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Original Article

Characterization of Calcined Jade and its immunomodulatory effect on macrophage isolated from Swiss albino mice

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ABSTRACT

Calcined Jade (CJ) is a metasilicate frequently used in traditional system of medicine as tonic to vital organs with several other pharmacological activities. X-ray powder diffraction (XRPD), inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectroscopy (AAS) and CHNS analyzer techniques were used to characterize CJ sample. CJ was administered orally to Swiss albino mice at a dose of 50, 75, 100 and 200 μg/kg body weight for 10 days and modulation of the macrophage mediated innate immune responses was studied. Flow cytometric analysis of TLR-2/4 on peritoneal macrophage revealed elevated expression of TLR-2 as compared to control. Significant increase in phagocytic activity was observed in peritoneal macrophage. The lymphoid organs weight and other toxicity parameters did not exhibit any harmful effect. To evaluate the presence of nanoparticles, CJ was dissolved in milli Q water, filtered and lyophilized. Transmission electron microscopic (TEM) analysis revealed the presence of spherical nanoparticles in CJ [14.7–142.0 nm dimension with average particle size of 64.6 nm]. In conclusion, we report stimulation of innate immune responses by CJ may partly be due to the formation of nanoparticles. Further experiments using isolated nanoparticles may further validate the role of nanoparticles.

1. Introduction

Metals and minerals have been utilized as component of a number of medicinal formulations since a long period in ancient health systems. These metals and minerals require greater scientific validation in terms of their efficacy, safety and pharmacokinetics before using for medication purposes. Therefore, further studies are required for the determination of the structures of these complex metal and mineral compounds. However, the examination is not limited to only determination of the structure of these metal and minerals. The crystalline structure, elements present, elemental groups as well as molecular structure, is ultimately essential to truly understand these metal and mineral compounds with respect to the safety of traditional medicines.[1] It is imperative for the practitioners of indigenous traditional systems of medicine including Ayurveda, Unani, Sidha and Homeopathy to update their methods of drug preparation according to the latest methods and techniques of structural analysis and use them as tools to validate efficacy and safety of the traditional medicines. In traditional system of medicine, variation in the collection process, timing and procedure of making calcined products of metals and minerals may yield same calcined product but with different qualities. In many cases, wrong manufacturing process may lead to production of inferior-quality products, which reduces efficacy and safety. In order to minimize variability and to strengthen the quality of indigenous products, standardization of a calcined product is essential.[2,3]

Calcined Jade (CJ) is a metasilicate rich traditional mineral compound used in the preparation of various herbo-mineral formulations which is in use since long and claimed to be effective in very small doses. It has been prescribed since many decades in a number of traditional preparations as rejuvenator or vitalizer which strengthens the vital organs of the body. It is useful in palpitation, dysentery, burning micturation hemorrhage. In combination with other medicines, Jade is prescribed in treating tuberculosis, jaundice, dyspepsia and urinary complaints.[4]

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Although several scientific studies have been carried out to answer structural analysis of other metals and minerals containing traditional calcined products but have not been studied on CJ. It is thus essential to prepare quality control parameters of this product. In the present research work, CJ has been analyzed with a focus of quality control parameters based on modern parameters. After analyzing the quality control parameters we also have studied the effect of CJ on modulation of macrophage activity in Swiss albino mice as animal model.

2. Materials and methods

2.1. Chemicals and reagents

The CJ used in our study was provided as a kind gift from late Professor M. S. Y. Khan (Department of Pharmaceutical Chemistry, Jamia Hamdard University, New Delhi, India). Fetal bovine serum (FBS), DMEM media, lipopolysaccharide (LPS, Type 055:B5 from *Escherichia coli*), penicillin-streptomycin, Phycoerythrin (PE)-labeled anti-TLR-4 and Alexafluor [AF]-647-labeled anti TLR-2 antibodies were purchased from BD Biosciences (San Diego, CA, USA). Goat RBCs were procured from local slaughter house. All other analytical grade fine chemicals were purchased from S.D. Fine Chemicals Ltd. (Mumbai, India).

