Anti-diabetic activity and safety assessment of Ayurvedic medicine, *Jasada bhasma* (zinc ash) in rats

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*Jasada bhasma* (zinc ash) is an extensively used Ayurvedic medicine for treating diabetes mellitus. The present communication presents yet unavailable comprehensive scientific data on its physico-chemical nature vis-à-vis anti-diabetic activity and toxicity profile. Zinc ash prepared by traditional method was found to consist of 200-500 nm sized particles, predominantly zinc oxide with hexagonal wurtzite crystal structure. The effective dose range of zinc ash in oral glucose tolerance tests performed using normoglycemic Wistar rats was found to be 3-30 mg/kg. Subsequently anti-diabetic activity was assessed in streptozotocin induced type 1 and type 2 diabetic rats. Four weeks treatment with zinc ash (1, 3, 10 mg/kg) resulted in improved glucose tolerance (16-19%), lowered blood glucose levels (20-33%) and reduced serum insulin levels (27-32%). Systemic absorption was assessed by single dose pharmacokinetic study where serum zinc levels were found to be elevated (3.5 folds) after oral administration of zinc ash. Acute and sub-acute toxicity tests demonstrated safety of zinc ash up to 300 mg/kg dose. 100 times the efficacy dose in rats. These findings, the first of their kind, provide concrete scientific evidence that justifies usage of zinc ash in diabetes treatment.

**Keywords:** Ayurveda, Diabetes, *Jasada bhasma*, Pharmacokinetics, Rats, Streptozotocin, Toxicity, Zinc ash

Indian system of medicine, Ayurveda uses several metal based preparations (*bhasmas*) for the treatment of diseases like anemia, jaundice, skin diseases, tuberculosis, sexual disorders, urinary disorders, tumors, colitis, osteoporosis, ischemia, arthritis and diabetes¹. In the texts of *Rasashastra* (a branch of Ayurveda dealing with metallic medicines), *bhasmas* of *Mandura* (iron), *Vanga* (tin), *Naga* (lead), *Tamra* (copper) and *Jasada* (zinc) have been mentioned for the treatment of diabetes². *Jasada bhasma* is cited for use in several other conditions including, anemia, neuromuscular diseases, eye diseasesand as a wound healing, anti-microbial and anti-aging agent³-⁵. However, very few studies investigating the anti-diabetic effects of *Jasada bhasma* are reported. An early anecdotal study showed anti-diabetic activity of *Jasada bhasma* in diabetic patients⁶. There are sporadic reports on reduction offasted glucose levels⁷, improved glucose tolerance⁸, anti-diabetic activity⁹ in rats treated with *Jasada bhasma*. In fact a modern version of *Jasada bhasma*, viz. zinc oxide nanoparticles have been thoroughly investigated for their anti-diabetic effect¹⁰. Taking an inspiration from this work, it was thought worthwhile to investigate *Jasada bhasma* for its anti-diabetic activity and toxicity assessment.

Several researchers have synthesized *bhasmas* and employed modern imaging techniques to determine the particle sizes¹¹-¹⁵. These studies suggested that *bhasmas*, when prepared by traditional methods, contain sub-micronic particles. Traditionally, *Jasada bhasmains administered orally with different vehicles such as honey, milk or ghee for a prolonged period of time to attain therapeutic effect. The lack of bioavailability data has resulted in the belief that it acts through local effects at the intestine, which needs to be validated experimentally. Presence of heavy metals in Ayurvedic medicines has been a cause of serious concern despite their traditional and widespread use¹⁶-¹⁸. It is necessary to prove their safety at therapeutic doses on the basis of well-established toxicological tests and models.

Clearly, there is a need for undertaking a systematic study on the physico-chemical characterization, anti-diabetic efficacy, oral bioavailability, and toxicity of *Jasada bhasma*. This communication describes detailed studies on a *Jasada bhasma* sample synthesized by traditional documented method. The study confirms sub-micronic nature of the *bhasma* and...
provides pharmacological evidence of its anti-diabetic activity in both type 1 and type 2 diabetic rats. Also, pharmacokinetic studies show systemic absorption of orally administered Jasada bhasma. Toxicological studies establishing safety up to 100 times the therapeutic dose are also described.

