Indian Journal of Traditional Knowledge
Vol. 10(4), October 2011, pp 716-720

Acute toxicity evaluation of some Unani drugs in crude and processed forms

Shamsi[*1] Shariq, Tajuddin[2] & Afaq[3] SH[1]

Department of Ilmul Saidla (pharmacy), National Institute of Unani Medicine, Bangalore 560091, Karnataka;
[2,3]Department of Ilmul Advia (Unani Pharmacology), Aligarh Muslim University, Aligarh 202002, UP;
Email: shariqaligs@gmail.com

Received 02.06.10; revised 12.01.11

Acute oral toxicity studies on the suspension of some Unani drugs *Azaraqi* (*Strychnos nux-vomica* Linn.) and *Sammulfar* (Arsenious oxide) were carried out on mice and compared to detoxified form. The effect of detoxifying process was assessed on the maximum tolerance dose of the test drugs gets affected during the treatment process. From the experimental studies it was evident that the LD-50 in detoxified form of both the test drugs found greater than crude materials.

Keywords: Toxicity, Crude drugs, Detoxified, *Strychnos nux-vomica,* Arsenious oxide

IPC Int. Cl.[8]: A01D 15/00, A01D 15/02, A01D 15/07, A01D 15/16, C01G 28/00

Azaraqi (*Strychnos nux-vomica* Linn.) and *Sammulfar* (Arsenious oxide), both are toxic drugs. *Strychnos nux-vomica* Linn. (Loganiaceae) is widely used in Unani medicine from very early times as mentioned in *Kitabul-Hashaish*[1]. It is grown extensively in southern Asian countries. The dried seeds of this plant improve blood circulation, and relieve rheumatic pain, also used in treating tumour and rheumatic arthritis[2]. *Azaraqi*, known in Indian drug, *Kuchla*[3] seeds have been used to treat paralysis[3,4,5]. Alkaloids, its main ingredients are mainly responsible for the pharmacological properties possessed by *nux-vomica*[6]. However, the toxic properties of this drug have limited the wider use.

The other drug, *Sammulfar* (Arsenious oxide), is considered as the king of poison, is now known to be a essential element, as totally arsenic-free diets, have been reported to cause growth retardation and adversely affect skin, hair and reproductive functions[7]. Arsenious oxide, recovered as a byproduct in the refining of copper [8] and popularly known as *sammulfar* (*Sankhiya*) in the Unani medicine, is an extremely poisonous substance previously used as an ingredient of weed killers, insecticides, and rat poisons[8, 9]. Although, poisonous in nature, it has been used as a medicine since time immemorial, now Arsenic trioxide (As_2O_3), is set to become the latest addition to the armoury for treating a rare form of leukaemia. Recently the FDA has announced the approval of Trisonex (Arsenic trioxide) for the treatment of acute promyelocytic leukaemia (APL), which affects approximately 2000 people each year in the USA[10]. In Unani System of Medicine Arsenious oxide has been used to treat malaria[11], diabetes[11], cancer[11], asthma[11, 12], scabies[12], syphilis[13], leprosy[13], arthritis[14], scorpion sting[15], pruritis[16].

In Unani system of Medicine, both the drugs are used in clinical practice after the processing. Though, such purificatory process has been carried by the Unani physicians but no attempt seems to find out the experimental basis to the claims especially in the changes and lessening of toxicological symptoms of their toxicity. Hence in the present study, the acute toxicological studies (LD-50) of *Azaraqi Ghair Mudabbar* (Crude nux vomica), *Azaraqi Mudabbar* (Detoxified nux vomica) and *Sammulfar Ghair Mudabbar* (crude arsenious oxide), *Sammulfar Mudabbar* (detoxified arsenious oxide) have been carried out with oral suspension of test drugs in gum acacia.

Methodology

The crude drugs were procured from an authentic source. The identity was confirmed by the voucher specimens (SC-0106/09-L and SC-0102/09) deposited in the Museum of Department of Ilmul Advia, Aligarh Muslim University, Aligarh.