2.2. Characterization of CJ

CJ sample was scanned on PAN analytical make X-pert powder diffractometer and 2 theta scan was adjusted from 10° to 100° using Ni filter, copper and potassium alpha radiation and sodium iodide scintillator.

Inductively coupled plasma-mass spectrometry (ICP-MS) analysis of CJ was done to quantify the presence of inorganic elements. Digested ash sample was analyzed using Perkin Elmer NexION 300D inductively coupled plasma-mass spectrometer. Sodium (Na), magnesium (Mg), iron (Fe) and potassium (K) were analyzed using Kinetic Energy Discriminator (KED) mode using helium gas while phosphorus (P) and sulfur (S) was analyzed using Dynamic Reaction Cell (DRC) mode using O_2 to remove polyatomic interferences.

Heavy metals content like lead (Pb), arsenic (As), cadmium (Cd) and mercury (Hg) were determined by atomic absorption spectroscopy in Agri Food Testing Laboratory at Punjab Biotechnology Incubator, Mohali, India.

The quantitative CHNS (carbon, hydrogen, nitrogen and sulfur) analysis of CJ sample was carried out on a Vario EL III CHNS elemental analyzer with sulfanilic acid as standard. Concentration of elements was calculated using the inbuilt software in CHNS elemental analyzer.[5]

2.3. Transmission electron microscopy

CJ sample was prepared for TEM analysis by dissolving the powdered CJ in milli Q water and filtered through whatmann filter paper number 1. Filtrate was lyophilized to remove excess of water and air dried. Further, nanoparticles of CJ recovered during the filtration process were again dissolved in milli Q water and followed by sonication in an ultrasonic bath. The sonicated sample was transferred to a carbon film supported by a copper grid. The images of the nanoparticles were taken on JEOL 2100F transmission electron microscope.

2.4. Animals and experimental design

All procedures in animal studies were carried out under a protocol approved by the University Animal Ethical Committee (Committee for the Purpose of Control and Supervision of Experiments on Animals). Male Swiss albino mice (weighing 20–25 gm, 6–8 weeks old) were obtained from University Animal House, Jamia Hamdard, New Delhi, India. Animals were quarantined for at least 1 week before the start of study. Five mice were housed per cage in plastic shoe box cages. Animal rooms were maintained at 18–26 °C with a relative humidity of 40–70% and a 12-hour light/dark cycle. The animals were kept on standard pellet diet, and *ad libitum* tap water access was provided. After being adapted to the environment for 1 week, the mice were randomly divided into five groups. Each experimental group consisted of five animals.

CJ was suspended in deionized water with 0.5% Sodium Carboxy Methyl Cellulose [Na CMC]. In the preliminary study, to determine the dose response curve, various concentrations of CJ were administered orally to mice. Based on the effect on level of IgM as analyzed using Plaque forming cell assay and Hemagglutination titer assay, CJ at the dose of 50, 75, 100 and 200 μg/kg body weight were selected for further study. The above dose concentrations were administered orally 0.1 ml/100 gm (v/w) body weight for 10 days in group II-V respectively. Group I was taken as control and received a similar volume of Na CMC (0.5%). Day 11 (24 h after final dosing) was considered as Day 0 in all subsequent protocols outlined below.

2.5. Flow cytometric analysis of macrophage surface antigen markers

On Day 0, Surface antigen markers (TLR-2 and TLR-4) were detected by flow cytometry in the peritoneal macrophages harvested by administering 10 ml of ice cold PBS in the peritoneal cavity of each mouse. Cells were washed thrice with stain buffer+FBS (BD Bioscience) and centrifuged at 300× g at 4 °C for 5 min. Cells were re-suspended in cold stain buffer+FBS to a final concentration of 10^6 cells/ml. The cell suspension was incubated with diluted (1:1000) fluorescent antibodies (stained with AF647-anti-TLR-2 and PE-anti-TLR-4) for 30 min on ice in dark chamber. Cells were washed twice with stain buffer+FBS to remove unbound antibodies. Cell pellet was re-suspended in 0.5 ml stain buffer+FBS and above mentioned surface antigen markers were analyzed in BD LSR II flow cytometer (BD Biosciences).[6]