Materials and Methods

Synthesis of Jasada bhasma sample—Zinc metal was subjected to the traditional method of shodhan (purification) and maaran (incineration). For shodhan, zinc was melted and poured in sesame oil (Sesamum indicum). On cooling, the mixture was filtered and the residue again melted and poured in oil. This procedure was repeated seven times. Similarly, seven cycles each were carried out using butter milk, cow urine and kudlati (aqueous extract of Dolichos biflorus, horse gram seeds). Finally, the molten zinc was poured in lime water in an earthen pot and kept for 3 h. Purified zinc so obtained was ground with equal parts of mercury until converted to pishtee form (amalgam). Lime juice was then added and further ground. Once, paste was converted to black colour, it was thoroughly washed with warm water and air dried. Paste was then ground with equal amount of pure sulfur and converted to powder. For maaran, the powder was transferred to earthen crucibles and sealed with clay. Calcination was then carried out by heating up to 500 °C in kukkat put (a pit in the ground, 9 × 9 × 9 inches in size). After overnight calcination, the product was taken out, ground and again calcined. This cycle was repeated 30 times to get the final product. The bhasma sample so obtained (zinc ash) was analyzed for size, shape and elemental composition using the following techniques.

Evaluation by classical tests of Ayurveda—The synthesized zinc ash was subjected to several tests mentioned in Ayurveda that evaluate the particle size, density and chemical stability of bhasmas. These tests include (a) Rekhaapoorntaw (particles in bhasma should be fine enough to lodge itself between the fine lines of fingers when rubbed), (b) Vaaritaratwa (bhasma when sprinkled should float on water and not settle down), (c) Nischandratwa (should not shine in sunlight), (d) Niruthatwa (should not form an alloy when burned on a thin silver sheet) and (e) Apunarbhawatwa (on heating with ghee, honey and borax; should not lead to reappearance of the source material).

Scanning electron microscopy (SEM) coupled with electron dispersive spectroscopy (EDS)—Powder suspensions of zinc ash (1 mg/mL) were spin coated on glass cover slips and imaged using Scanning Electron Microscope (Model JSM-7001, JEOL, Tokyo, Japan) after gold sputtering. Spot EDS of the particles was performed to analyze the elemental composition.

High resolution transmission electron microscopy (HRTEM) coupled with selective area electron diffraction (SAED)—Powder sample was sprinkled on adhesive carbon tape, placed on copper grid and visualized using Transmission Electron Microscope (Model Tecnai-G2, FEI, Hillsboro, USA). SAED was carried out to gather information on crystal structure.

X-ray diffraction (XRD)—Additional information on morphology and chemical composition was obtained from XRD studies. Powder XRD was performed using desktop X Ray diffractometer (Model MiniFlex II, Rigaku, Tokyo, Japan) with Cu Ka radiation (K value 0.9 and 1.5405 A°) and 20 ranging from 10-80°.

Atomic absorption spectroscopy (AAS)—Zinc ash sample was acid treated and its zinc content estimated using Atomic Absorption Spectrometer (Model A Analyst 800, Perkin Elmer, Waltham USA).

Other chemicals—Streptozotocin, glibenclamide and pioglitazone were purchased from Sigma Aldrich Corporation, St. Louis, USA. Carboxymethyl cellulose (CMC) used for preparation of zinc ash suspensions was purchased from Merck, Darmstadt, Germany. Glucose used in oral glucose tolerance test (OGTT) was procured from HiMedia Laboratories Limited, Mumbai, India.

Experimental animals—All animal experiments were performed after the approval of the Institutional Animal Ethics Committee. Adult Wistar rats of both sexes (~ 8 weeks old, weighing 200-250 g) were used in the experiments. In case of studies in type 2 diabetic rats, five day old pups that were injected streptozotocin were allowed to grow until 12 weeks of age and then included in the study. Rats of different groups and sexes (~ 8 weeks old, weighing 200-250 g) were housed in different polypropylene cages. Standard laboratory pellet feed and purified drinking water were provided ad libitum.

Temperature of the experimental room was maintained at 22±3 °C. Relative humidity was controlled between 30-70% and 12:12 h L:D cycle was maintained. Oral dosing was performed on body weight basis using an 18 gauge oral feeding needle.

