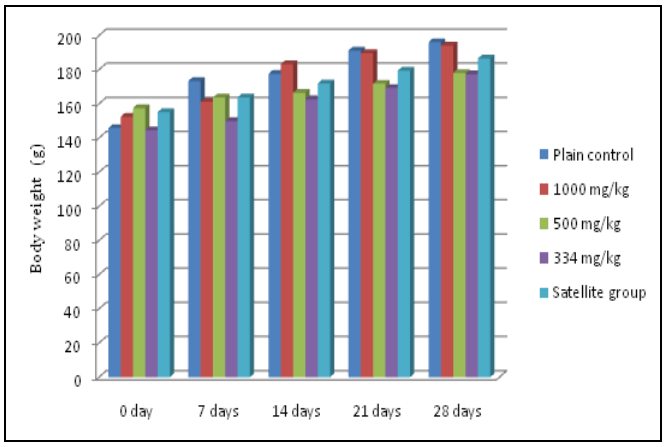


**FIG. 1: BODY WEIGHT OF RATS TREATED WITH HYDROALCOHOLIC EXTRACT OF MAJOON HAJRUL YAHOOD FOR 28 DAYS**

**TABLE 3: FOOD AND WATER CONSUMPTION OF RATS TREATED ORALLY WITH HYDROALCOHOLIC EXTRACT OF MAJOON HAJRUL YAHOOD FOR 28 DAYS**

| Groups | Food | water |
|---|---|---|
| Plain control | 12.81 ± 3.52 | 20.58 ± 8.15 |
| 1000 mg /kg | 12.11 ± 3.24 | 23.35 ± 5.91 |
| 500 mg/kg | 11.76 ± 2.60 | 21.94 ± 6.51 |
| 333.33 mg/kg | 12.10 ± 1.51 | 22.24 ± 6.96 |
| Satellite group | 12.31 ± 2.01 | 21.13 ± 7.97 |

**TABLE 4: EFFECTS OF HYDROALCOHOLIC EXTRACT OF MAJOON HAJRUL YAHOOD ON BLOOD PARAMETERS OF RATS AFTER 28 DAY OF TREATMENT**

| Hematological parameters | Control | 1000 mg/kg | 500 mg/kg | 333.33 mg/kg | Satellite group |
|---|---|---|---|---|---|
| **Male** | | | | | |
| Hemoglobin (%) | 13.09 ± 0.64 | 13.44 ± 0.66 | 13.84 ± 0.73 | 12.98 ± 0.63 | 13.34 ± 0.64 |
| Total RBC ($10^6$ /$mm^3$) | 6.84 ± 0.37 | 6.44 ± 0.45 | 6.99 ± 0.50 | 6.61± 0.32 | 6.49 ± 0.26 |
| WBC ($10^3$ /$mm^3$) | 12.02 ± 0.49 | 13.46 ± 1.65 | 12.44 ± 1.74 | 13.02 ± 048 | 6.49 ± 0.26 |
| Platelets ($10^3$ /$mm^3$) | 418.6 ± 28.09 | 387.8 ± 75.49 | 415.8 ± 43.16 | 434.4 ± 42.34 | 406.2 ± 39.55 |
| HCT (%) | 41.64 ± 2.02 | 37.18 ± 1.70 | 38.06 ± 2.68 | 36.86 ± 1.90 | 38.10 ± 1.83 |
| MCV ($\mu m^3$) | 54.8 ± 0.73 | 54.4 ± 0.24 | 53.8 ± 0.73 | 53.8 ± 0.86 | 53.6 ± 0.51 |
| MCH (pg) | 20.48 ± 0.46 | 19.9 ± 0.49 | 20.1 ± 0.48 | 19.82 ± 0.22 | 20.46 ± 0.36 |
| MCHC (g/dl) | 30.658 ± 1.54 | 33.14 ± 2.09 | 33.78 ± 2.34 | 34.42 ± 1.70 | 34.01 ± 1.91 |
| **Female** | | | | | |
| Hemoglobin (%) | 12.66 ± 0.52 | 12.32 ± 0.37 | 12.44 ± 0.56 | 12.58 ± 0.43 | 12.08 ± 0.32 |
| Total RBC ($10^6$ /$mm^3$) | 6.72 ± 0.22 | 5.71 ± 0.13 | 5.86 ± 0.21 | 5.73 ± 0.43 | 5.63 ± 0.24 |
| WBC ($10^6$ /$mm^3$) | 11.14 ± 1.25 | 9.62 ± 1.05 | 10.62 ± 0.91 | 10.88 ± 0.85 | 10.08 ± 0.38 |
| Platelets ($10^6$ /$mm^3$) | 444.8 ± 78.94 | 423.2 ± 40.99 | 408.6 ± 21.44 | 455.6 ± 36.47 | 413.4 ± 38.54 |
| HCT (%) | 34.104 ± 1.91 | 33.02 ± 2.67 | 33.108 ± 0.70 | 33.26 ± 1.22 | 32.92 ± 1.65 |
| MCV ($\mu m^3$) | 55.8 ± 0.66 | 57.4 ± 1.29 | 56.6 ± 0.93 | 57.2 ± 2.15 | 55.4 ± 0.87 |
| MCH (pg) | 20.3 ± 0.80 | 20.76 ± 0.54 | 20.98± 0.65 | 20.19 ± 1.43 | 21.48 ± 0.85 |
| MCHC (g/dl) | 31.64 ± 2.55 | 30.04 ± 1.31 | 31.84 ± 0.78 | 31.34 ± 0.89 | 31.02 ± 0.94 |

