



THERAPEUTIC POTENTIAL OF JAGGERY AGAINST SILICA NANOPARTICLES INDUCED TOXIC MANIFESTATIONS IN SPRAGUE-DAWLEY RATS

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ABSTRACT

The protective effects of aqueous extract of Jaggery were evaluated against silica nanoparticles (SNPs) induced toxic manifestations in liver, kidney and lung tissues. Male albino rats were orally treated with Jaggery (400 and 800 mg/kg) for 7 days, after intraperitoneal administration of SNPs (50 mg/kg) for 6 weeks. SNPs administration significantly increased the levels of serum alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, serum alkaline phosphatase, urea, uric acid, creatinine and lipid peroxidation level in tissues, whereas substantial decreases were observed in levels of glutathione, alkaline phosphatase, glucose-6-phosphatase, superoxide dismutase, catalase enzymatic activities in liver, kidney & lung and albumin level in serum. Treatment of Jaggery has shown significantly restoration in the studied parameters toward normal value. Thus, Jaggery can be used to reduce tissues toxicity and may serve as an alternative medicine in the treatment of hepatic, renal and pulmonary tissues etiology.

KEYWORDS: Silica nanoparticles, Jaggery, Superoxide dismutase, Glutathione and Catalase



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INTRODUCTION

Recent progress in nanotechnology increases the use of nano-sized silica (SiO₂) as a promising material. Silica nanoparticles (SNPs) have been found extensive applications in biomedical and biotechnological fields such as medical diagnostics, gene therapy, biomolecules detection, bioimaging, DNA and drug delivery¹⁻². Due to the widespread application of SNPs, exposure in humans may occur via inhalation, ingestion, dermal penetration and injection³. Inhaled ambient ultrafine particles can be found in heart, bone marrow, liver, kidney and even central nervous system. SNPs exposure is associated with different disorders including systemic sclerosis, emphysema, rheumatoid arthritis, dermatomyositis, glomerulonephritis, vasculiti and cardiovascular disease⁴, while certain crystalline silica polymorphs may cause silicosis and lung cancer⁵. LD₅₀ (Lethal dose) of silica through intraperitoneal route is 400 mg/kg of body weight⁶. Numerous previous studies on nanoparticles toxicity reported that oxidative stress is one of the most important toxicity mechanisms related to exposure to nanoparticles⁷. SNPs can reportedly cause inflammatory responses, hepatotoxicity as well as fibrosis⁸. Despite the development of several therapies against SiO₂ toxicity (including whole-lung lavage, steroids, tetrandrine drugs and aluminium powder inhalation)⁹, none can reverse or slow the disease process. Novel approaches for the prevention and treatment of silica-induced toxicity are needed. In recent years, plant and plant products have been the main focus in the search for nutraceuticals to combat oxidative stress induced diseases. Jaggery is a traditional unrefined non-centrifugal sugar made from sugarcane juice (*Saccharum officinarum*) which is widely consumed in Asia, Africa, Latin America and the Caribbean. It contains an enormous polyphenols, carotene, protein, carbohydrate, vitamins and minerals. It has also been prescribed in various diseases like jaundice, breathlessness and kidney problems¹⁰. Jaggery possesses a variety of pharmacological properties such as anti-

inflammatory, antioxidant, anticarcinogenic and cytoprotective¹¹. Previous studies have revealed that Jaggery has remarkable beneficial effect on pulmonary system against environmental toxicants¹². Jaggery can counter the genotoxic effects induced by arsenic in vivo¹³. Earlier studies on Jaggery showed that Jaggery feeding helps to improve the body defence mechanism and does not appear to have any negative toxicological effects¹⁴. Thus, the present study was undertaken to evaluate the therapeutic efficacy of Jaggery against silica nanoparticles induced toxic manifestations.

MATERIALS AND METHODS

(i) Chemicals and Drugs

Silica nanoparticles (10-20nm) were obtained from Sigma-Aldrich (USA). All chemicals were procured from Sigma-Aldrich (USA), E-Merck (Germany), Ranbaxy Pvt. Ltd. and BDH Company (India). Loaves of Jaggery were procured from an authentic dealer (Sai Nath Broker, Dabra) Gwalior, Madhya Pradesh (India).

(ii) Preparation of Doses and Treatments

The silica nanoparticles were administered at a dose of 50 mg/kg, i.p. with vehicle (normal saline, 0.9%)¹⁵. Aqueous suspension of Jaggery (400 and 800 mg/kg, p.o.) was prepared in gum acacia (1%)¹⁶.