Single dose OGTT study in normoglycemic rats—Four groups of rats (n=5) were fasted for 18 h.
Subsequently, zinc ash suspension made in 0.5% aqueous solution of CMC was administered to treatment group rats (final dosage 30, 100 and 300 mg/kg). Untreated ‘control’ group rats were dosed with CMC solution (0.5%, 1 mL/kg). Blood samples (~ 5 µL) were drawn from retro-orbital plexus under ether anesthesia, after 1 h and glucose levels were checked using glucose monitor (Accuchek Active, Roche Diagnostics, Manheim, Germany). Each of the rats was then given an oral glucose load of 1.5 g/kg (time of administration being noted as ‘0’ min). Blood samples (~ 5 µL) were then drawn at 30, 60 and 120 min intervals and glucose levels recorded.

Repeated dose study in normoglycemic rats—Daily oral administration of CMC (1 mL/kg) and zinc ash (3, 10, and 30 mg/kg) was carried out to control and treatment group rats (n=5) for 14 days and blood glucose levels were estimated on 14th day. The rats were then fasted for 18 h after which zinc ash was administered and OGTT performed (as described above).

Induction of diabetes in rats—Type 1 diabetes was induced by injecting streptozotocin (45 mg/kg)in the tail vein of adult rats. For induction of type 2 diabetes, streptozotocin (90 mg/kg) was injected intraperitoneally to 5 day old rat pups. Blood glucose levels were checked one week later (type 1 model) or at 12 weeks of age (type 2 model). Animals showing hyperglycemia were selected and grouped (n=6, uniformly distributed over glucose levels ~250-500 mg/dL). Glibenclamide the dose of 5 mg/kg and pioglitazone at the dose of 30 mg/kg were used as positive controls in studies using type 1 and type 2 diabetic rats, respectively.

Studies in diabetic rats—Rats of non-diabetic and diabetic control groups were administered CMC solution (0.5%, 1 mL/kg). Treatment group rats received zinc ash at different doses of 1, 3 and 10 mg/kg. Positive control group rats received either glibenclamide (5 mg/kg) or pioglitazone (30 mg/kg). Rats were dosed orally on body weight basis, once daily for 4 weeks. On day 28, blood samples (~ 500 µL) were drawn from non-fasted rats and glucose levels estimated. Serum was separated by centrifugation, stored and analyzed later for insulin levels using rat insulin ELISA kit (Millipore Corporation, Billerica, USA). Next day, fasted rats (18 h) were subjected to OGTT (as described above). Blood glucose levels were recorded at all the time points. Blood samples (~ 500 µL) drawn at 0 min time point were centrifuged, serum separated and stored until analysis of insulin levels. Serum triglycerides (TG) and non-esterified fatty acids (NEFA) levels were later estimated using standard kits (Randox Laboratories, Crumlin, UK). Next day, rats were euthanized using ether over anesthesia. Liver, kidney, spleen, heart, muscle and pancreas were collected and stored in 10% formal saline for further processing. Later, sections (~5 µm thick) were cut, fixed on slides, stained using hematoxylin and eosin, and observed under the microscope for histopathological changes.

Dialysability study—Zinc ash was suspended in simulated gastrointestinal buffer (0.1 N HCl, pH 1.6) or intestinal buffer (phosphate buffered saline, pH 6.8) and dispersion transferred to a dialysis membrane bag. The bag was placed in a beaker containing simulated blood buffer (0.9% saline, pH 7.4) and shaken for 30 min or 2 h. The concentrations of zinc in the beaker as well as dialysis bag i.e. the dialyzed and undialyzed fraction respectively, were measured by AAS. Percent dialysability was calculated by the formula:

\[ D(\%) = \frac{\text{dialyzed}}{(\text{dialyzed} + \text{undialyzed})} \times 100 \]

Single dose pharmacokinetic study—Adult Wistar rats were divided into 2 groups of 3 male and 3 female rats each. Control and treated rats were orally administered CMC solution (0.5%, 1 mL/kg) and zinc ash (300 mg/kg) respectively. Blood samples (~ 800 µL) were drawn through retro-orbital plexus under ether anesthesia at 0, 0.5, 1, 2, 4, 8 and 24 h time points after dose administration. Serum was separated and analyzed for zinc levels by AAS. After 24 h time point blood collection, anesthetized animals were immediately euthanized and tissues (liver, kidney, heart, spleen, pancreas, muscle, adipose and brain) were collected. Tissue samples were acid digested and processed to estimate zinc content by AAS.

