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**Original Article**

# Quality Control of *Majoon-e-Nisyan* and its Acute Oral Toxicity Study in Experimental Rats

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**ABSTRACT**

The clinical condition Amnesia causes difficulty in learning new information and the inability to recall past events. It is primarily concerned with recent memory loss.

*Majoon-e-Nisyan* (MJN) is a polyherbal Unani formulation, present in a semi-solid form. It is widely used potent drug of the Unani System of Medicine (USM) for treating *Nisyan* (amnesia). In the present study polyherbal Unani formulation, MJN has been studied for its quality control and acute toxicity. Standardization (quality control) of drugs deals with drug identity, drug quality and purity determination. Standardization of MJN had been done as per the Unani pharmacopoeial parameters approved by World Health Organization (WHO) - Pharmacognostical parameters, Physico-chemical parameters, high-performance thin-layer chromatography (HPTLC), microbial load, aflatoxin, and heavy metals. Solvents and chemicals used in the study were of analytical grade and used instrument were calibrated. By conducting an acute oral toxicity study in rats, the safety of MJN was assessed. The limit test method of OECD guideline 425 was followed in the study. Results of standardization and standard operating procedures (SOPs) for preparation of MJN may serve as the standard reference in the future. The data generated in the study for the quality control of MJN proved the quality of formulation and shows that MJN is not toxic in rats following acute dosing up to 2000 mg/kg bw. The data obtained in the paper for MJN may be used as a standard guideline for preparation of the formulation which can save time, cost, and resources for future research endeavours.

**Keywords** Acute toxicity*, Majoon-e-Nisyan, Unani, Nisyan,* Amnesia

## 1. INTRODUCTION

In current study, MJN was studied in detail for its macroscopic characters and physic-chemical parameters which are important for identification and quality control of Unani medicines respectively (single drugs as well as compound formulation).

Amnesia is clinically manifested by difficulty in learning new information and the inability to recall past events. (Kumar RD *et al.,* 2013). In this condition recent memory loss occurs. The initial symptom of dementia syndrome is often amnesia which is characterized by multiple cognitive deficits. (Goldman L *et al.,* 2001) The incidence of Transient global amnesia (TGA) has been reported to be 5.2-10 per 100,000 per year, and 23.5-32 per 100,000 per year among 50 years and older persons (Kremen S *et al.,* 2019). All the raw materials in the compound were taken dried and powdered separately and passed through sieve no. 80 and boiled on slow heat after adding a specified quantity of honey, 0.1 % of citric acid is added, and a homogenous product is prepared called *Majoon* as mentioned in Unani pharmacopeia of India (Anonymous, 2010).

*Majoon-e-Nisyan* (MJN) have six ingredients, which are- *Kundur* (*Boswelia serrate* Roxb.), *Waj* (*Acoruscalamus* L.), *Saad Koofi* (*Cyperusrotundus* L.), *FilfilSiyah* (*Piper nigrum* L.), *Zanjabeel* (*Zingiberofficinale* Rosc.) and *Asl-asli* (Honey) (Anonymous, 2010).

These ingredients of MJN possess hot and dry temperament which are antagonist to the temperament (*Mijaz*) of the morbid material which causes *Galeez-balgham* (Thick *Phlegm*) possessing *Barid Ratab* (cold and wet) *Mijaz* (temperament). MJN have *Muqawwi-e-Dimagh* (cerebral tonic), *Munaqqi-e-Dimagh* (cerebro-evacuant), *Muqawwi-e-A'sab* (nervine tonic) and *Munaqqi-e-Balgham* from brain (phlegmatic cerebro-evacuant) properties (Anonymous, 2009; Anonymous, 2010).

The potent efficacy of ingredients of MJN in memory impairment has been proved in various clinical and pre-clinical studies. *Kundur* (*Boswelia serrata*) has been reported for beneficial effects on AlCl3-induced AD (Alam M *et al.,* 2010).

The neuro-protective activity of the plant *Waj* (*Acorus calamus*) has been reported on Alzheimer's type of dementia (Amit K, Vandana; 2013).Combine extract of *Saad Koofi* (*Cyperus rotundus* L.) and *Zanjabeel* (*Zingiber officinale* Rosc.) (Malhotra S, Singh AP; 2013) has shown the improvement in memory impairment.