* Corresponding author

Detoxification of Azaraqi: *Azaraqi* was detoxified by the method given in National Formulary of Unani Medicine. The seeds were covered with yellow clay (*Peeli mitti*) in an earthen pot for 10 days, and irrigated daily. After 10 days seeds were taken out, washed and boil the seeds in milk, then the outer cover were removed with the help of sharp knife. The embryos were also removed. The remaining parts were first dried in shade and then powdered[17].

Detoxification of Sammulfar: *Sammulfar* was detoxified by the method given in National Formulary of Unani Medicine. *Sammulfar* were powdered and equal quantity of lemon juice (w/v) was put in a mortar and pestle, and mixed with heavy motion till the lemon juice is absorbed. This process was repeated 7 times upto the stage till it will be a fine powder, and finally the content was spread in a tray to dry[17].

Swiss Albino mice (20-25 gm) of either sex were used for this experiment. The animals were given standard pellet diet and given tap water ad libitum. The experiments were performed in a quiet room with an ambient temperature of 22 ± 2°C. The animal house was illuminated from 6-18 hrs daily. A pilot study with 2 mice to each dose of test drugs was carried out to find out the approximate dose producing 50% mortality.

Swiss albino mice were divided into 6 groups of 10 animals each. The animals were kept on fasting for 10 hrs after that the powdered material of the test drugs in the form of suspension in the gum acacia were administered in graded quantities (mg/kg B wt or gm/kg B wt) singly by oral route, respectively. Following the administration of the test drugs all the animals were kept in a cage singly and observed continuously for 2, 6 and 24 hrs for mortality. The number of animals dying within 24 hrs in each group was recorded[18].

This method employs cumulative values. It is assumed that an animal killed by a certain dose would have been killed by a larger dose, and that a surviving animal would have survived a smaller dose.

The cumulative survivors are recorded in the Tables by adding successive entries, of the survived animals, upward. Last group is neglected, since doses smaller than the greatest allowing all animals to survive have no significance, and doses larger, the least causing all animals to survive have no significance. The percentage survival for both doses adjacent to the LD 50 is computed, and then the proportionate distance from 50 % is computed, and multiplied by the logarithm of the proportionate dose increment. This product is added to the logarithm of the LD-50 and the LD-50 so computed[18, 19].

Results

Acute toxicological studies of crude raw materials and detoxified materials of both drugs (*Azaraqi* and *Sammulfar*) have conducted to ascertain the maximum tolerance dose of the drugs by adopting the oral route. The study design was single dose administration starting from the dose corresponding to clinical dose.

For determining the toxic doses (LD-50) the graded quantities of the test drugs were administered orally to mice, and the doses producing death in nearly 50% of animals were noted and calculated. The LD-50 for the crude raw material of *Azaraqi* was calculated as 250 mg/kg B wt (Table 1) and for *Sammulfar* as 40 mg/kg B wt (Table 2). The LD-50 for the detoxified material of *Azaraqi* was calculated as 514mg/kg B wt. and for detoxified *Sammulfar* as 55 mg/kg B wt. The doses used the number of animals dead and survived and the percentage survival has been depicted in Tables 1 & 2. The results are presented in Figs 1-4.

Discussion

Azaraqi was detoxified with milk and *Sammulfar* with *Aab-e-Limun*. Though such purificatory processes have been carried by the Unani physicians, but no attempt seems to find out the experimental basis to the claims especially in the changes and lessening of toxicological symptoms of their toxicity. In view of this consideration the scientific appraisal of *Tadbir-e-Advia* has prescribed, in relation to two most important poisonous drugs, viz *Azaraqi* and *Sammulfar*. The effect of detoxifying process was assessed on the basis as to whether the maximum tolerance dose in the test drugs. Acute toxicological studies of crude raw materials (untreated) and detoxified (treated) materials, both drugs (*Azaraqi* and *Sammulfar*) have been conducted to ascertain the maximum tolerance dose of the drugs by adopting the oral route (corresponding to human use) in animals. The study design was single dose administration starting from the dose corresponding to clinical dose. The LD-50 for the untreated materials of *Azaraqi* and *Sammulfar* were calculated as 250 mg / kg B wt and 40 mg / kg B wt, respectively, whereas the LD-50 for

