Comparative toxicity study of various dosage forms of *Sammul far* (Arsenic trioxide) in mice

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*Sammul far* (Arsenic trioxide) is a poison but its use in Unani medicine as a therapeutic agent is common, of course, after subjecting it to various processes of detoxification. Two forms of processed Arsenic, i.e. simply processed by grinding it along with certain other drugs (Mudabbar/detoxified form), and by burning it at a very high temperature and getting its oxide (*Kushta*/calcined form) are widely used by Unani physicians. However, these two forms have not been studied scientifically for their toxic potential or pharmacological and therapeutic profile. Therefore, a study was designed to evaluate acute and sub acute toxicity of *Sammul far* processed by different methods in albino mice with an aim to prepare their toxicity/safety profile and find the safest dosage form through their inter comparison. The data obtained from the study were analyzed by using *Kruskal Wallis* with Dunn’s multiple pair comparison test, and ANOVA non- repeated measure. A thermogram was also prepared by recording the heat pattern during the course of preparation of *Kushta* by conventional method (as described in Unani literature) and was standardized for preparation of *Kushta* by a furnace. The study revealed Mudabbar form to be more toxic than the calcined form. Further, *Kushta* prepared by furnace was safer than that prepared by classical method.

**Keywords:** Sammul far, Kushta, Mudabbar, Thermogram, Toxicity

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Metals and drugs of mineral origin by default, and many plant drugs that are known for their toxic effect are not used as such as therapeutic agent because of their ability to induce toxic effects even at therapeutic dose level. Rather, they are subjected to various mechanical and chemical processes in order to remove the elements of toxicity at all or downgrade them to a level at which the drug can be safely used. Some of the drugs are ignited/incinerated/calcined at a high temperature so as to ascertain the plausibility of their safe use. The incinerated material is technically known as *Kushta* (Calx) - a Persian word meaning killed1, containing carbonate or oxide in finely divided form. The product has high dissolution rate and ability to get absorbed in the body in a very short period; therefore a small amount of *Kushta* induces quick onset of action and high magnitude of effect.

Arsenic trioxide though a notorious poison has been in use as therapeutic agent for at least 2400 yrs and continued to be used in Unani medicine2 as general tonic, hematopoietic, blood purifier, cardio tonic, stomachic, aphrodisiac, antipyretic and antimicrobial agent3-9 after processing and detoxifying it. Presently, its use in human is not allowed in Modern medicine because of the reservations of physicians about its likely toxic effect. Though, its compounds have been studied extensively for toxicity10-17 but little attention has been paid to study its therapeutic efficacy of its modified/ calcined form in spite of its extensive use in Unani medicine since long. Thus, an element of doubt always remains about its safety. Therefore, in present study we subjected the modified/calcined form of Arsenic trioxide to acute and chronic toxicity studies so as to determine its safety profile and safe dose range.

In Unani medicine, *Kushta* is prepared by classical method, i.e. putting the test drug along with some herbs and sometimes other minerals as specified in a sealed pot of earthenware known as *Boota* which is kept in a pit filled with cow dung cakes ignited to produce heat of a high degree required to burn the drug completely. This classical method is time tested,
proven and considered better, but the procedure is unarguably less sophisticated, relatively subjective, time taking and complex. Therefore, it was thought reasonable to use modern techniques to prepare the Kushta. Hence, in present study we also prepared Kushta in a muffle furnace after extrapolating the Thermogram recorded in classical method. Further, since Sammul Far is also used in Mudabbar (detoxified) form but without incineration, therefore a sample of this was also prepared. All the three samples were used for comparative acute and sub acute toxicity studies.

Materials and methods
The proposal for the experiment was approved by IAEC of National Institute of Unani medicine, Bangalore vide Reg. No. 9531C/06/ CPCSEA, August 2008. The experiment was undertaken in the laboratory of Department of Ilmul Advia, National Institute of Unani medicine, Bangalore.

Arsenic Trioxide was procured from Nice Pvt. Ltd. Kerala, India, while Alum and Lemon were purchased from local market of Bangalore, India.

Healthy albino mice of both sexes, weighing 25-30 gm were obtained from Central Animal House Facility of National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore.