2.6. Analysis of nitrite production

Peritoneal macrophages were collected by the above given method and suspended in complete DMEM medium supplemented with 10% fetal calf serum (Sigma–Aldrich, USA). Adhered cells were incubated at 37 °C for 24 h in presence or absence of positive stimulator of nitric oxide production (LPS, 5 μg/ml) (LPS from *E. coli* 055:B5, Sigma, USA). Culture soup was collected for the estimation of NO by Griess reagent. Briefly, 59 μl of cell supernatant mixed with 1% sulphanilamide, 0.1% naphthylethylene diamine and 2.5% H_3PO_4 were incubated at room temperature for 10 min to form a chromophore. The absorbance was read at 550 nm and NO was measured using $NaNO_2$ as standard.[7]

2.7. Phagocytosis

Phagocytic rate and index was analyzed according to the protocol given by Sano et al. (2003) with slight modifications.[8] Briefly, on Day 0, mice were intra-peritoneally injected with 0.5 ml of 5×10^6 gRBCs and were euthanized 1 h later. The fluid of abdominal cavity was collected to make a smear and incubated at 37 °C for 30 min in a wet box fixed with 95% ethanol. The cell smear was stained with Wright–Giemsa dye and the numbers of macrophages ingesting gRBC out of a total of at least 100 cells were calculated by

direct visual enumeration using a light microscope. The phagocytic rate (PR) and phagocytic index (PI) were calculated as follows:

PR (%) = [(Number of macrophages ingesting gRBC)/(Total number of macrophage)] × 100

PI = Total number of ingested gRBC/Number of macrophage ingesting gRBC

2.8. Lymphoid organ weight and cellularity

The lymphoid organ weight and cellularity of mice administered with CJ for 10 days was determined. The relative organ weights (organ weight/100 g body weight) of spleen and thymus were determined for each mouse. Single cell suspension was prepared in HBSS from bone marrow, spleen and thymus for cell count.

2.9. Everted gut sac analysis to determine cellular uptake of CJ

Everted gut sac analysis was done to evaluate the intestinal absorption of CJ sample by the following method with slight modification.[9] Wistar rats (male, 8 weeks) were fasted for 12 h (*ad libitum* access to water) prior to the experiment. The rats were anesthetized and sacrificed. The whole small intestine was isolated and gently flushed with Krebs-ringer buffer (pH 7.0). A 6–8 cm segment of the small intestine was removed and everted over a silicone tube. The bottom portion was tied with thread and the segment was filled with 0.6–0.8 ml of Krebs-ringer buffer (pH 7.0). The filled segment was then placed in 2.5 ml of either Krebs-ringer buffer only (control group) or in CJ (200 μg/mL) in Krebs-ringer buffer and incubated at 37 °C for 45 min. After incubation, the solution on the serosal side of the segment was collected. Optical density of each solution was taken at 230 nm to determine the concentration of CJ.

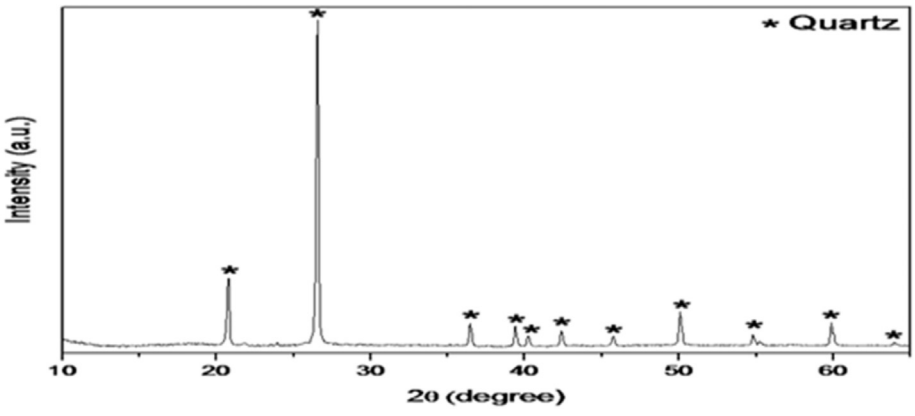
2.10. Statistical analysis

The statistical analysis of all the treatment groups and the significance of differences between treated and control groups was determined using GraphPad prism software (Dunnet student's t-test) and $p < 0.05$ was chosen as the level of significance.