All data are reported as the mean ± SEM, analyzed by ANOVA one way followed by Tukey's multiple comparisons

**TABLE 5: EFFECTS OF HYDROALCOHOLIC EXTRACT OF MAJOON 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 group |
|---|---|---|---|---|---|
| **Male** | | | | | |
| Glucose (mg/dl) | 108.63 ± 3.15 | 106.89 ± 7.30 | 114.71 ± 5.81 | 115.45 ± 4.74 | 103.64 ± 4.65 |
| Total cholesterol (mg/dl) | 137.05 ± 9.62 | 125.47 ± 23.14 | 126.80 ± 19.63 | 138.48 ± 8.63 | 134.27 ± 31.67 |
| Urea (mg/dl) | 38.5 ± 2.45 | 39.01 ± 2.72 | 40.23 ± 3.63 | 40.03 ± 2.94 | 37.94 ± 3.72 |
| Creatinine (mg/dl) | 1.14 ± 0.23 | 0.98 ± 0.10 | 1.056 ± 0.14 | 1.01 ± 0.16 | 1.18 ± 0.15 |
| SGOT (U/L) | 31.85 ± 3.61 | 31.93 ± 2.52 | 29.13 ± 2.96 | 29.13 ± 1.97 | 31.98 ± 2.76 |
| SGPT (U/L) | 37.34 ± 2.80 | 35.72 ± 3.43 | 37.47 ± 3.34 | 38.11 ± 8.87 | 34.60 ± 1.72 |
| Albumin (g/dl) | 3.37 ± .036 | 2.92 ± 0.31 | 2.58 ± 0.22 | 2.66 ± 0.19 | 2.32 ± 0.34 |
| Total protein | 6.01 ± 0.51 | 4.88 ± 0.58 | 6.12 ± 0.44 | 5.84 ± 0.43 | 5.06 ± 0.44 |
| ALP (g/dl) | 132.12 ± 5.80 | 133.2 ± 12.04 | 114.2 ± 4.82 | 134.6 ± 12.78 | 122.2 ± 7.23 |
| Total bilrubin (mg/dl) | 1.045 ±0.27 | 0.81 ± 0.11 | 0.92 ± 0.22 | 0.84 ± 0.11 | 0.87 ± 0.19 |
| Sodium (mEq/L) | 137.47 ± 4.01 | 114.07 ± 11.24 | 120.13 ± 2.06 | 115.39 ± 1.04 | 130.77 ± 13.03 |
| Potassium (mEq/L) | 4.09 ± 0.34 | 4.64 ± 1.02 | 4.96 ± 0.60 | 5.08 ± 0.38 | 3.76 ± 0.21 |
| **Female** | | | | | |
| Glucose (mg/dl) | 112.83 ± 5.60 | 115.02 ± 10.98 | 115.88 ± 8.09 | 119.91 ± 5.11 | 116.96 ± 3.59 |
| Total cholesterol (mg/dl) | 132.47 ± 11.75 | 130.63 ± 0.94 | 132.50± 9.45 | 132.37 ± 6.52 | 130.95 ± 2.75 |
| Urea (mg/dl) | 37.02 ± 1.58 | 33.25 ± 4.34 | 34.03 ± 2.14 | 36.89 ± 2.42 | 37.94 ± 3.46 |
| Creatinine (mg/dl) | 0.99 ± 0.14 | 0.92 ± 0.09 | 0.89 ± 0.09 | 0.91 ± 0.10 | 0.94 ± 0.21 |
| SGOT (U/L) | 33.11 ± 2.06 | 31.41 ± 2.45 | 31.61 ± 1.44 | 32.97 ± 2.96 | 32.92 ± 2.17 |
| SGPT (U/L) | 37.62 ± 1.36 | 33.40 ± 1.44 | 35.47 ± 3.10 | 37.85 ± 1.45 | 35.94 ± 2.89 |
| Albumin (g/dl) | 3.75 ± 0.299 | 2.96 ± 0.34 | 3.19 ± 0.39 | 3.68 ± 0.41 | 3.13 ± 0.48 |
| Total protein | 4.98 ± 0.43 | 4.91 ± 0.67 | 5.76 ± 0.63 | 5.28 ± 1.08 | 5.49 ± 0.48 |
| ALP (g/dl) | 134.99 ± 2.59 | 133.2 ± 5.04 | 132.4 ± 13.66 | 130.6 ± 2.46 | 131.6 ± 3.08 |
| Total bilrubin (mg/dl) | 0.70 ± 0.17 | 0.74 ± 0.25 | 0.64 ± 0.18 | 0.65 ± 0.15 | 0.74 ± 0.12 |
| Sodium (mEq/L) | 134.63 ± 3.75 | 130.47 ± 6.42 | 133.43 ± 8.92 | 128.63 ± 3.31 | 135.67 ± 4.60 |
| Potassium (mEq/L) | 4.37 ± 0.34 | 3.54 ± 0.40 | 4.32 ± 0.34 | 4.3 ± 0.27 | 3.74 ± 0.37 |