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The *Piper nigrum* (*Filfil Siyah*) prevents the nerve degeneration and alleviates the associated neuro-psychological symptoms in animal models of Alzheimer's disease (Majeed, Prakash L; 2010).

## 2. MATERIAL AND METHODS

**Collection and authentication of crude ingredients of the formulation**

The raw drugs of the study formulation MJN were availed from the local Hyderabad market and GMP Certified Pharmacy Section of NRIUMSD, Hyderabad. All the crude drugs were identified and authenticated by Botanist of SMP Unit of NRIUMSD, Hyderabad. The identified drugs were preserved in the Museum of NRIUMSD, Hyderabad with Voucher Specimen Numbers as; *Waj* (SMPU/CRI-HYD 13574), *Filfil siyah* (SMPU/CRI-HYD 13575), *Sad koofi* (SMPU/CRI-HYD 13576), *Kundur* (SMPU/CRI-HYD 13577) and *Zanjbeel* (SMPU/CRI-HYD 13578).

**Table 1.** Ingredients of *Majoon-e-Nisyan* (Anonymous, 2006; Anonymous, 2010).

| S. No. | Drug Name | Scientific Name | Parts Used | Quantity |
|---|---|---|---|---|
| 1 | Kundur | *Boswellia serrate* Roxb. | Resin | 60 g |
| 2 | Waj | *Acorus calamus* L. | Rhizome | 60 g |
| 3 | Sad Kufi | *Cyperus rotundus* L. | Rhizome | 60 g |
| 4 | Zanjabeel | *Zingiber officinale* Rosc. | Rhizome | 30 g |
| 5 | FilfilSiyah | *Piper nigrum* L. | Fruit | 30 g |
| 6 | Asl-Asli | *Apis mellifera* L. | Secretions of plants (floral nectar) | 800 g |

**Preparation of the formulation**

Study formulation was prepared in the GMP certified Pharmacy Section of *National Research Institute of Unani Medicine for Skin Disorders*, Hyderabad as per the formula and procedure mentioned in National Formulary of Unani Medicine (NFUM) and Unani Pharmacopoeia of India (Anonymous, 2010).

The raw ingredients were examined by naked eye and cleaned properly for any foreign materials. All the ingredients mentioned in the above table were taken, dried and powdered separately, filtered through the sieve (no 80). Quantity of honey was taken as per the composition, 0.1% citric acid was added and boiled on slow flame. *Qiwam* of 75% consistency was prepared and filtered through the muslin cloth. Powder of all the ingredients were added in the hot *Qiwam* and mixed vigorously to prepare the homogenous formulation. Allowed it to cool at room temperature and stored in tightly closed containers to protect it from light and moisture (Anonymous, 2010).

**Evaluation of organo-leptic Properties**

Organo-leptic characters- colour, odour, smell, taste and appearance of the formulation carried out with naked eye, and were observed as per the standard schedule.

**Physico-chemical evaluation**

The Physico-chemical parameters were carried out on MJN in the Drug standardization Research Unit (DSRU) of *National Research Institute of Unani Medicine for Skin Disorders (NRIUMSD)*, Hyderabad. Various parameters like Determination of Total Ash, acid insoluble ash (Afaq SH *et al.*, 1994; Wallis TE, 2004; Anonymous, 2006; Anonymous, 2010), Alcohol soluble matter, Water soluble matter (Anonymous, 2006; Anonymous, 2010), pH of 1% aqueous solution, reducing sugar and non-reducing sugar (Anonymous, 2010; Anonymous, 2006), Microbial Load (Anderson J M, 1975;Gunn B A, 1977; Hansen W, Yourassawsky E J;1984;Greenberg A E, 1985; Rambach A, 1990;Forbes *et al.*, 1998; Eyo AA,2001), aflatoxin analysis (Anonymous, 2010; Liu Y, Wu F; 2010), heavy metals were analyzed. Thin layer chromatography (TLC), High performance Thin layer chromatography (HPTLC) was performed for developing the fingerprint profile.