Acute toxicity study—Wistar rats were distributed into groups of five animals each. Zinc ash was administered orally (30 and 300 mg/kg doses) to treatment group rats, while control rats received CMC solution (0.5%, 1 mL/kg). Behaviour, clinical signs and mortality were monitored at 0.5, 1, 2, 4 and 8 h intervals post dosing on the first day. Subsequently, all parameters were monitored at least once a day for 14 days. Any signs of abnormal behaviour or toxicity seen were scored (on a scale of 0 to 5) and recorded. Body weight was recorded on days 1, 7, 14 and 15 after dosing. On day 15, rats were euthanized and major organs viz. liver, kidney, spleen and heart were observed for any gross pathological changes. Organs were then collected, their weights recorded; and stored
in formal saline to be processed later for histopathological examination.

*Sub-acute toxicity study*—Adult Wistar rats were distributed into groups of five animals each. CMC solution (0.5%, 1 mL/kg) or zinc ash (30 and 300 mg/kg) was orally administered, once daily to rats for 28 days. On day 28 of treatment, blood samples (~ 500 µL) were drawn from retro-orbital plexus and serum collected. Biochemical parameters viz. serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), creatinine and urea were measured using standard diagnostic kits (Ranbaxy Laboratories, Gurgaon, India). On day 29, animals were euthanized, major organs collected, weighed and stored in formal saline for later histopathological examination.

*Statistical analyses*—Data are presented and analyzed using GraphPad Prism software. The mean and standard error of mean (S.E.M.) values of each group are shown in the tables and graphs. Area Under Curve (AUC) values were calculated from OGTT graphs. Statistical significance was tested by One Way Analysis of Variance (ANOVA) followed by Dunnett’s Multiple Comparison Test.

**Results**

*Evaluation by classical tests of Ayurveda*—Compliance to tests evaluating the particle size, density and chemical stability of *bhasmas* as mentioned in standard Ayurvedic texts indicated complete conversion of metal to oxide form and desired size reduction.

*Scanning electron microscopy (SEM) coupled with electron dispersive spectroscopy (EDS)*—SEM of zinc ash showed 200 to 500 nm sized particles. EDS data showed 29.8% zinc content in the sample. Other elements viz. oxygen (34.3%), sodium (15.2%), sulfur (13.3%) and potassium (7.5%) were also present. Toxic metals viz. mercury, lead, arsenic, aluminium, antimony and bismuth were not detected.

*High resolution transmission electron microscopy (HRTEM) coupled with selective area electron diffraction (SAED)*—TEM image (Fig. 1a) of zinc ash also showed 200 to 500 nm sized particles, more or
less spherical. HRTEM image (Fig. 1b) clearly showed the lattice arrangement of atoms. Fast Fourier Transformation (FFT) of the HRTEM image (Fig. 1c) and SAED showed atoms (brighter spots) arranged in hexagonal pattern suggesting wurtzite structure of zinc oxide.

**X-ray diffraction (XRD)**—The XRD pattern of zinc ash is presented in Fig. 1D. Zinc ash showed four peaks at 31.9, 36.4, 47.7 and 56.5; in agreement with standard zinc oxide (JCPDS data no. 75-0576); indicating (100), (101), (102) and (110) phase structure. Three other peaks seen at 27.0, 28.7 and 30.7 can be attributed to the presence of hexagonal zinc sulfide (JCPDS data no 75-1547).

**Atomic absorption spectroscopy (AAS)**—Zinc ash sample was found to contain 70.7% zinc, corresponding to 88% zinc oxide.

**Single dose OGTT study in normoglycemic rats**—Results obtained showed that treatment with zinc ash (100, 300 mg/kg) caused significant suppression of glucose levels at 30, 60 and 120 min time points (Fig. 2a) suggesting improved glucose tolerance. Reduction in AUC values was also seen; statistically significant at 100 and 300 mg/kg dose (Fig. 2b).

**Repeated dose study in normoglycemic rats**—Zinc ash treatment (at all the doses) resulted in significant reduction of nonfasted blood glucose levels as compared to control group (Fig. 2c). Fasted glucose levels were not altered with treatment. Lack of effects on fasted glucose levels in normoglycemic condition suggested no risk of hypoglycemia. In OGTT conducted at the end of treatment, zinc ash showed modest reduction of AUC values (Fig. 2d).