All data are reported as the mean ± SEM, analyzed by ANOVA one way followed by Tukey's multiple comparisons

**TABLE 6: RELATIVE ORGAN WEIGHT (g/100g OF BODY WEIGHT) OF RATS TREATED WITH HYDROALCOHOLIC EXTRACT OF MAJOON HAJRUL YAHOOD FOR 28 DAYS**

| | Plain Control | 1000 mg /kg | 500 mg/kg | 333.33 mg/kg | Satellite group |
|---|---|---|---|---|---|
| **Male** | | | | | |
| Liver | 5.33 ± 0.18 | 5.31 ± 0.44 | 5.40 ± 0.43 | 5.24 ± 0.14 | 5.20 ± 0.49 |
| Kidney | 1.06 ± 0.09 | 0.93 ± 0.06 | 1.06 ± 0.10 | 0.99 ± 0.031 | 0.96 ± 0.08 |

| | | | | | |
|---|---|---|---|---|---|
| Spleen | 0.54 ± 0.12 | 0.48 ± 0.04 | 0.52 ± 0.03 | 0.53 ± 0.04 | 0.44 ± 0.04 |
| Heart | 0.39 ± 0.01 | 0.40 ± 0.05 | 0.44 ± 0.02 | 0.41 ± 0.04 | 0.37 ± 0.03 |
| Stomach | 0.66 ± 0.07 | 0.54 ± 0.04 | 0.64 ± 0.04 | 0.68 ± 0.02 | 0.60 ± 0.07 |
| Lungs | 0.91 ± 0.22 | 1.25 ± 0.19 | 1.41 ± 0.16 | 1.35 ± 0.13 | 1.39 ± 0.17 |
| **Female** | | | | | |
| Liver | 5.08 ± 0.24 | 4.85 ± 0.17 | 4.41 ± 0.18 | 4.35 ± 0.11 | 4.75 ± 0.16 |
| Kidney | 1.24 ± 0.08 | 1.14 ± 0.07 | 0.91 ± 0.01 | 0.97 ± 0.14 | 1.00 ± 0.02 |
| Spleen | 0.53 ± 0.05 | 0.53 ± 0.04 | 0.58 ± 0.05 | 0.58 ± 0.03 | 0.45 ± 0.05 |
| Heart | 0.46 ± 0.02 | 0.39 ± 0.03 | 0.44 ± 0.02 | 0.40 ± 0.02 | 0.41 ± 0.01 |
| Stomach | 0.67 ± 0.07 | 0.69 ± 0.05 | 0.74 ± 0.06 | 0.69 ± 0.05 | 0.69 ± 0.04 |
| lungs | 1.49 ± 0.05 | 1.26 ± 0.05 | 1.20 ± 0.10 | 1.18 ± 0.07 | 1.23 ± 0.12 |

All data are reported as the mean ± SEM, analyzed by ANOVA one way followed by Tukey's multiple comparisons