Table 1—LD-50 of *Azaraqi Ghair Mudabbar* and *Mudabbar*

Group	Doses		No of animals (Dead)		No of animals (Survived)		Cumulative value						% Survival	
							Dead		Survived		Total			
	AG	AM	AG	AM	AG	AM	AG	AM	AG	AM	AG	AM	AG	AM
1	400	750	10	10	0	0	10	10	25	27	35	37	71.4	73
2	300	650	7	6	3	4	17	16	25	27	42	43	59.5	63
3	250	550	5	5	5	5	22	21	22	23	44	44	50	52.3
4	150	450	3	2	7	8	25	23	17	18	42	41	40.5	43.9
5	100	350	0	0	10	10	25	23	10	10	35	33	28.6	30.3
6	100	350	0	0	10	10	25	23	-	-	-	-	-	-

Azaraqi Ghair Mudabbar: AG *Azaraqi Mudabbar:* AM
No of animals in each group = 10
Calculated LD-50: *Azaraqi Ghair Mudabbar* = 250 mg/kg; *Azaraqi Mudabbar* = 514 mg/kg

Table 2—LD-50 of *Sammulfar Ghair Mudabbar* and *Mudabbar*

Group	Doses		No of animals (Dead)		No of animals (Survived)		Cumulative value						% Survival	
							Dead		Survived		Total			
	SG	SM	SG	SM	SG	SM	SG	SM	SG	SM	SG	SM	SG	SM
1	50	65	10	10	0	0	10	10	25	25	35	35	71.4	71.4
2	45	60	7	7	3	3	17	17	25	25	42	42	59.5	59.5
3	40	55	5	5	5	5	22	22	22	22	44	44	50.0	50.0
4	35	50	3	3	7	7	25	25	17	17	42	42	40.5	40.5
5	15	20	0	0	10	10	25	25	10	10	35	35	28.6	28.6
6	15	20	0	0	10	10	25	25	-	-	-	-	-	-

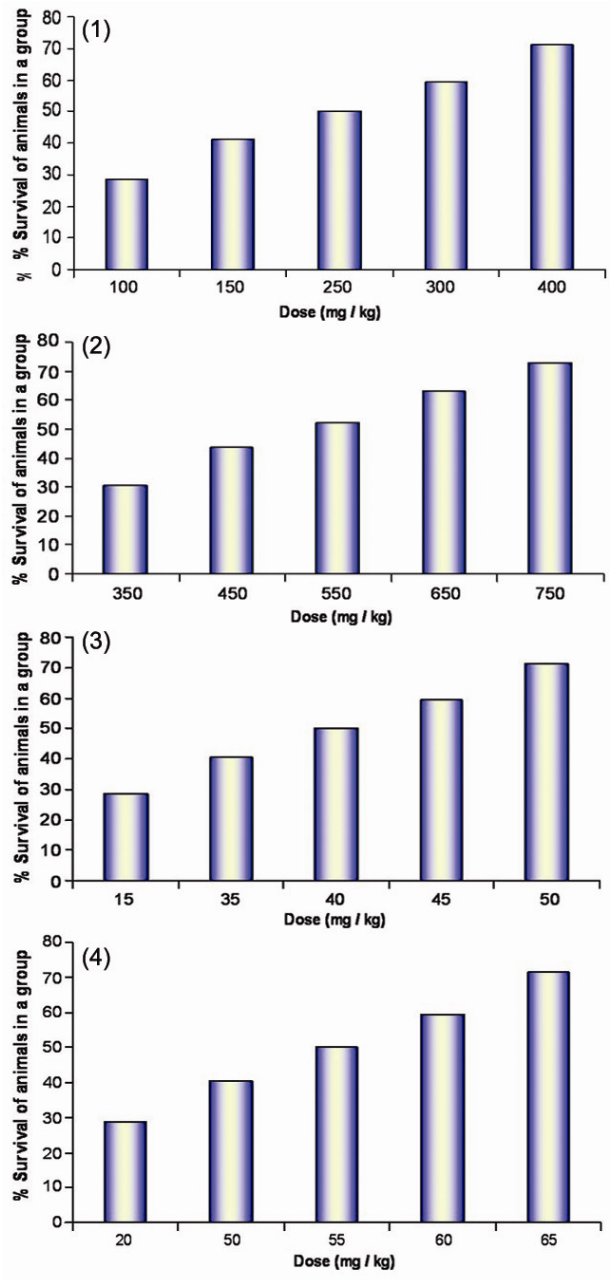
Sammulfar Ghair Mudabbar: SG *Sammulfar Mudabbar:* SM
No of animals in each group = 10
Calculated LD-50: *Sammulfar Ghair Mudabbar* = 40 mg/kg; *Sammulfar Mudabbar* =55 mg/kg