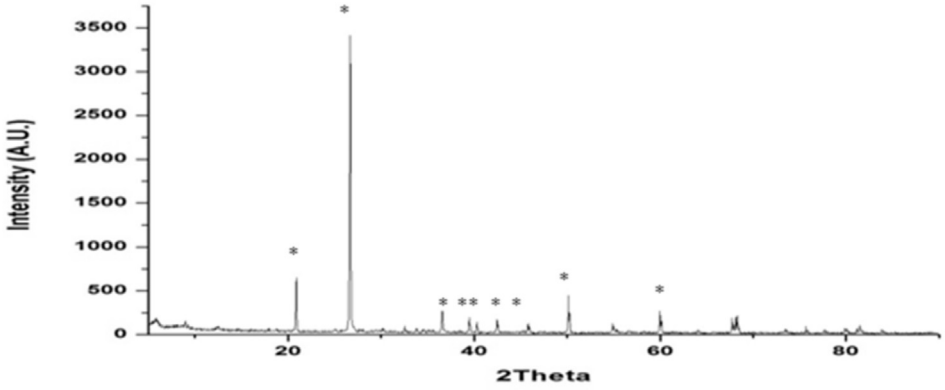
3. Results

3.1. Characterization of CJ

Quartz was detected as major mineral present in the CJ determined by XRPD analysis. The characteristic peaks of quartz found to be appear at position d = 4.26 Å (2θ = 20.85), d = 3.34 Å (2θ = 26.66), d = 2.45 Å (2θ = 36.56) and d = 2.28 Å (2θ = 39.49) (Fig. 1a). The peaks of CJ appeared at position d = 4.25 Å (2θ = 20.87), d = 3.34 Å (2θ = 26.66), d = 2.45 Å (2θ = 36.53), d = 2.28 Å (2θ = 39.44), d = 2.23 Å (2θ = 40.26), d = 2.12 Å (2θ = 42.42), d = 1.97 Å (2θ = 45.80) and d = 1.81 Å (2θ = 50.19)



(a). XRD pattern of Quartz.



(b). XRD pattern of calcined Jade [CJ].

Fig. 1. The XRD analysis of CJ nanoparticle sample was done on PAN analytical make X-pert powder diffractometer.

(Fig. 1b). Comparative analysis of the peak values of Quartz and CJ showed that the peak values of both were lying on the similar positions which confirms the presence of quartz as chief mineral in CJ. Quartz is the silicate of magnesium and calcium, suggesting that the variety of CJ used in our study was nephrite.[10] The peak positions which are similar in CJ and quartz are indicated with stars in Fig. 1a and b.

3.2. The elemental analysis of CJ

Inorganic elemental composition of CJ was determined by ICP-MS (ICP-MS, Perkin Elmer NexION 300D). The maximum dose of CJ administered to the mice was 200 μg/day, that contains 0.32 μg Na/day, 1.3 μg K/day, 5.26 μg Mg/day, 8.14 μg Fe/day, 14.1 μg P/day and 23.9 μg of S/day (Table 1). The quantity of the above elements was found to be below the acceptable tolerable daily intake limit established by food and nutrition board, USA.[11]

AAS analysis revealed that CJ contained 129.89 mg/kg Pb, 10 mg/kg As and 0.4 mg/kg Hg (Table 1). A tolerable acceptable daily intake value of these heavy metals has been established by the Food and Agricultural Organization/World Health Organization Joint Expert Committee on Food Additives (Table 1).[12] The maximum dose of CJ administrated to the mice in this study was 200 μg/kg body weight for 10 days, which contain 3 ng/day of Pb, 0.008 ng/day of Hg and 0.2 ng/day of As. Cd content was not detected in CJ. The data suggest that Pb, Hg and As content do not cross the limit of acceptable tolerable daily intake.

The purity of CJ was determined using CHNS analyzer. The CHNS content were 0.408% (C), 0.302% (H), 0.061% (N) and 0.078% (S), respectively which revealed that only 0.85% CJ content was organic material, while the rest of the 99.15% content was inorganic material (Table 1).