(iii) Maintenance of Animals and their Feeding

Adult male albino rats of Sprague–Dawley strain (180±10 g body weight) were selected from departmental animal facility where they were housed in polypropylene cages under uniform husbandry conditions (25 ± 2°C temp, 60-70% relative humidity, 12 h photoperiod). Animals were fed on commercially available standard animal diet (Pranav Agro Industries Ltd., New Delhi, India) and drinking water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee for the Purpose of Control and Supervision of

Experiments on Animals (CPCSEA/501/01/A), India.

(iv) Experiment Design

Thirty adult male rats were divided into five groups of six animals each. The animals were administered SNPs at the dose of 50 mg/kg, i.p. for 6 weeks followed by different doses of Jaggery (400 and 800 mg/kg, p.o.) after 24h of toxicant administration for 7 days. Jaggery *per se* group was administered at the dose of 800 mg/kg orally.

- Group I: Control (Vehicle only).
- Group II: Jaggery *Per se* (800 mg/kg, orally for 7 days).
- Group III: SNPs (50 mg/kg, i.p.) for 6 weeks.
- Group IV: SNPs (as in group III)+ Jaggery (400 mg/kg, p.o. for 7 days).
- Group V: SNPs (as in group III)+Jaggery (800 mg/kg, p.o. for 7 days).

(v) Processing Samples for Biochemical Analysis

Twenty four hours after the last treatments, blood was collected by puncturing the retro-orbital venous sinus (retro-orbital plexus) under mild anesthesia¹⁷. Blood was allowed to clot for 30 min at room temperature, and then the clot was gently detached from the wall of the test tube with the help of very thin sterilized needle. The test tubes were centrifuged for 20 minutes at 554×g to harvest serum, which was stored at -20 °C until analysis. For other assays, the tissues were homogenized with a Remi Motor homogenizer (RQ-122) using a glass tube and a Teflon pestle, in different media according to the variable tested. Liver tissue homogenates (10% w/v 0.15 M KCl) were prepared for lipid peroxidation. Homogenates (5% w/v) were prepared in 0.25 M sucrose solution for the estimation of reduced glutathione, and homogenates (5% w/v) were prepared in hypotonic solution (0.008% NaHCO₃) for enzymatic assay.

(vi) Blood Biochemical Assay

Various blood biochemical parameters were measured, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT)¹⁸, serum alkaline

phosphatase (SALP) (Kit No. 117677), lactate dehydrogenase (LDH) (Kit No. 117602), urea (Kit No. 117618), uric acid (Kit No. 117623), creatinine (Kit No. 103385) and albumin (Kit No. 1118275) were assessed by kit methods as per instructions provided by the company (E-Merck, Germany).

(vii) Tissue Biochemical Assay

Lipid peroxidation (LPO) was determined by measuring thiobarbituric acid reactive substances (TBARS) in liver, kidney and lung¹⁹. Reduced glutathione (GSH) level was determined by dithionitro benzoic acid (DTNB) in liver, kidney and lung²⁰. Superoxide dismutase (SOD)²¹, catalase²² and alkaline phosphatase (ALPase)²³ activities were also determined in liver, kidney and lung tissues. Glucose-6-phosphatase (G-6-Pase) in liver tissue was also determined²⁴.

(viii) Statistical Analysis

Results are presented as mean ± S.E.M. of six animals used in each group. Data were subjected to statistical analysis through one-way analysis of variance (ANOVA) taking significant at 5% level of probability followed by Student's t-test taking significant at $P \leq 0.05$ ²⁵.

RESULTS

1. Blood Biochemical Assay

Administration of SNPs induced significant increase in the enzymatic activities of ALT, AST, LDH and SALP ($P \leq 0.05$) as compared to the control group. Oral administration of Jaggery at two different doses (400 and 800 mg/kg, p.o.) showed significant recoupment in a dose dependent manner ($P \leq 0.05$) (Table 1). Significant elevation was observed in urea, uric acid, creatinine and decline in albumin after silica intoxication. Treatment with Jaggery at two different doses significantly reversed their level towards control. The 800 mg/kg dose of Jaggery revealed more significant therapeutic effectiveness ($P \leq 0.05$) as compare to 400 mg/kg dose (Table 1).