**Heavy metals Analysis** (Anonymous, 2010)

Heavy Metals analysis of MJN was done by Atomic Absorption Spectro-photometry at Drug Standardization Research Institute (DSRI), Ghaziabad. In this determination of Lead, Cadmium, Arsenic, Mercury and Copper was done. The obtained result is mentioned in table 5.

**TLC (Thin layer chromatography)** (Anonymous, 2010; Anonymous, 2006)

Thin layer chromatography of chloroform extract of MJN was done on a pre-coated TLC plate. Sample preparation: Extract of 5 g powder of MJN was mixed with 100 ml of chloroform and extracted for 30 min. The extract was filtered and concentrated to 5 ml then obtained sample obtained was used for thin layer chromatography and the chloroform extract was applied on the TLC plate.

*Procedure:* 10 µl of chloroform extract was applied as 10 mm bands on silica gel "G" plate and the plate was placed in Toluene: Ethyl acetate: Methanol (7:2:1) and $R_f$ was calculated.

*Detection:* Under UV 366nm the TLC plate showed seven major spots at $R_f$ values 0.18 (blue), 0.32 (blue), 0.40 (blue), 0.50 (blue); 0.64 (blue), 0.71 (light yellow), 0.78 (light blue), and under UV 254nm shows nine spots at $R_f$ values 0.17, 0.25, 0.37, 0.42, 0.51, 0.62, 0.68, 0.78, 0.88 (all black).

*Method conditions:*

Make of HPTLC instrument: Desaga *Sarstedt Gruppe* (Germany)
Development chamber: 20 X10 cm, Twin-trough chamber
Stationary phase: Pre coated silica gel 60 F254
Aluminum plates: (Merck, KgaA, Germany)
Plate thickness : 0.2 mm
Plate size : 200 x 100 mm
Distance from starting : 20 mm

Distance from bottom : 10 mm
Volume applied : 5 µl
Band length : 10 mm
Distance between tracks : 20 mm
Development distance : 80 mm
Solvent used : HPLC grade
Extract storage vials : 5 ml glass vials
Mobile phase : Toluene: Ethyl acetate: Methanol = 7: 2: 1, v/v/v

**Evaluation of Acute toxicity**
Safety of MJN was assessed by conducting acute oral toxicity study in rats. The study was performed as per limit test method of OECD guideline 425.

***Experimental Animals***
Wistar rats (100 ± 20 g, about 6 weeks old) were procured from Edara Research Foundation, Hyderabad, India. The selected female rats were nulliparous and non-pregnant. Rats were individually housed in transparent cages in the air-conditioned room maintained at the temperature of 22°C ± 3°C and relative humidity of 30-70%, with a 12:12 h light/dark illumination cycle. CPCSEA guidelines of laboratory animal care were followed throughout the experiment. Protocol of the study was approved by the Institutional Animals Ethics Committee vide Protocol No CRIUM/IAEC/2017/01/P09 (CPCSEA, 2018). Animals were provided with standard pellet feed (SDS Diet) and drinking water ad libitum, unless stated otherwise. Animals were acclimatized to the laboratory conditions for one week before using them for experiment (CPCSEA, 2018) in Animal House of *NRIUMSD*, Erragadda, Hyderabad.

***Acute toxicity study***
The study formulation (MJN) was evaluated for the acute toxicity as per OECD guideline 425 following oral administration of a single dose and observation for 14 days. Limit test as per guideline was performed in rats at a single dose of 2000 mg/kg bw of MJN. Rats were fasted overnight prior to dosing. Suspension of MJN was prepared in mortar pestle using 0.3% aqueous carboxy-methyl cellulose. One female rat was orally administered with 2000 mg/kg bw of MJN using stainless steel feeding cannula. That rat was survived; therefore, two additional rats were administered with 2000 mg/kg bw, 48-h after the dosing of first rat. Since both additional rats survived, therefore, all the three animals were carried to full 14-days observation without dosing to further animals (OECD, 2008).