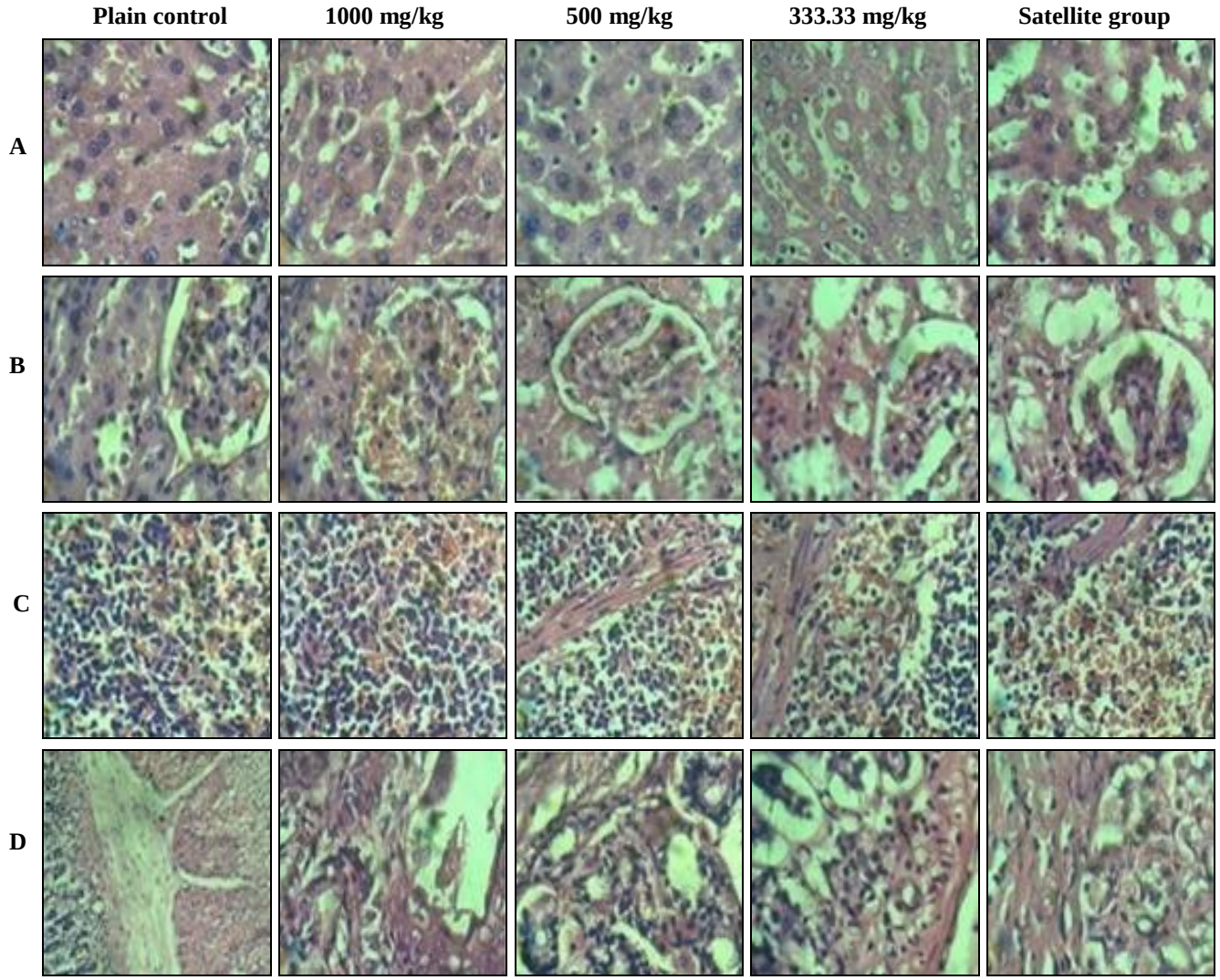


**FIG. 2: HISTOPATHOLOGY H AND E STAIN 40× OF RAT TISSUES OF HYDRO ALCOHOLIC EXTRACT OF MAJOON HAJRUL YAHOOD TREATED GROUPS' RATS AFTER 28 DAYS (A) LIVER, (B) KIDNEY, (C) SPLEEN AND (D) STOMACH**