Kushta Sammul far-1(KSCM) was prepared by the method as prescribed in National Formulary of Unani Medicine, India. Arsenic trioxide and Alum were taken in the ratio of 1:2. Two small earthen pots of uniform size were taken that served as boota. Arsenic trioxide and alum were spread layer by layer in one pot and the other pot was put over the first. Joints of both pots were sealed with mud smeared cloth known as Gille Hikmat. A 60 × 60 × 60 cm pit was dug and the preparation was placed inside the heap of 2.5 kg cow dung cake placed in the pit. Other 2.5 kg cow dung cake was put over it so that the boota would remain in the middle. A Thermocouple (an electronic instrument assembled for recording of temperature) was inserted into the pit placing the tip close to the boota so as to measure minute by minute temperature. The cakes were ignited and the temperature variance recorded from this point of time till whole of the cow dung cake burnt to the ashes. The temperature variance was recorded. A Thermogram was obtained which was applied in Muffle Furnaces method for preparing Kushta Sammul far - 2(KSMF). Sammul far Mudabbar (SFM) was prepared by triturating Sammul far with lemon juice, 5 ml for 10 gm of Sammul far, till the juice dried up completely. It was repeated for 7 times. The three dosage forms viz. KSCM, KSMF and SFM were also subjected to Atomic Absorption Spectrometer for estimation of elemental Arsenic.

The dose for mice to determine LD50 was calculated by multiplying 1.5 gm human lethal doses described in Unani literature by conversion factor of 12, which was found to 360 mg/kg. Therefore, 180 mg/kg (1/2 of the lethal dose) was selected for acute toxicity study. Further higher doses were calculated by Miller’s formula by multiplying the previous dose by 1/2 to calculate the next higher doses. For sub acute toxicity studies the doses were selected on the basis of ED50 (14.42 mg, 14.2 mg and 1.72 mg for KSCM, KSMF and SFM respectively). Further doses were calculated by using Miller’s formula.

Acute toxicity test was carried out by the method of Manna et., Twenty four albino mice weighing 25-30 gm of both sexes (equal sex ratio) were divided into 4 groups consisting 6 animals each. They were kept under controlled condition of temperature (25±2 °C) and humidity 44-55 and were given pellet food and water ad libitum; a 12 hrs day and night cycle were maintained. Group I served as control while group II, III and IV were used for determination of LD50 of KSCM, KSMF and SFM, respectively. The animals were fasted over night and the three test samples were administered orally after dissolving them in 0.3% of Carboxy methyl cellulose sodium salt in 100 ml of water. The animals were observed for respiratory and CNS symptoms, behavioral changes, and LD50.

Sub acute toxicity test was carried out by the method described by Ghosh. Albino mice of both sexes weighing 25-30 gm of both sexes (equal sex ratio) were divided into four groups consisting of 6 animals each. The animals of each group were dosed for two weeks at multiple dose of ED50 calculated by Miller’s formula as describe earlier. On the 15th day of treatment animals were sacrificed under thiopentone anesthesia by serving neck vessels aseptically. Blood was collected from each animal for preparing smears for DLC. A sample was kept under refrigeration (4 °C) for separation of serum which was used for RFT (Serum Creatinine and Blood Urea) and LFT (SGOT and SGPT). Portions of kidney and liver were collected in 10% formalin solution for histopathological examinations. The body weight of animals was determined before and after treatment.
The data obtained from the study were analyzed by using Kruskal Wallis with Dunn’s multiple pair comparison test, ANOVA non-repeated measure. The difference in the mean scores of various groups was considered significant at P < 0.05.

Results

In the group administered KSCM the first response of decreased motor activity was observed at 341mg/kg and half of the animals of this group died at the dose of 2896.40 mg/kg. The group administered KSMF showed the first response of decreased motor activity at the dose of 331.20 mg/kg. No animal died in this group up to the dose of 8439.20 mg/kg. No further dosing was possible in this group. In the group administered SFM, the first response of decreased motor activity was observed at 200 mg/kg. At the dose of 341.20 mg/kg half of the animals died in this group (Table 1).