2. Tissue Biochemical Assay

Various biochemical parameters were performed in liver, kidney and lung tissues. A significant increase was observed in the level of LPO in liver, kidney and lung after SNPs intoxication, when compared with the control group ($P \leq 0.05$). Treatment with Jaggery at two different doses (400 and 800 mg/kg, p.o.) reversed the oxidative stress significantly towards control by inhibiting LPO in dose dependant manner ($P \leq 0.05$). Significant reduction ($P \leq 0.05$) was seen in GSH content in liver and kidney after SNPs intoxication. Post treatment of Jaggery was very effective in restoring the glutathione content which had been substantially decreased by SNPs (Table

2). Both the doses of Jaggery improved the GSH level in liver, kidney and lung tissues. However, therapy at 800 mg/kg was very effective. In our study, significant reduction was seen in SOD, CAT, ALPase and G-6-Pase level after SNPs administration ($P \leq 0.05$) (Tables 2-3). Analysis of variance showed significant recovery in the level of SOD, CAT, G-6-Pase and ALPase enzymes after administration of Jaggery at both the doses (400 and 800 mg/kg), but most effective restoration was observed at 800 mg/kg dose. No adverse effects were found in the tissue biochemical parameters after *per se* treatment of Jaggery.

Table 1
Effect of Jaggery on various blood biochemical variables against SNPs induced alterations

Treatments	AST (IU/L)	AST (IU/L)	LDH (IU/L)	SALP (IU/L)	Albumin (g/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Group I	66.0 ± 3.6	45.0 ± 2.4	44.0 ± 2.4	192 ± 10.6	3.7 ± 0.20	27.0 ± 1.4	1.7 ± 0.093	0.18 ± 0.009
Group II	66.8 ± 3.6	45.8 ± 2.5	42.8 ± 2.3	190 ± 10.5	3.6 ± 0.19	28.0 ± 1.5	1.8 ± 0.099	0.19 ± 0.010
Group III	107 ± 5.9 [#]	111 ± 6.1 [#]	95.0 ± 5.2 [#]	301 ± 16.6 [#]	2.5 ± 0.13 [#]	89.0 ± 4.9 [#]	3.2 ± 0.176 [#]	0.42 ± 0.023 [#]
Group IV	87.0 ± 4.8* (48.7%)	77.0 ± 4.2* (51.5%)	70.0 ± 3.8* (49.0%)	252 ± 13.9* (44.9%)	3.0 ± 0.16* (41.6%)	57.0 ± 3.1* (51.6%)	2.4 ± 0.132* (53.3%)	0.29 ± 0.016* (54.1%)
Group V	78.0 ± 4.3* (70.7%)	62.0 ± 3.4* (74.2%)	59.0 ± 3.2* (70.5%)	233 ± 12.8* (62.3%)	3.3 ± 0.18* (66.6%)	48.0 ± 2.6* (66.1%)	2.1 ± 0.116* (73.3%)	0.25 ± 0.013* (70.8%)
ANOVA	16.6 [@]	55.9 [@]	42.9 [@]	14.8 [@]	8.78 [@]	84.7 [@]	26.8 [@]	47.2 [@]
F- Value								

Data are mean ± S.E.; N = 6; ANOVA [@]= Significant, # SNPs vs Control; * SNPs + Therapy vs SNPs at $P \leq 0.05$. Abbreviations: SNPs= Silica nanoparticles; AST= Aspartate aminotransferase; ALT= Alanine aminotransferase; LDH= Lactate dehydrogenase; SALP= Serum alkaline phosphatase; %= Percent protection.

Table 2
Effect of Jaggery treatment on various tissue biochemical variables after SNPs induced alterations

Treatments	LPO (n mole of MDA/mg protein)			GSH (μ mole/g)			G-6-Pase (μ mole Pi/ min/g liver)
	Liver	Kidney	Lung	Liver	Kidney	Lung	Liver
Group I	0.31 ± 0.01	0.44 ± 0.02	0.48 ± 0.02	7.4 ± 0.40	7.3 ± 0.40	7.8 ± 0.43	6.10 ± 0.33
Group II	0.33 ± 0.01	0.46 ± 0.02	0.50 ± 0.02	7.3 ± 0.40	7.21 ± 0.40	7.7 ± 0.42	5.96 ± 0.32
Group III	0.59 ± 0.03 [#]	1.70 ± 0.09 [#]	0.90 ± 0.04 [#]	5.9 ± 0.32 [#]	5.7 ± 0.31 [#]	6.3 ± 0.34 [#]	3.80 ± 0.21 [#]
Group IV	0.46 ± 0.02* (46.4%)	1.10 ± 0.06* (47.6%)	0.69 ± 0.03* (50.0%)	6.7 ± 0.37* (53.3%)	6.5 ± 0.35* (50.0%)	7.0 ± 0.38* (46.6%)	5.07 ± 0.28* (55.2%)
Group V	0.41 ± 0.02* (64.2%)	0.90 ± 0.04* (63.4%)	0.61 ± 0.03* (69.0%)	6.9 ± 0.38* (66.6%)	6.9 ± 0.38* (75.0%)	7.3 ± 0.40* (66.6%)	5.29 ± 0.29* (64.7%)
ANOVA	26.7 [@]	100 [@]	26.6 [@]	2.98 [@]	3.77 [@]	2.74 [@]	11.71 [@]
F- Value							