3.3. Presence of nanoparticles in CJ

TEM analysis was carried out to determine the particle size of CJ. The shape of nanoparticle was spherical and the size of the particle ranges from 14.7 to 142.0 nm with average size of 64.6 nm (Fig. 2A). The size distribution analysis of CJ nanoparticles revealed that majority of nanoparticles were in between the range of 10–30 nm (Fig. 2B), indicating that the CJ fulfils the criteria of a nano-drug in terms of particle size.[13]

3.4. Effect of CJ on macrophage surface markers

The peritoneal macrophages isolated 24 h after the final dose administration of CJ to hosts without any antigenic stimulation were used to assess the expression of surface TLR-2 and TLR-4 markers using flow cytometry. Expression of macrophages surface markers TLR-2 in peritoneal macrophages increased at 50 (16.4%), 75 (40.5%) and 100 (38.6%) μg/kg body weight as compared to control (3.3%). TLR-4 was found to be decreased at all dose, 50 (3.3%), 75 (2.8%), 100 (1.7%), and 200 (11.9%) μg/kg body weight, as compared to control (16.6%) (Fig. 3(a–e)). The maximal enhancement in the expression of TLR 2 positive cells was at 75 μg/kg body weight (40.5%), while maximal reduction in expression of TLR-4 was reported at 100 μg/kg body weights (1.7%). The double positive cells slightly increased as compared with control.

3.5. Effect of CJ on nitrite production and phagocytic activity

No significant change in the level of nitrite production in peritoneal macrophages was observed (Table 2). However effect of CJ on phagocytic rate and phagocytic index was found to be increased at all dose level, but significant increase in phagocytic rate and phagocytic index was found at 100 μg/kg body weight ($p < 0.05$) (Table 2). The maximum increase in phagocytic rate and phagocytic index was observed at a dose of 100 μg/kg body weight ($p < 0.05$).

3.6. Effect of CJ on relative lymphoid organ weight, cellularity, and toxicity

Oral administration of CJ caused a significant increase ($p < 0.05$) in spleen weight at dose of 75 μg/kg body weight (Table 2). The size and weight of the thymus also increased significantly ($p < 0.05$) at 50 and 75 μg/kg body weight (Table 2). There was a significant increase ($p < 0.01$) in bone marrow and spleen cellularity at dose of

Table 1
Inorganic elements, heavy metals and CHNS content of CJ.

Induced coupled Pl induced coupled plasma mass spectrometry			
Inorganic elements	% Content in CJ	WHO recommended value/day (gm)	Content in 200 μg of CJ given to mice/day
Na	0.16	2.40 gm/day	0.32 μg/day
Mg	2.63	0.35 gm/day	5.26 μg/day
K	0.69	3.50 gm/day	1.30 μg/day
Fe	4.07	0.04 gm/day	8.14 μg/day
P	7.5	4.00 gm/day	14.1 μg/day
S	11.95	Not specified	23.9 μg/day
Atomic absorption spectrometry			
Heavy metals	Results	WHO permissive daily limit for 25 gm mouse	Content in 200 μg of CJ given to mice/day
Lead	129.89 mg/kg	1.2 μg/day	3 ng/day
Mercury	0.4 mg/kg	0.24 μg/day	0.008 ng/day
Arsenic	10 mg/kg	0.6 μg/day	0.2 ng/day
Cadmium	Not detected	–	–
CHNS analysis			
Weight of CJ (mg)	Element	Content (%)	C/N Ratio
7.7900	Nitrogen	0.061	6.731
	Carbon	0.408	
	Sulfur	0.078	
	Hydrogen	0.302	

Digested Calcined Jade sample was analyzed for inorganic elemental content using Perkin Elmer NexION 300D induced coupled plasma mass spectrophotometer (ICP-MS). Heavy metals like lead, arsenic, cadmium and mercury in the CJ nanoparticles was determined by atomic absorption spectroscopy. Content of carbon, hydrogen, nitrogen and sulfur were determined by CHNS elemental analyzer using inbuilt software.