Polymorphonuclear leukocyte, Lymphocyte, Monocyte and Eosinophil counts in the group administered KSCM, were found to be 52.5 (42, 55), 47 (36, 56), 5 (1, 10) and 1 (1, 3), respectively. Whereas the group treated with KSMF, these were found as 28.5 (19, 54), 64 (38,78), 3 (1, 10) and 1 (1, 4). In the group administered SFM, the corresponding values were estimated as 32.5 (17, 46), 60 (40, 76), 5 (2, 8) and 2 (0, 7), respectively. The effect of all the three forms of Sammul far on body weight was found statistically non significant. Serum Creatinine in plain control group was estimated to be 0.699 ± 0.03 mg/dl, whereas in the groups treated with KSCM, KSMF and SFM it was found to be 0.871 ± 0.09 mg/dl and 0.689 ± 0.02 mg/dl and 0.736 ± 0.10 mg/dl, respectively. Serum urea in plain control group was estimated to be 53.51 ± 1.77 mg/dl, whereas in the groups treated with KSCM, KSMF and SFM it was estimated as and 60.25 ± 5.18 mg/dl and 61.65 ± 4.50 mg/dl, respectively. SGOT in plain control group was estimated to be 229.95 (145.30, 244.90) mg/dl, whereas in the groups treated with KSCM, KSMF and SFM it was estimated to be 293.80 (280.00, 313.30) mg/dl, 132.50 (129.20, 152.90) mg/dl, 112.55 (109.50, 121.80) mg/dl and 229.95 (145.30, 244.90) mg/dl, respectively. SGPT in plain control group was estimated to be 59.13 ± 1.75 mg/dl, where as in the groups treated with KSCM, KSMF and SFM it was found to be 60.92 ± 3.01mg/dl, 75.71 ± 4.92 mg/dl and 64.70 ± 0.77 mg/dl, respectively(Table 2).

Elemental Arsenic in KSCM, KSMF and SFM estimated by AAS were found to be 6.388 ± 0.711, 3.623 ± 1.327 and 48.386 ± 11.275, respectively (Table 3).

In KSCM group, histopathological findings showed congestion in the kidney. In KSMF the histopathological findings showed mild blood vessel congestion. The stroma showed mild to moderate degree of edema and it was sparsely infiltrated by inflammatory cells. In SFM the histopathological finding showed acute glomerulonephritis with interstitial lymphocytic infiltrate and focal areas of ischemic necrosis in the tubules (Figs 1-6).

Discussion

The study demonstrated that Kushta prepared by classical method (KSCM) is safer than the non-kushta form (but processed for detoxification) of Arsenic while that prepared by furnace (KSMF) is even more safe than the former one. This is evident from the toxicity studies of the three samples as the toxic effect caused by the three samples has been found of different intensity and that caused by Kushta form was significantly lower than the non-kushta form of Arsenic. Further, KSMF induced lower toxicity as compared to KSCM suggesting that the preparation of Kushta by furnace is safer than prepared by conventional method. Both acute and sub acute toxicity studies demonstrated that KSMF is

| Table 1 – Lethality with dosage variation of Sammul far in acute toxicity study |
|---|---|---|---|---|
| Dose (mg) | Plain Control | KSCM | KSMF | SFM |
| 5 | 6/0 | 6/0 | 6/0 | 6/0 |
| 8.53 | 6/0 | 6/0 | 6/0 | 6/3 |
| 14.56 | 6/0 | 6/0 | 6/0 | --- |
| 24.85 | 6/0 | 6/0 | 6/0 | --- |
| 42.41 | 6/0 | 6/0 | 6/0 | --- |
| 72.41 | 6/0 | 6/3 | 6/0 | --- |
| 123.60 | 6/0 | --- | 6/0 | --- |
| 210.98 | 6/0 | --- | 6/0 | --- |

Numerator denotes live and denominator denotes died animals, means dose not administered

| Table 2 – Estimation of elemental Arsenic by Atomic Absorption Spectrometer in different Arsenic forms |
|---|---|
| Groups | Elemental Arsenic(ppm) |
| KSCM | 6.388 ± 0.711 |
| KSMF | 3.623 ± 1.327 |
| SFM 48 | 386 ± 11.275 |
much safer as it was tolerated to higher doses in comparison to other dosage forms.

The study clearly demonstrated that both the methods of preparation of *kushta* are useful and are capable of rendering the Arsenic safe; however, the furnace method has little advantage over the conventional method. These findings were supported further by the AAS findings that revealed that KSMF contained lower amount of Arsenic as compared to KSCM. The amount of Arsenic found in SFM was significantly high but lower than that contained in crude form of Arsenic. Thus, it is evident that the various methods of detoxification used by Unani physicians to make the Arsenic safe are rational though the level of safety is different. It is likely the physicians may have adjusted the therapeutic efficacy of the drug at different dose levels.