**Studies in type 1 diabetic rats**—Zinc ash treatment showed suppression of glucose levels in OGTT and reduction of AUC values (~16%, Fig. 3a) suggesting improved glucose tolerance. Glibenclamide treatment also showed suppression of glucose peaks in OGTT. Type 1 diabetic rats exhibited hyperglycemia under fasted as well as non-fasted conditions. Treatment

![Figure 2](image-url)
with zinc ash resulted in dose dependent reduction of nonfasted glucose levels (~ 20% at 10 mg/kg dose, Fig. 3b). Glibenclamide treatment resulted in modest reduction of nonfasted blood glucose levels. A significant reduction in the fasted circulating glucose levels (~ 33%) was seen at 3 and 10 mg/kg doses of zinc ash (Fig. 3c). Nonfasted serum insulin levels decreased with zinc ash treatment (~ 32% at 10 mg/kg dose, Fig. 3d) as compared to diabetic control group. No effect was seen on fasted insulin levels after zinc ash treatment. Glibenclamide showed modest increase in nonfasted as well as fasted insulin levels, as is expected in the case of type 1 diabetic rats.

Body weight, feed intake and lipid parameters of type 1 diabetic rats are shown in Table 1. Diabetic control rats displayed polyphagia and polydipsia as compared to nondiabetic rats. Treatment with zinc ash or glibenclamide did not affect feed intake in diabetic rats. Nondiabetic rats gained ~ 3 times more body weight than diabetic rats. Body weight was reduced in zinc ash (10 mg/kg) group, albeit not statistically significant. Glibenclamide treatment showed body weight neutrality. In case of lipid parameters, no significant changes were noticed in serum TG levels of diabetic control and zinc ash treated rats. Also, the escalated NEFA levels in diabetic rats could not be normalized by zinc ash treatment. As expected, neither TG nor NEFA levels were reduced by glibenclamide treatment. Microscopic examination of tissue sections revealed kidney damage and pancreatic islet necrosis in diabetic control group as compared to nondiabetic control group. Tissue sections of zinc ashtreated groups did not reveal any further histopathological changes.

**Studies in type 2 diabetic rats**—Treatment with zinc ash resulted in suppression of glucose peaks in OGTT suggesting improved glucose tolerance. Significant reduction of AUC values (~19%) was seen in zinc ash (3 mg/kg) group (Fig. 4a). A significant reduction in nonfasted and fasted glucose levels (~20% and ~25% respectively at 10 mg/kg dose, Fig. 4b and 4c) was also seen after zinc ash treatment, comparable to pioglitazone (~21% and ~18%...
respectively). Further, zinc ash treatment showed trend towards decrease in nonfasted insulin levels (~27% at 10 mg/kg dose, Fig. 4d), similar to pioglitazone. Fasted insulin levels were not affected by zinc ash treatment. These results suggested possible insulin sensitizing effects of zinc ash in diabetic rats.

As can be seen in Table 1, type 2 diabetic rats were dyslipidemic as compared to non-diabetic rats. Zinc ash treatment (10 mg/kg) resulted in modest reduction