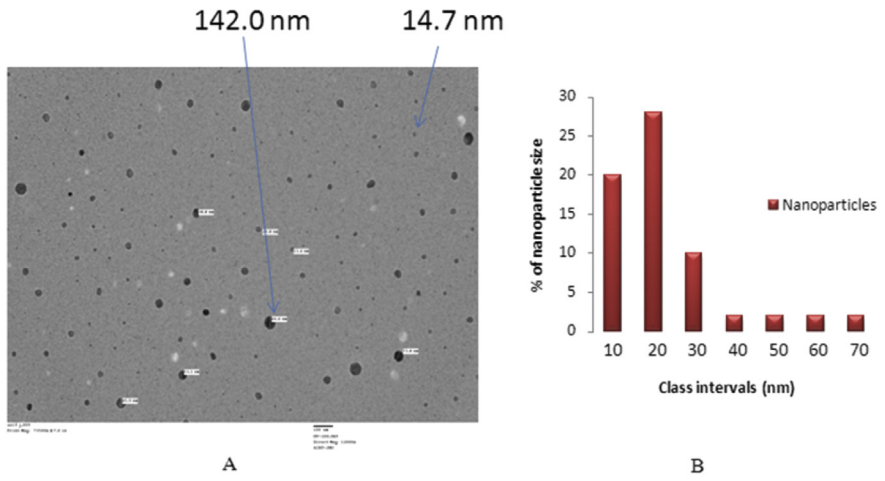


Fig. 2. The TEM analysis of CJ was done to determine the particle size.

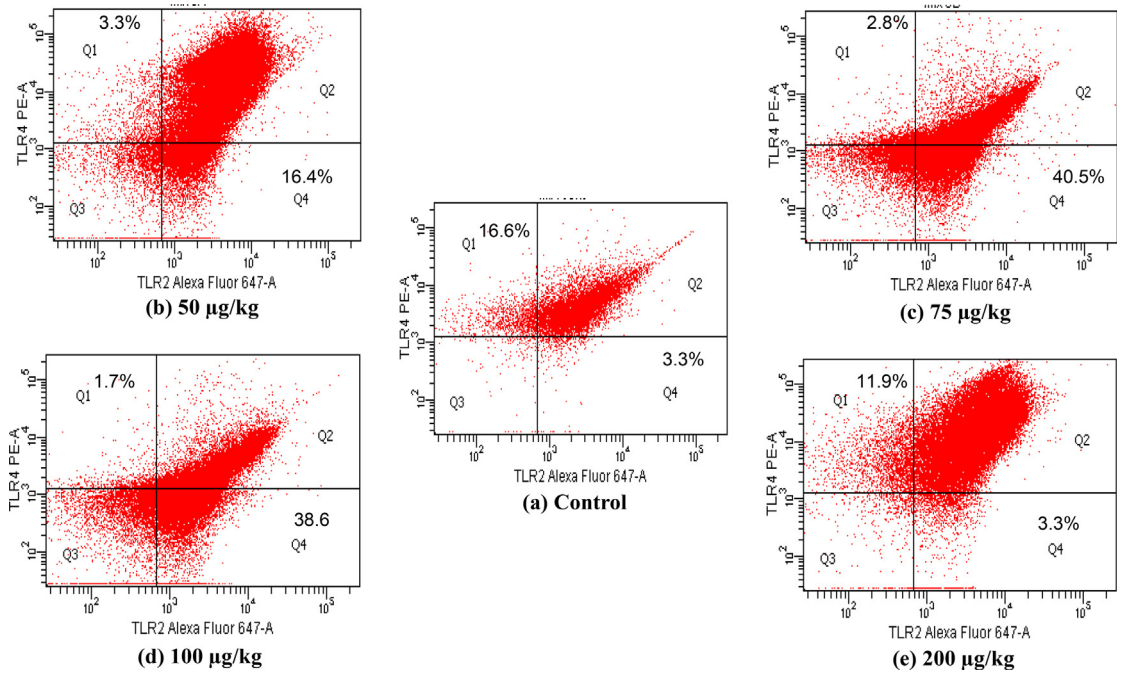


Fig. 3. Flow cytometric analysis of peritoneal macrophage surface antigen TLR-2/4. Staining of peritoneal macrophages was done with surface markers TLR-2 (Alexa flour 647-conjugated monoclonal antibody) and TLR-4 (PE-conjugated monoclonal antibody). (a) Control; (b) 50 µg/kg dose; (c) 75 µg/kg dose; (d) 100 µg/kg dose; (e) 200 µg/kg dose.