the detoxified materials of *Azaraqi* and *Sammulfar* were calculated as 514 mg / kg B wt and 55 mg / kg B wt, respectively.

The maximum tolerance dose in the detoxified test drugs is almost due to the extraction of some portion of alkaloid (s) by the milk in *Azaraqi*, whereas, in case of *Sammulfar*, Arsenic trioxide dissolved in the *Aab-e-Limun.*

The phenomenon of diffusing marker compounds of *nux-vomica* in the milk on boiling may be explained that the milk contains more than 82% of water[20]. The water of milk may facilitate the release of marker compounds in milk as strychnine and brucine contents of *nux-vomica* are reported to be dissolved into water. In case of *Sammulfar*, Arsenious oxide depleted when treated with *Aab-e-Limun* because when the Arsenious oxide comes in contact to any acid, it reacts and form the salt, e.g. with Hcl it become Arsenic chloride and with citric acid it become arsenic citrate. It may possible that the chloride or citrate salt of Arsenic is less toxic than its oxide forms, that is why the *Tabib* are detoxifying it in acid. It also appeared from the acute toxicity studies that the LD-50 was found more in case of *Sammulfar Mudabbar* with *Aab-e-Limun*. It is a matter of research to set the toxicity limit of oxides and salts.

It is obvious from the tabulated results that detoxified (*Mudabbar) nux vomica* seed and *Sammulfar* become less toxic as the LD-50 is found more in comparison to untreated (*Ghair Mudabbar).* The detoxified test drugs have higher toxic doses than those of untreated drugs. This perhaps may be due to low concentration of poisonous alkaloid, viz. strychnine and brucine in the *nux vomica* seeds and Arsenic in the *Sammulfar. Shoba* and *Thomas* have reported the LD-50 value for untreated *Strychnos nux-vomica* as 237 mg/kg B wt in mice[21]. This value is very much similar to the value as observed the LD-50 value for *Strychnos nux-vomica* (*Azaraqi Ghair Mudabbar*) as 250 mg/kg B wt in mice. A review of the Registry of toxic effects of chemical substances shows that the LD-50 value for Arsenic trioxide (*Sammulfar*) was 39.4 mg/kg P O in animals [22]; this



Figs 1-4—(1) LD-50 of *Azaraqi ghair Mudabbar;* (2) LD-50 of *Azaraqi Mudabbar;* (3) LD-50 of *Sammulfar ghair Mudabbar;* and (4) LD-50 of *Sammulfar Mudabbar* in *aab-e-Limun*

value is almost similar with our values as we observed the LD-50 value for Arsenic trioxide (*Sammulfar*).

It was also observed that both the detoxified test drugs are well tolerated or non toxic upto a higher dose levels as compared with the low dose levels of untreated drugs. The survival rate of the animals in the groups treated with the detoxified drug materials was also high. The death of animals occurred more quickly in the groups treated with crude raw materials of test drugs and most of the animals died within 2 hrs as compared to the groups treated with the detoxified drugs.