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Body weight gain (g)</th>
<th>Feed intake (g/day)</th>
<th>Serum triglycerides (mg/dL)</th>
<th>Serum NEFA (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic Control</td>
<td>A 43.8 ± 5.6</td>
<td>19.7 ± 0.8&quot;</td>
<td>61.0 ± 1.2</td>
<td>0.35 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>B 36.2 ± 3.0&quot;</td>
<td>18.3 ± 1.8</td>
<td>25.5 ± 4.0</td>
<td>0.35 ± 0.0*</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>A 14.8 ± 7.4</td>
<td>27.8 ± 0.9</td>
<td>59.7 ± 6.3</td>
<td>0.60 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>B 10.2 ± 4.8</td>
<td>23.9 ± 4.1</td>
<td>37.6 ± 2.4</td>
<td>0.55 ± 0.1</td>
</tr>
<tr>
<td>Zinc ash (1 mg/kg)</td>
<td>A 17.7 ± 15.3</td>
<td>29.2 ± 1.6</td>
<td>65.5 ± 9.4</td>
<td>0.58 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>B 5.0 ± 4.9</td>
<td>21.2 ± 3.9</td>
<td>37.2 ± 3.8</td>
<td>0.67 ± 0.1</td>
</tr>
<tr>
<td>Zinc ash (3 mg/kg)</td>
<td>A 16.6 ± 11.0</td>
<td>26.8 ± 1.2</td>
<td>57.7 ± 5.3</td>
<td>0.56 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>B 13.0 ± 3.3</td>
<td>23.3 ± 4.1</td>
<td>40.8 ± 1.4</td>
<td>0.72 ± 0.1</td>
</tr>
<tr>
<td>Zinc ash (10 mg/kg)</td>
<td>A -11.2 ± 6.5</td>
<td>27.1 ± 1.2</td>
<td>63.6 ± 9.6</td>
<td>0.50 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>B 8.5 ± 4.0</td>
<td>22.3 ± 1.8</td>
<td>31.3 ± 3.4</td>
<td>0.70 ± 0.0</td>
</tr>
<tr>
<td>Glibenclamide (5 mg/kg)</td>
<td>A 18.0 ± 4.7</td>
<td>26.0 ± 1.1</td>
<td>56.6 ± 3.0</td>
<td>0.54 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>B 29.7 ± 5.5&quot;</td>
<td>23.3 ± 2.3</td>
<td>24.7 ± 2.1&quot;</td>
<td>0.64 ± 0.0</td>
</tr>
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</table>

ZA = zinc ash, NEFA = non esterified fatty acids

$P$ values: *< 0.05, **< 0.001, when compared to diabetic control, as analyzed by One Way ANOVA followed by Dunnett’s test.

![Fig. 4](image_url) — Anti-hyperglycemic activity of zinc ash (ZA) in type 2 diabetic rats. NDC, DC and Pio indicate nondiabetic control, diabetic control and pioglitazone. [(a) AUC values of OGTT; (b) nonfasted blood glucose levels; (c) fasted blood glucose levels; (d) nonfasted serum insulin levels. $P$ values: *< 0.05 and **< 0.001 when compared with diabetic control, as analyzed by One Way ANOVA followed by Dunnett’s test]
of TG levels. Pioglitazone treatment, as expected, resulted in significant TG reduction. Neither zinc ash nor pioglitazone could result in reduction of NEFA levels. Type 2 diabetic rats too displayed lower body weight gain despite polyphagia as compared to non-diabetic rats. Pioglitazone treatment significantly increased the body weight gain (known side effect of thiazolidinediones). Zinc ash treatment did not result in any significant effect on body weight gain and feed intake. Further, zinc ash treatment did not result in any histopathological changes as compared to diabetic control group.

Dialysability study—The cumulative release (under acidic and basic conditions) of zinc ions from zinc ash was to the tune of 30%. The undialyzable component of zinc ash was >50% suggesting that under in vivo conditions, zinc ashsplits into two phases, the soluble or ionic zinc and the insoluble or particulate zinc.

Single dose pharmacokinetic study—Zinc ash treatment resulted in higher serum zinc concentration (~ 3.5 mg/L) as compared to control (~ 1 mg/L, Fig. 5). Maximum concentration ($C_{\text{max}}$) was attained at 2 h time point, after which zinc levels declined and came back to basal values within 4 h. No significant change was seen in tissue zinc levels of control and zinc ash treated groups.

Acute toxicity study—No abnormal behaviour, clinical signs or mortality was seen in any of the treated rats indicating safety after single oral administration of high dose of zinc ash. There was no significant difference in body weight gain of zinc ash treated group rats as compared to control group rats. No significant effect on relative organ weights was seen in any of the treatment groups as compared to control group. No gross pathological changes were seen after single dose administration of zinc ash. Major organs (liver, kidney, heart and spleen) appeared normal in rats of zinc ash treated groups after histological examination.

Sub-acute toxicity study—No significant effect was seen on body weight gain or relative organ weights of rats treated with 30 and 300 mg/kg doses of zinc ash. No significant effect was seen on SGOT or SGPT activity (Fig. 6a and 6b) suggesting no major hepatotoxicity or cardiotoxicity. Serum creatinine (Fig. 6c) and urea levels were also not altered after four weeks treatment with zinc ash. No major histopathological changes were observed (Fig. 7) in zinc ash treated groups as compared to control group. These results indicate no risk of organ damage after multiple administrations of high doses of zinc ash.