All three rats were observed for clinical signs and symptoms of toxicity for 14 days. Observations were made at least once during first 30 minutes with special attention during first 4 hours on the day of dosing and once daily thereafter for the total of 14 days. Body weights were recorded prior to dosing and weekly thereafter. Feed intake was recorded at weekly interval. All the three rats were sacrificed after 14-days observation period and were subjected to necropsy. All external and internal lesions were carefully observedand recorded. Internal organs were collected and weighed, and data was recorded. As, no toxic lesions occurred in any organ, no tissue sample was subjected to histo-pathological investigation

## 3. RESULTS

***Standardization***

**Organo-leptic properties**
Organo-leptic evaluation of MJN (in three different batches) was carried out.
MJN was found to be semisolid, blackish-brown in colour, characteristic odour, and sweetish-bitter in taste.


**Fig.1** *Majoon-e-Nisyan*

**TLC (Thin layer chromatography)**
***$R_f$ values***
Under UV 366nm the TLC plate showed seven major spots at $R_f$ values 0.01 (blue), 0.12 (blue), 0.44 (blue), 0.50 (blue); 0.60 (blue), 0.74 (light yellow), 0.85 (light blue), and under UV 254nm shows nine spots at $R_f$ values 0.01, 0.13, 0.21, 0.43, 0.54, 0.65, 0.70, 0.76, 0.86 (all black).The data was represented in the table (2 A,B).HPTLC fingerprint profile of chloroform extract is shown in Fig.2 A&B. The graphical representation was shown in the figure 3 (A, B). Hence these spots show specific finger prints of the study formulation. These data may be used as a reference in future.

**Table 2A**. Peak list of chloroform extract at UV 366nm

| Peak no | Y-Pos | Area | Area % | Height | $R_f$ value |
|---|---|---|---|---|---|
| 1 | 9.9 | 327.14 | 3.72 | 183.61 | 0.01 |
| 2 | 17.3 | 95.96 | 1.09 | 61.23 | 0.12 |
| 3 | 31.5 | 51.40 | 0.58 | 20.41 | 0.31 |
| 4 | 40.8 | 64.41 | 0.73 | 26.51 | 0.44 |
| 5 | 45.1 | 612.44 | 6.96 | 217.61 | 0.50 |
| 6 | 52.0 | 166.82 | 1.90 | 102.25 | 0.60 |
| 7 | 56.1 | 5709.76 | 64.93 | 1207.83 | 0.65 |
| 8 | 62.2 | 1081.90 | 12.30 | 584.79 | 0.74 |
| 9 | 66.8 | 454.18 | 5.16 | 146.38 | 0.80 |
| 10 | 70.2 | 146.83 | 1.67 | 62.03 | 0.85 |
| 11 | 80.6 | 83.20 | 0.95 | 34.18 | 0.99 |

**Table 2B.** Peak list of chloroform extract at UV 254nm

| Peak no | Y-Pos | Area | Area % | Height | $R_f$ value |
|---|---|---|---|---|---|
| 1 | 10.0 | 1099.45 | 4.44 | 453.77 | 0.01 |
| 2 | 18.3 | 1244.30 | 5.02 | 245.97 | 0.13 |
| 3 | 24.0 | 952.71 | 3.85 | 297.01 | 0.21 |
| 4 | 34.6 | 3009.55 | 12.15 | 803.51 | 0.36 |
| 5 | 40.3 | 2315.33 | 9.35 | 806.95 | 0.43 |
| 6 | 48.2 | 3786.61 | 15.29 | 668.30 | 0.54 |
| 7 | 52.0 | 421.47 | 1.70 | 331.41 | 0.60 |
| 8 | 55.9 | 3886.31 | 15.69 | 1076.58 | 0.65 |
| 9 | 59.3 | 1628.15 | 6.58 | 636.49 | 0.70 |
| 10 | 64.0 | 3130.61 | 12.64 | 626.15 | 0.76 |
| 11 | 71.1 | 903.05 | 3.65 | 294.47 | 0.86 |
| 12 | 76.4 | 1187.02 | 4.79 | 298.00 | 0.94 |
| 13 | 79.9 | 1197.78 | 4.84 | 380.91 | 0.98 |

**Microbial Load analysis**

Total microbial plate count (TPC) was found to be 10 x 103, 86 x102 and 98 x 102 in three samples respectively which is much lower than WHO permissible limit. *Salmonella Spp*., *Escherichia coli* and Total Yeast and Mould count were found to be absent. Hence MJN is not harmful for consumption. The data of microbial load analysis was represented in the table 3.