Conclusion

Present study substantiates that detoxified toxic drugs lose elements of toxicity upto certain limits. For that very reason classical Unani Physicians allowed used of certain toxic drugs only after subjecting them to detoxification process to avoid any untoward adverse effects.

Acknowledgement

The authors are grateful to Prof K M Y Amin, Incharge Pharmacology section, Department of Ilmul Advia for suggestions and help received from him time to time.

References

1 Discorides, *Kitabul Hashaish*, MSS, Vol 2, (Ilmul Advia seminar, A.M.U Aligarh), 70CE, 305.
2 Yin W, Wang T, Yin F & Cai B, Analgesic and antiinflammatory properties of brucine and brucine N-Oxide extracted from seeds of *strychnos nux-vomica*, *Ethnopharmacol*, 88 (2003) 205-214.
3 Attar HZ, *Ikhtiyarat-e-Badiyee*, (Matba Munshi Naval Kishore, Kanpur), 1888, 13-14.
4 Halim A, *Mufridat-e-Azizi*, 4th edn, (Saheet-e-Mandir Press Ltd, Lucknow), 1948, 8 &58.
5 Anonymous, *The Unani Pharmacopeia of India*: Ministry of Health and Family Welfare, Vol 2, Part 1, (Deptt of Ayush, Govt of India, New Delhi), 2007, 15-16.
6 Yin W, Deng X-K, Yin F-Z, Zhang X-C & Cai B-C, The Cytotoxicity induced by brucine from the seed of *Strychnos nux-vomica* proceeds via apoptosis and is mediated by cyclooxegenase 2 and caspase 3 in SMMC 7221 cells, *Food Toxicol*, 45 (2007) 1700-1708.
7 Siddiqui R & Vohora SB, Analgesic activity of Arsenic preparations used in unani-tibb, *Indian Drugs*, 37 (2000) 274-278.
8 Anonymous, *New Standard Encyclopedia*, Vol 2, (Standard Educational Corporation, Chicago), 1983, 631.
9 Kumar S & Muley PR, Precolumn chelation with 7-bromo-8-quinolinol for simultaneous determination of anti cancer drug-Arsenic Trioxide and its impurities by RPHPLC, *Indian Drugs*, 43 (2006) 993-996.
10 Bradley D, Therapeutic needs revive arsenic compound, *Pharmaceuti Technolo,* 3 (2000) 401.
11 Ghani N, *Khazeenat-ul-Advia*, Vol 1, (Matba Munshi Nawal Kishore, Lucknow), 1921, 840-844.
12 Kareem N, *Makhzan-ul-Advia*, Vol 1, (Matba Munshi Nawal Kishore, Kanpur), 1880, 497-498.
13 Khan MA, *Muheet-e-Azam,* Vol 1, (Matba Nizami, Kanpur), 1313H, 98.
14 Hakim MA, *Bustan-ul-Mufridat*, (Idara Taraqqee, Urdu Publication, Lucknow), 1343H, 202.

15 Momin MH, *Tohfat-ul-Momineen*, (Matba Hasani), 1272H, 161.

16 Antaki DA, *Tazkira-e-ulil-Albab (Arabic)*, (Azhari Press, Egypt), 1343H, 176.

17 Anonymous, *National Formulary of Unani Medicine*, English Edition, Part I, (Ministry of Health and Family Welfare, New Delhi), 1983, 319.

18 Turner RA, *Screening Methods in Pharmacology*, Vol 1, (Academic Press, New York), 1965, 62-63,302-304.

19 Reed LJ & Muench H, A simple method of estimating fifty percent end points, *Am Hygiene*, 27 (1938) 493-497.

20 Nadkarni KM, *The Indian Materia Medica*, Vol 1, 3rd edn, (Popular Book Depot, Bombay 7, Dhootapapeshwar Prakashan Ltd, Panvel), 2000, 1175-1181.

21 Shoba FG & Thomas M, Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhea, *Ethnopharmacol*, 76 (2001) 73-76.

22 Anonymous, Scientific discussion (European Medical Agency), 2004, 6.