Discussion

Although bhasmas have proved their clinical efficacy in Ayurveda, there are several issues that limit their acceptability in modern medicine. Firstly,
the synthesis procedures are laborious, time consuming and often difficult to interpret from ancient texts. Secondly, commercial preparations may not be authentic as standards for manufacture and quality control are not yet properly defined. These lacunae tend to permit sale/availability of sub-standard products in the market (with impurities such as heavy metals) often causing undesirable toxicity effects upon prolonged use.25

In the present work Jasada bhasma (zinc ash) was synthesized as per standard procedures mentioned in Ayurveda and subjected to classical tests.26,27 Besides these tests detailed characterization of the preparation was carried out using modern analytical and imaging techniques to determine its physical and chemical nature and to detect heavy metal contaminants, if any. SEM of the zinc ash sample used in the present study revealed 200 to 500 nm particles in the preparation, confirming its sub-micronic nature. HRTEM analysis also established the sub-micronic nature of zinc ash. The FFT image showed well defined spots, confirming the single crystalline nature of zinc ash. The spot pattern can be assigned to the hexagonal phase of zinc oxide.28 Bhowmick et al.29 reported zinc oxide as nanoparticles in the filtered fraction of Jasada bhasma whereas the unfiltered fraction contained micron sized particles. However, nanoparticle could not be detected in the present zinc ash sample. EDS of zinc ash sample used in the present study revealed the presence of zinc and oxygen suggesting formation of zinc oxide. Detection of sulfur in the sample indicated the possibility of zinc sulfide formation. No traces of mercury were detected. The EDS results were in agreement with XRD data that showed peaks corresponding to zinc oxide and zinc sulfide. Nonetheless, lesser amount of sulfur as compared to oxygen suggested zinc oxide as the predominant component. Several researchers have used atomic absorption spectroscopy (AAS) to estimate metal content in bhasma samples.14,30 Zinc oxide content in the present sample worked out to 88% (estimated as zinc by AAS) which is higher than reported 69.5% by Shubha and Hiremath.31 These results in conjunction with imaging and EDS data suggest optimal oxidization as a result of incineration process confirming that the zinc ash preparation consisted of sub-micronic particles, predominantly of zinc oxide.

During the synthesis process of zinc ash, ingredients such as sesame oil (Sesamum indicum), butter milk, cow urine and kulathi (aqueous extract of Dolichos biflorus, horse gram seeds) were employed. Some of these ingredients, viz. sesame oil, cow urine, and horse gram seeds have been reported to possess anti-diabetic activity.32–34 It must be mentioned, however, that any organic remnants of these ingredients would be oxidized during the incineration process and hence are not likely to contribute to any biological activity.

Preliminary range finding studies of zinc ash were carried out in normoglycemic rats. Based on the dose used in Ayurveda practice, 30 mg/kg was used as the
starting dose. To evaluate dose dependent effects, two higher doses (100 and 300 mg/kg) were also tested. Results of single dose OGTT study demonstrated similar improvement of glucose tolerance at 100 and 300 mg/kg doses of zinc ash, suggesting saturation of efficacy. Therefore, repeated dose study was carried out at lower doses of 3, 10, 30 mg/kg. Modest improvement in glucose tolerance at lower doses suggested beneficial effects on multiple dosing.