75 µg/kg body weight (Table 2). The cellularity of thymus significantly increased ($p < 0.05$) at 100 µg/kg body weight (Table 2).

3.7. Intestinal absorption of CJ particles

Intestinal absorption of CJ sample was evaluated using everted gut sac method. Everted gut sac analysis revealed that the absorption of CJ sample from the mucosal side to the serosal side of the sacs was significantly greater after incubation for 45 min (Fig. 4). This suggested that CJ sample is absorbed through the intestine and that the surface property of the particles is one of a determinant of the degree of absorption. Tissue viability is a limiting factor in everted gut sac analysis; however, the viability and metabolic activity of intestinal tissue has been reported as being retained for approximately 2 h under physiological conditions.[9]

4. Discussion

Metasilicates of many minerals are being used as medication in traditional system of medicines since centuries despite the presence of the minute quantity of heavy metals. However, this field of research has been largely neglected due to associated suspicion of heavy metal toxicity. In the present work, we thoroughly characterized Jade, a metasilicate of magnesium and calcium which is used in a significant number of formulations in traditional Indian system of medicine and then assessed its immunomodulatory potential. Jade is most frequently used in the calcined form that has been hypothesized to contain nanoparticles which might be responsible to enhanced biological activities. However, till date, very little experimental validation has been done to confirm the presence of nanoparticles and their biological impact.

Table 2
Effect of CJ on innate immunity and toxicity parameters.

Immune Responses	Group I: Control	Group II: JA (50 μg/kg body weight)	Group III: JB (75 μg/kg body weight)	Group IV: JC (100 μg/kg body weight)	Group V: JD (200 μg/kg bwt)
Innate immune response					
Nitric oxide induction					
Un-stimulated	9.23 ± 0.379	9.34 ± 0.432	9.68 ± 0.350	9.91 ± 0.296	9.51 ± 0.350
LPS stimulated	17.23 ± 0.363	17.17 ± 0.283	17.69 ± 0.499	18.05 ± 0.254	17.46 ± 0.337
Phagocytic rate	18.3 ± 2.02	23.6 ± 1.85	32.0 ± 2.51	41.3 ± 2.60*	38.6 ± 2.02
Phagocytic index	1.03 ± 0.018	1.08 ± 0.032	1.09 ± 0.022	1.20 ± 0.069*	1.08 ± 0.021
Lymphoid organs wt. (gm/100 gm body weight)					
Spleen	0.250 ± 0.019	0.318 ± 0.046	0.378 ± 0.014*	0.317 ± 0.023	0.322 ± 0.030
Thymus	0.129 ± 0.060	0.179 ± 0.024*	0.159 ± 0.002*	0.163 ± 0.011	0.144 ± 0.004
Cellularity of lymphoid organs (Mean ± S.E. × 10^6)					
Spleen	169.8 ± 23.8	126.9 ± 18.8	381.8 ± 38.6*	333.0 ± 65.8	280.0 ± 55.2
Thymus	124.3 ± 9.1	208.0 ± 42.68	217.0 ± 11.57*	229.7 ± 24.3*	148.8 ± 22.4
Bone marrow	8.01 ± 1.2	15.8 ± 2.0	27.4 ± 4.1**	15.9 ± 2.6	15.2 ± 3.1

Animals were sacrificed 24 h after the administration of CJ for 10 days at different concentration. NO induction is expressed as absorbance at 550 nm by un-stimulated and LPS stimulated macrophages. Macrophage phagocytic response is expressed as assessed by phagocytic rate and phagocytic index. Organ weights were recorded and calculated as gm/100 gm body weight. A single cell suspension of bone marrow, thymus and spleen was prepared in HBSS. Cells were counted using Neubauer chamber. Data represent mean ± S.E. (n = 5). *$p < 0.05$, **$p < 0.01$, when compared to control.