**Aflatoxin analysis**

Detections of aflatoxin B1, B2 and G1, G2 were found to be absent. Hence it is safe to use. The data of aflatoxin analysis was represented in the table 4.

**Heavy metal analysis**

The analysis of heavy metals in MNJ, no traces of heavy metals were found, hence it is not hazardous for health. The data is given in the table 5. Proper *virechana karma* leads to clearance in all the *Srotasas* (channels of body), freshness in the sense organs, lightness in the body, improvement in *Agni* (metabolism) and attains disease-free status (Caraka Samhita, Siddhi Sthana, 1/17).

Improvement in the status of *Agni* brings out improved health status, enhancement of *Ojas*, and improved quality of life. *Agni* is supposed to be the pillar upon which the health and longevity of a person are dependent. When the *Agni* is not in equilibrium, i.e. either *Tikshna* (hyperfunction) or *Manda* (hypofunction) or *Vishama* (sometimes hyperfunction sometimes hypofunction) the state of normalcy is disturbed and the individual suffers from various diseases and if *Agni* stops functioning, it leads to death of the individual. Hence, the *Agni* is alleged to be the basic reason for health and longevity (Caraka Samhita, ChikitsaSthana, 15/3-4).

**Table 3.** Microbial load Contamination

| S.No | Parameter analyzed | Results | | | Permissible limits as per WHO |
|---|---|---|---|---|---|
| | | Sample-I | Sample-II | Sample-III | |
| 1. | Total microbial plate count (TPC) | $10 \times 10^3$ | $98 \times 10^2$ | $86 \times 10^2$ | Not more than $10^5$ / g |
| 2. | *Salmonella Spp.* | Nil | Nil | Nil | Nil |
| 3. | *Escherichia coli* | Nil | Nil | Nil | Nil |
| 4. | Total Yeast &Mould count | Nil | Nil | Nil | Not more than $10^3$ / g |

**Table 4.** Aflatoxin Contamination

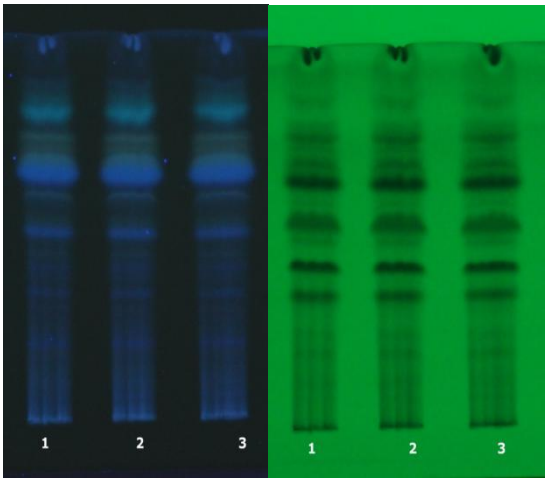
| S. No | Parameter analyzed | Results | | | Permissible Limits as per WHO |
|---|---|---|---|---|---|
| | | Sample-I | Sample-II | Sample-III | |
| 1. | B1 | Nil | Nil | Nil | Not more than 0.50 ppm |
| 2. | B2 | Nil | Nil | Nil | Not more than 0.10 ppm |
| 3. | G1 | Nil | Nil | Nil | Not more than 0.50 ppm |
| 4. | G2 | Nil | Nil | Nil | Not more than 0.10 ppm |

**Table 5.** Heavy metals Contamination

| S. NO. | Parameters Analyzed | Results | WHO Permissible Limits |
|---|---|---|---|
| 1. | Lead – (Pb) | ND | 10 ppm |
| 2. | Cadmium – (Cd) | ND | 0.3 ppm |
| 3. | Arsenic – (As) | ND | 3.0 ppm |
| 4. | Mercury – (Hg) | ND | 1.0 ppm |