After completing theraing finding studies in normoglycemic rats, zinc ash was evaluated in type 1 as well as type 2 diabetic rats (models of insulin deficiency and insulin resistance, respectively). The observed improved glucose clearance in OGTT can be attributed to two major known mechanisms. Firstly, zinc ash could inhibit intestinal alpha-glucosidase enzyme and thereby reduce glucose absorption as reported by Ueda et al. for anorgano-zinc complex. Secondly, increased glucose-stimulated insulin secretion by zinc could result in better glucose disposal. Zinc accumulates in pancreatic beta cells due to beta cell specific zinc transporter and results in increased glucose-stimulated insulin secretion. Similar mechanisms may be operative in the case of zinc ash. Further, improved glucose clearance in OGTT after multiple dosing and the fact that these results were comparable to pioglitazone treatment suggest insulin sensitization as a possible mechanism. In the present study, a reduction in serum insulin levels was also seen after zinc ash treatment, similar to pioglitazone. These results also suggest that zinc ash possibly works as an insulin sensitizer. Reduction in nonfasted as well as fasted glucose levels was seen in zinc ash treated type 1 (insulin deficient) and type 2 (insulin resistant) diabetic rats, suggesting that multiple mechanism may be involved. Zinc can be known to regulate glucose metabolism; enhance insulin half-life; and play a role in insulin secretion. Further, zinc ions are reported to enhance insulin signaling and action that might have contributed to control of nonfasted hyperglycemia. Zinc is also reported to regulate glucagon secretion from pancreatic alpha cells. As a result, glucagon stimulated hepatic pathways viz. glycogenolysis and gluconeogenesis would be suppressed in fasting state that might have contributed to reduced fasted blood glucose levels observed in the present study. However, further experimentation is necessary to identify the targets of action.

It has been argued that the process of bhasmikaran (synthesis of bhasma) results in size reduction thereby facilitating absorption and assimilation in the body. Particle solubility especially plays an important role in bioavailability. Solubility of zinc ash was evaluated by using a simple dialysis experiment. Researchers have not used this technique for bhasmas although reports on nanoparticle dissolution exist. It is expected that only ions can pass through a dialysis membrane of 12 KD cut-off value. In the present study, detection of zinc in the dialyzed fraction indicates that zinc ash gets ionized in aqueous media. It was found that only about 30% of the total zinc could get dialyzed suggesting that under in vivo conditions zinc ash is encountered as zinc ions as well as particulates, after oral administration and hence the extent of their uptake in the stomach and intestine would be crucial. It is highly unlikely that zinc, in any form, would be absorbed from the stomach. The primary site of exogenous zinc absorption is the intestine, where it occurs through the apical surface of enterocytes. The soluble fraction of zinc ash could get immediately absorbed from the intestine and enter circulation through specific transporters (e.g., zinc transporter 1) that are abundant along basolateral membranes of enterocytes. This could result in the initial spurt of zinc in blood as observed in the present study. The uptake of particles from the GI tract is governed by both their size and surface characteristics. Thus smaller, typically nanometric particles have been shown to exhibit increased uptake.

In the present study serum zinc levels plummeted rapidly after 2 h of oral administration of zinc ash. These results clearly suggest that a major portion of the particulate fraction of zinc ash is not taken up through the intestine and is probably excreted in the feces. Nevertheless, serum zinc levels were elevated in pharmacokinetic study, confirming oral absorption of zinc ash.

It is widely believed that metals are toxic limiting their use in medicine. However, amongst the several trace elements, zinc is probably the safest; virtually nontoxic to living organisms. It is the only element that is neither cytotoxic nor systemically toxic, carcinogenic, mutagenic, or teratogenic. The reported oral LD₅₀ of zinc salts is 237-623 mg/kg in rats. In the present study, toxic effects were not seen at doses that were 10 and 100 times the optimal therapeutic dose (3 mg/kg) of zinc ash, clearly indicating its safety.

Several zinc complexes have been recognized as potential candidate drugs by modern medicine. However, poor oral bioavailability and toxicity concerns associated with their usage remain as
formidable problems yet to be resolved satisfactorily. Thus development of zinc-based anti-diabetic drugs has lagged behind, despite their tremendous potential. Over this background, Jasada bhasma (zinc ash) appears to be a clever way invented by Ayurveda for delivering zinc to treat diabetes and other diseases.

Conclusions

Ayurvedic medicine Jasada bhasma (zinc ash) comprises of sub-micronic particles, predominantly of zinc oxide. Anti-diabetic activity of Jasada bhasma has been pharmacologically validated using both type 1 and type 2 diabetes rat models. Even though the preliminary findings suggest that the anti-diabetic activity of zinc ash might be due to insulin sensitizing effects, further studies to identify the targets of action need to be carried out. Pharmacokinetic data confirm systemic absorption of zinc ash after oral administration. The study also suggests that the preparation is free of heavy metals and is safe for use even at 100 times the efficacy dose. To the best of our knowledge, this is the first comprehensive study of its kind validating/endorsing the extensive use of zinc ash preparation in Ayurveda for the treatment of diabetes.

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References