The histological findings clearly demonstrated that SFM is toxic as it caused liver damage and produced the features of acute hepatitis. Similarly, it induced renal injury that amounted to acute nephritis. Development of hepatitis or nephropathy after

<table>
<thead>
<tr>
<th>Groups</th>
<th>Polymorph (Median and range)</th>
<th>Lymphocyte (Median and range)</th>
<th>Monocyte (Median and range)</th>
<th>Eosinophil (Median and range)</th>
<th>DLC (median and range)</th>
<th>SGOT (Median range)</th>
<th>SGPT (Median range)</th>
<th>Serum creatinine (Median range)</th>
<th>Blood urea (Median range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain control</td>
<td>27(19.45)</td>
<td>70(56.78)</td>
<td>2(2.4)</td>
<td>1(1.1)</td>
<td>229.95 (145.30,244.90)</td>
<td>59.13±1.75</td>
<td>0.699±0.03</td>
<td>53.51±1.77</td>
<td></td>
</tr>
<tr>
<td>KSCM</td>
<td>52.5(42.55)</td>
<td>47(36.56)</td>
<td>5(1.10)</td>
<td>1(1.3)</td>
<td>132.50 (129.20,152.90)</td>
<td>60.92±3.01</td>
<td>0.871±0.09</td>
<td>68.82±6.82</td>
<td></td>
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<tr>
<td>KSMF</td>
<td>28.5(19.54)</td>
<td>64(38.78)</td>
<td>3(1.10)</td>
<td>1(1.4)</td>
<td>112.55 (109.50,121.80)</td>
<td>75.71±4.92</td>
<td>0.689±0.02</td>
<td>60.25±4.18</td>
<td></td>
</tr>
<tr>
<td>SFM</td>
<td>32.5(17.64)</td>
<td>60(40.76)</td>
<td>5(2.8)</td>
<td>2(0.7)</td>
<td>293.80* (280.00,313.30)</td>
<td>64.70±0.77</td>
<td>0.736±0.10</td>
<td>61.65±4.50</td>
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Test used: Kruskall Wallis test with Dunn’s Pair comparison test for DLC, SGOT
Test used for SGPT: Kruskall Wallis with Dunn’s multiple pair comparison test
* p<0.05, as compared with control, + _ p<0.05 as compared with KSMF

Fig. 1–Histopathology of Kidney of KSCM group; Fig. 2–Histopathology of Liver of KSCM group; Fig. 3–Histopathology of Kidney KSMF group; Fig. 4–Histopathology of Liver of KSMF group; Fig. 5–Histopathology of Kidney of SFM group; Fig. 6–Histopathology of Liver of SFM group.
administration of drug is a very serious sign of toxicity. Such a drug should definitely be avoided or some further modification be made to make it suitable for use.

In a study LD50 in mice has been shown to be 26 mg/kg26,27 which is even higher than the dose of SFM, i.e. required to induce LD50 (341.20 mg/kg). It suggests that not only the processing of drug by burning it at higher temperature brings the concentration of Arsenic low but even the detoxification is also responsible to lower the concentration of Arsenic and thereby reducing the element of toxicity significantly. Some other toxicity studies on Arsenic showed variable doses causing LD50 according to the species of Arsenic for example arsin-3 mg/kg, arsenite-14 mg/kg, arsenate-20 mg/kg, monomethylarsenic acid-700-1800 mg/kg, dimethylarsenic acid-700-2600 mg/kg, arsenobetaine and arsenuocholine >10000 mg/kg can induce LD50. In another study the LD50 of monomethyl arsenic acid has been shown to be 916 mg/kg dymethyl arsenic acid 448 mg/kg, trimethyl arsenic oxide 5500 mg/kg, arsenuocholine 6500 mg/kg and arsenobetaine 4260 mg/kg26,27. The study does not match to any of the values mentioned above because in our study concentration of Arsenic low but even the administration of drug is a very serious sign of toxicity. Such a drug should definitely be avoided or some further modification be made to make it suitable for use.

This is probably one of the earliest reports depicting the pattern of gradual heat acceleration, the peak and its descend during the course of preparation of Kushta. The Thermogram prepared through this study will help to standardize the operational procedure as well as the product. This study will also pave the way for further studies of intermediates and the finished products of Kushta Sammul far and other calcined materials used in Unani medicine.

Conclusion

It can be concluded that the classical method of preparation of Kushta is inarginably useful method by which an effective and safe dosage form can be prepared. However, switching over to other techniques that are specific, safe and entail least complexity is desirable. On the basis of the findings it can be said that Kushta can also be prepared with the help of muffle furnace which was found in present study to be safer even than that prepared by classical method by using the Thermogram prepared by us. Use of Sammul far Mudabbar as therapeutic agent should be avoided.

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