**Table 6.** Physico-chemical Parameters

| Parameter | Total ash (% *w/w*) | Acid insoluble ash (% *w/w*) | Alcohol soluble matter (% *w/w*) | Water soluble matter (% *w/w*) | pH 1% aqueous solution | Reducing sugar | Non -reducing sugar |
|---|---|---|---|---|---|---|---|
| BATCH 1 | 1.4410 | 0.4564 | 64.7828 | 72.1799 | 5.44 | 35.6671 | 0.6777 |
| | 1.4281 | 0.4774 | 65.5975 | 73.2592 | 5.55 | 37.1229 | 0.8430 |
| | 1.3537 | 0.5196 | 65.2032 | 74.0077 | 5.57 | 36.3805 | 0.6777 |
| BATCH 2 | 1.3305 | 0.4167 | 66.4092 | 71.8625 | 5.45 | 37.5962 | 0.5738 |
| | 1.2756 | 0.4598 | 67.0024 | 70.2239 | 5.37 | 36.8443 | 0.5512 |
| | 1.4288 | 0.6018 | 66.4128 | 73.9132 | 5.37 | 36.1218 | 0.5469 |
| BATCH 3 | 1.3601 | 0.5068 | 67.6385 | 74.4145 | 5.60 | 37.4863 | 0.7121 |
| | 1.3140 | 0.3833 | 67.3904 | 71.9968 | 5.68 | 36.7340 | 0.6842 |
| | 1.2923 | 0.5065 | 67.5323 | 75.5191 | 5.61 | 36.0137 | 0.5452 |
| Mean ±SD | 1.3582 ± 0.0618 | 0.4809± 0.0633 | 66.4410± 1.0509 | 73.0418± 1.6173 | 5.5155± 0.1115 | 36.6629± 0.6693 | 0.6457± 0.1005 |