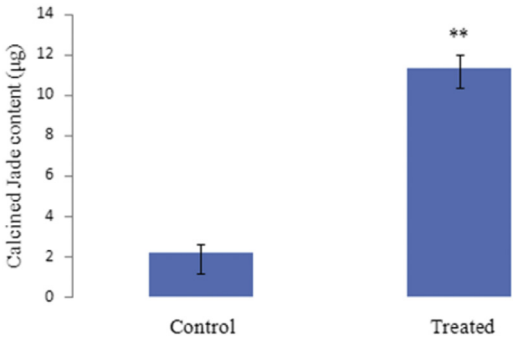


Fig. 4. Measurement of intestinal absorption of CJ in rat using everted gut sac method combined with spectrometry. Intestinal sacs were incubated in solutions of the indicated CJ sample (200 μg/mL) for 45 min. Values are expressed as mean ± S.E.M ($n = 5$). **$p < 0.01$ compared with the control group.

The present study revealed the presence of nanoparticles in CJ, with an average size of 64.6 nm, as determined by TEM. The formation of nanoparticles in CJ takes place during the elaborate process of calcination. This interesting result indicates the usage of nanoparticles as a form of medication since ancient times. It is known that nano size materials lead to enhanced absorbability, adaptability and digestibility.[14–16]

Macrophages are primary cells of innate immunity and interact first with foreign invaders. Upon activation, macrophages engulf the foreign invader, process it and present to T and B lymphocytes which in turn synthesize various cytokines and antibodies against the foreign antigen to eliminate them.[17] Oral administration of CJ was found to activate macrophages as indicated by the significant increase in phagocytic rate and phagocytic index. Also, an increased expression of TLR-2 and decreased expression of TLR-4 was observed. Toll like receptors (TLRs), are protein molecules on the surface of antigen presenting cells (APC), having potential to interact with the extracellular material and induce appropriate downstream signaling in the cellular compartment. TLR activation leads to antimicrobial activity and synthesis of inflammatory cytokines. TLR activation leads to either MyD88-dependent pathway which promotes inflammatory response or TIR domain-containing adaptor inducing interferon (TRIF) β-dependent pathway which promotes T-cell activation. Lower expression of TLR-4 may be due to inhibitory effect of CJ on inflammatory response as TLR-4 is mainly responsible for the MyD88-dependent inflammatory response. The estimation of inflammatory cytokine IL-1β level and the level of NO production by stimulated peritoneal macrophages isolated from CJ treated mice, as compared to control, also points towards this conclusion. In cells, TLR-2 promotes primarily two types of pathways, STAT-1-dependent pathways that promote Th1 cell-based responses or STAT-6-dependent pathways that promote Th2 cell activation.[18] Significant enhancement in the level of Th-1 cytokines indicates that CJ may be responsible for the induction of STAT-1 dependant pathway which leads to the activation of Th-1 directing immune response (Data not shown).

The findings reported in this study suggest that oral administration of CJ for 10 days at 50–200 μg/kg body weight in male Swiss albino mice leads to apparent changes in the overall health of the animal. An increase in bone marrow cellularity indicates effect on hematopoietic stem cells of bone marrow.[19] The increase in thymus and spleen weight, which represent primary and secondary organs of the immune system, suggests an immunopotentiating effect of CJ. The increase in thymus weight might have been partly due to the stimulating effect of CJ on the maturation of lymphocytes and bone marrow hematopoietic cells that ultimately home in thymus. CJ did not affect the body weight or survival suggesting that it is well-tolerated by the mice.

5. Conclusion

The study points out towards the capacity of CJ to be a multipotent inducer of immune responses by way of stimulating non-specific and specific immune mechanisms of the host. Jade in calcined form has been prescribed since centuries by practitioners of traditional systems of medicine. However, this study tempts us to state that nanoparticles generated in the traditional process of calcination cause the enhanced immunopotentiating effect and traditional formulations using CJ, even as a component may be improved upon by isolating and using nanoparticles formed during the process of calcination as opposed to the native calcined product. This study might be considered as a small step towards providing a thrust to the field of alchemy to generate newer avenues to research the potential of traditional mineral formulations used since centuries.

Conflict of interest

The authors report no competing financial interest and are responsible for the content and writing of the paper, which is the explicit work of authors under the overall supervision of FK.

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