**DISCUSSION:** In developing countries, botanicals have become famous in healthcare, and some have been falsely considered as safe because they are natural in origin. However, there is a lack of data on the toxicological profile and adverse effects of these compounds [31]. Although botanicals are widely considered to be of lower risk compared with synthetic drugs, they are not completely free from the possibility of toxicity or other adverse effects. Thus, toxicological evaluation of plants derived products, including extracts, forms an essential part of scientific validation of medicinal plants [32]. The toxicological evaluation of the hydroalcoholic extract of Majoon hajrul yahood

was performed to evaluate the risks of acute and sub-acute administration since it is widely used for therapeutic purposes. The antiurolithiatic property of this formulation makes it imperative to be used for a long period of time; hence there is a dire need to establish its safety profile. Acute toxicity is usually an initial study performed: to serve as the basis for classification and labeling, to provide initial information on the mode of toxic action of a substance, to help arrive at a dose of a new compound, to aid in the identification of the further range of doses in animal studies and also for calculating the therapeutic index of drugs and chemicals [31, 33]. Administration of hydroalcoholic extract of Majoon hajrul yahood in rats does not cause death. Also, there were no significant changes in water and food consumption in any animals given a single dose of Majoon hajrul yahood (300, 2000, and 5000 mg/kg) when compared with the control group. Furthermore, necropsy of the vital organs after 14 days showed no change in any organ. Subacute toxicity showed no death or any behavioral changes after treated with test extract at the doses 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.

The hematopoietic system is one of the most susceptible targets for toxic compounds and an important index of physiological and pathological status in man and animal [30, 34]. In the hematological analysis, there were no statistically significant differences observed in parameters between the groups. This indicates that the subacute administration of the major hajrul yahood does not produce ant toxic effects on the hematopoietic system and is safe. After some exposure to potentially toxic substances, there will be changes in body and organ weights, which are a clear indication of damage caused by the substance test [30, 34]. Body weights of each animal were carefully recorded on the day of starting the experiment and thereafter at every week till the completion of the experiment.

The weight of the organs is used as markers of the 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 [30, 35].

In the present study, no statistically significant difference was observed between relative wet weights of the organ examined (liver, kidney, spleen, and stomach), among groups in **Table 5**. This indicates that the multiple doses of the formulation did not affect the organ-to-body weight ratio and macroscopic characteristics like appearance, color, size.

The liver plays a major role in xenobiotics metabolism. As a result, it is usually affected by toxicants. Hepatocellular damage and functional capacity of the liver are evaluated by determining some enzymes and proteins in **Table 4**. The functional capacity of the kidney was evaluated *via* the determination of the serum urea and creatinine levels [1]. Clinical biochemistry tests are used in toxicology studies to generate data that are crucial for evaluating altered organ function or major toxic effects in tissues in experimental animals [33].

Specifically, effects on kidney and liver should be performed and under certain circumstances may provide useful information [30]. In this study, there was no statistical difference in the liver or renal parameters. Several classes of pharmacologically important phytochemicals like saponins, tannins, flavonoids, *etc*., were detected in the preliminary screening of Majoon hajrul yahood. In addition, the sum effect of the extract could not have been due to one phytoconstituents, but rather a combination of each constituent [32]. *Legenaria vulgaris* and *Cucumis melo* on of the ingredients of the tested formulation possess hepatoprotective and nephroprotective activities. Previous studies reported that plant extracts rich in tannins, flavonoids can lead to the relaxation of the smooth muscle of the urinary tract, which could facilitate the expulsion of stones from the kidney and diminished the size of calculi in rats [36]. Flavonoids and phenolic compounds exhibit inhibitory effects against multiple microorganisms and are the natural antioxidants with antimutagenic and anti-inflammatory properties [37, 38].

Histopathological analysis of organs of treated animals, both macro and microscopically, is the basis of a safety assessment. In this study, the macroscopic analysis, the extract at all doses tested, produced no changes in the animals' vital organs in the qualitative analysis.

Similarly, in histology, there were no findings suggestive of toxic effects **Fig. 2**. These results proved to be consistent with biochemical analysis, confirming the safety of using the hydroalcoholic extract of Majoon hajrul yahood.

**CONCLUSION:** In summary, the NOAEL in the present study was 5000 mg/kg in acute toxicity study. Findings from this investigation provide valuable information on the acute and sub-acute toxicity profiles of hydroalcoholic extract of Majoon hajrul yahood in Wistar rats. These results might be helpful in future pre-clinical and clinical studies. Moreover, further, long term and special toxicity studies are essential.

**ACKNOWLEDGEMENT:** Authors are thankful to the Ex-director of NIUM, Professor. Mohd. Zulkifle and present director Professor. Abdul Wadud for providing the best possible facilities to carry out this research work. We also acknowledge Professor. Ghulamuddin Sofi for his help in the statistical analysis of data.

**CONFLICTS OF INTEREST:** Nil

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**How to cite this article:**
Makbul SAA and Jahan N: Acute and sub acute toxicity study of hydroalcoholic extract of unani formulation: (majoon hajrul yahood) used in urolithiasis. Int J Pharm Sci & Res 2020; 11(11): 5694-02. doi: 10.13040/IJPSR.0975-8232.11(11).5694-02.

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