**At UV 366 nm** **At UV 254 nm**

**Fig.2 A&B** HPTLC fingerprint profile of chloroform extract of *Majoon-e-Nisyan* at 366 nm and 254nm (L to R)

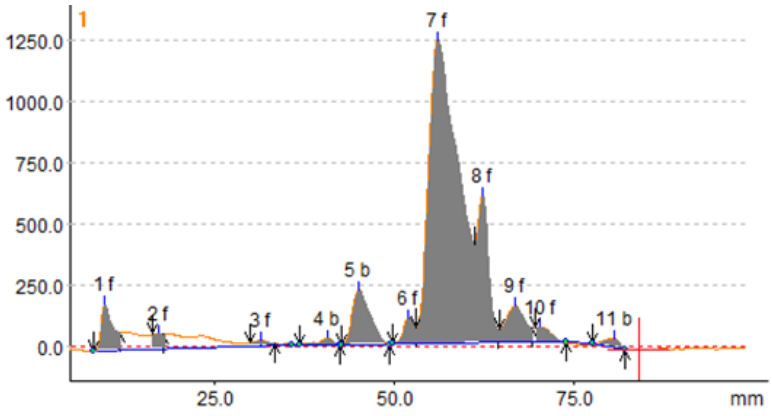



**Fig.3A** Densitogram of chloroform extract at UV 366 nm

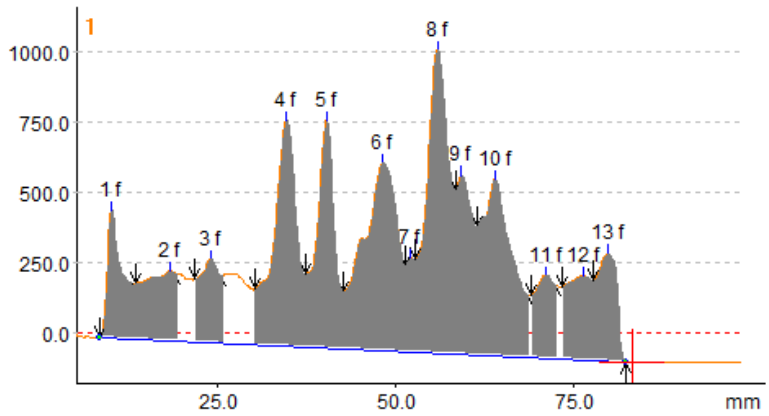



**Fig.3B** Densitogram of Chloroform extract at UV 254 nm

**TOXICITY STUDY**

In acute toxicity study, there were no deaths either on the day of treatment or throughout the 14-day post-treatment observation period. No signs of systemic toxicity were observed throughout the observation period. Individual body weights and feed consumption are given in Table-7. None of the animals lost body weight and all rats showed expected gains in body weight over the study period. All the three animals survived until the scheduled necropsy on Day-15 and no abnormalities were noted on gross necropsy. Haematology and biochemistry data are shown in Table-8 and 9, respectively. Hence, we can elicit that this study formulation is not acutely toxic to human beings but still further repeated dose toxicity study is advised.

**Table 7.** Body weight and feed consumption Data of MJN treated rats (Acute toxicity)

| Study Day | Sex | Dose (mg/kg bw) | Weight in gram | | | Weight of Feed in gram | | |
|---|---|---|---|---|---|---|---|---|
| | | | F1 | F2 | F3 | F1 | F2 | F3 |
| 1st Day | Female | 2000 | 120 | 133.6 | 129 | 18 | 18.2 | 16.8 |
| 7th Day | Female | 2000 | 159.4 | 175.1 | 176.1 | 19.2 | 16.5 | 18.3 |
| 14th Day | Female | 2000 | 174.5 | 190.1 | 194.6 | 20.5 | 17.5 | 19.1 |

**Table 8.** Summary of haematology of MJN treated rats (Acute toxicity)

| | | | |
|---|---|---|---|
| Hb (Hemoglobin) (gm %) | 13.4 | 13.1 | 13.3 |
| RBC (Million/mm3) | 6.6 | 6.5 | 7.7 |
| WBC (/mm3) | 2800 | 2600 | 3000 |
| Platelet (lakhs/mm3) | 3.5 | 3.1 | 5.9 |
| HCT (%) (hematocrit) | 40 | 35 | 44 |
| Neutrophil (%) | 2 | 2 | 3 |
| Lymphocyte (%) | 94 | 93 | 94 |
| Eosinophil (%) | 2 | 2 | 1 |
| Monocyte (%) | 2 | 2 | 2 |

**Table 9**- Summary of Biochemistry of MJN treated rats (Acute toxicity)

| Parameters | 2000 mg/kg bw.F1 | 2000 mg/kg bw.F2 | 2000 mg/kg bw.F3 |
|---|---|---|---|
| Glucose (fasting) mg/dl | 77 | 86 | 92 |
| AST (IU/L) | 158 | 165 | 148 |
| ALT (IU/L) | 61 | 51 | 69 |
| Bilirubin (mg/dL) | 0.29 | 0.24 | 0.31 |
| ALP (IU/L) | 72 | 59 | 103 |
| Total Protein (g/dL) | 6.1 | 5.5 | 6.1 |
| Albumin (g/dL) | 5.2 | 4.4 | 4.4 |

| | | | |
|---|---|---|---|
| Globulin (g/dL) | 0.9 | 1.1 | 1.7 |
| BUN (mg/dL) | 20.1 | 17.2 | 19.1 |
| Creatinine (mg/dL) | 0.7 | 0.7 | 0.6 |
| Uric acid mg/dL) | 2.3 | 2.2 | 2.2 |
| Sodium ions (mmol/L) | 135 | 136 | 137 |
| Potassium ions (mmol/L) | 4.1 | 5.2 | 7.4 |
| Chloride ions (mmol/L) | 103 | 100 | 98 |
| T.Calcium ions (mmol/L) | 2.1 | 2.5 | 2.4 |

## 4. DISCUSSION

**Standardization of MJN**

Authenticity and quality control is a must for evaluating safety and accurate efficacy of the herbal drugs. In USM, these are done as per the technical guidelines issued by WHO for herbal drugs. The study drug MJN plays a vital role in the treatment of many neurological conditions mainly amnesia (Fan T P *et al.,* 2012). In this study detailed evaluation of organo-leptic characters, physicochemical parameters and preclinical acute toxicity study has been done. organo-leptic characters of MJN is semi solid in consistency, blackish brown in colour, peculiar specific odour and bitter sweetish in taste (Anonymous, 2010).

Physico-chemicals parameters performed in this study are-
Extractive values (weights of the extractable chemical constituents of crude drug) of the MJN in different solvents (alcohol, water) are found to be within standard limit. (Anonymous, 2006; Anonymous, 2010)

- pH value measures the concentration of hydrogen ions which indicates the purity of the drugs. In present study it is found to be within normal limits (Lachman L *et al.,* 1991; Goodman, Gilman, 2001).

- Ash value determines the correct identity and cleanliness (absence of foreign matters in the drug). Total Ash value and acid (10 % dilute Hcl) insoluble ash value are found to be within standard range (Afaq SH *et al.,* 1994; Wallis TE, 2004; Anonymous, 2006; Anonymous, 2010).

- Reducing sugars indicates the capability of reducing the oxidizing agents in alkaline solution while non reducing sugars cannot do the same and in the present study reducing sugars are in standard range (Anonymous, 2010; Anonymous, 2006).

- HPTLC gives clear idea about the presence of adulteration by variation in the number of spots in TLC plate in the formulations which is highly helpful in testing the quality and purity of the drugs (Anonymous, 2010; Anonymous, 2006).

- Microbial load in the drugs are very hazardous to the consumers, hence its analysis is mandatory in quality control process. In the present study microbial count of three samples was evaluated and found to be 10 x 103/g, 98 x 102/g and 86x102/g, which is within the permissible limit recommended by WHO. Total fungi and pathogens like E.coli, Salmonella, moulds and total-yeast are absent or nil in the present study Formulation, which is favourable for the consumers (Anderson J M, 1975; Gunn B A, 1977; Hansen W, Yourassawsky E J;1984; Greenberg A E, 1985; Rambach A, 1990; Forbes *et al.,* 1998; Eyo AA, 2001).

- Heavy metals and aflatoxins are highly unsafe for human beings, so its estimation is carried out in the present study and it is found to be within the limits mentioned by WHO (Anonymous, 2010; Liu Y, Wu F; 2010).

- In preclinical acute toxicity study of MJN at the dose of 2000 mg/kg bw of MNJ as 0.3% aqueous CMC suspension, there found to be no toxic sign and symptoms and mortality. LD50 has come to higher than 2000 mg / kg-bw. Hence it is proved that MJN is not acutely toxic preclinically at above mentioned dose.

## 5. CONCLUSION

In the present work, a classical Unani formulation, *Majoon-e-Nisyan*, has been studied for its standardization, safety, and efficacy. The formulation is reported to be very effective and has been used in the Unani system for the management of amnesia and related disorders for a long time. The data presented in the study may serve as a standard reference for identification and quality control of MJN by various parameters approved by WHO for quality control of polyherbal compound formulations. The present findings of this formulation will provide fingerprint data through physicochemical parameters along with heavy metals, microbial load, and Aflatoxin contaminations, which in turn will be helpful to pharmaceutical industries and other research organizations. MJN may be further studied in-vitro and clinical trials in detail for the determination of its property and efficacy towards amnesia and its related disorders. However, no scientific data is available in terms of its safety in animals. Therefore, a toxicity study has been undertaken in experimental animals. Before conducting the pre-clinical studies, this formulation has been standardized as per the recommended guidelines of WHO for herbal drugs and procedures mentioned in Unani Pharmacopoeia of India with almost all recommended parameters. The data generated for the "Standardization of MJN"- can serve as a 'standard' for future research endeavors and save time, cost, and resources. Acute toxicity study conducted as per the limit test method of OECD guideline-425 revealed no toxic sign and symptoms or mortality in any of the treated rats at the dose of 2000 mg/kg BW of MJN. Based on the acute study data, the oral LD50 of the MJN in rats was estimated to be greater than 2,000 mg/kg body weight.

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## CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

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