Data are mean ± S.E.; N = 6; ANOVA [@]= Significant, # SNPs vs Control; * SNPs + Therapy vs SNPs at $P \leq 0.05$. Abbreviations: SNPs= Silica nanoparticles; LPO = Lipid per oxidation; GSH = Reduced Glutathione; G-6-Pase= Glucose-6-phosphatase; % = Percent protection.

Table 3
Effect of Jaggery treatment on various tissue biochemical variables after SNPs induced alterations

Treatments	CAT ($\mu\text{mole of H}_2\text{O}_2/\text{min}/\text{mg protein}$)			SOD ($\mu\text{mole}/\text{min}/\text{mg protein}$)			ALPase ($\text{mg Pi}/100\text{g}/\text{min}$)		
	Liver	Kidney	Lung	Liver	Kidney	Lung	Liver	Kidney	Lung
Group I	66.0 \pm 3.6	78.0 \pm 4.3	72.0 \pm 3.9	56.0 \pm 3.0	60.0 \pm 3.3	58.0 \pm 3.2	72.0 \pm 3.9	2078 \pm 114	410 \pm 22.6
Group II	64.6 \pm 3.5	77.1 \pm 4.2	70.7 \pm 3.9	55.0 \pm 3.0	59.3 \pm 3.2	57.0 \pm 3.1	71.1 \pm 3.9	2076 \pm 114	408 \pm 22.5
Group III	41.0 \pm 2.2 [#]	40.0 \pm 2.2 [#]	41.0 \pm 2.2 [#]	32.0 \pm 1.7 [#]	30.0 \pm 1.6 [#]	39.0 \pm 2.1 [#]	51.0 \pm 2.8 [#]	1410 \pm 77.9 [#]	205 \pm 11.3 [#]
Group IV	53.0 \pm 2.9* (48.0%)	59.0 \pm 3.2* (50.0%)	56.0 \pm 3.0* (48.3%)	44.0 \pm 2.4* (50.0%)	44.0 \pm 2.4* (46.6%)	48.0 \pm 2.6* (47.3%)	60.0 \pm 3.3* (42.8%)	1760 \pm 97.2* (52.3%)	320 \pm 17.6* (56.0%)
Group V	58.0 \pm 3.2* (68.0%)	67.0 \pm 3.7* (71.0%)	62.0 \pm 3.4* (67.7%)	49.0 \pm 2.7* (70.8%)	50.0 \pm 2.7* (66.6%)	52.0 \pm 2.8* (68.4%)	65.0 \pm 3.5* (66.6%)	1920 \pm 106* (76.3%)	366 \pm 20.2* (78.5%)
ANOVA	12.3 [@]	22.2 [@]	16.6 [@]	16.3 [@]	24.1 [@]	8.91 [@]	7.13 [@]	8.73 [@]	23.0 [@]
F- Value									

Data are mean \pm S.E.; N = 6; ANOVA @= Significant, # SNPs vs Control; * SNPs + Therapy vs SNPs at P \leq 0.05.

Abbreviations: SNPs= Silica nanoparticles; CAT= Catalase; SOD= Superoxide dismutase; ALPase= Alkaline phosphatase; % = Percent protection.

DISCUSSION

With the rapid development and extensive application of silica nanoparticles in biomedical and biotechnological fields, the toxicity of SNPs attracts more attention. Studies demonstrated that SNPs induced toxicological effects mainly on lung, liver, spleen and kidney tissues²⁶. ALT, AST, ALP and LDH are well known diagnostic indicators of hepatic injury. Increased levels of these enzymes in serum of SiO₂ nanoparticles treated animals (6 weeks exposure) have been attributed to hepatic structural damage because these enzymes are normally localized to the cytoplasm and are released into the circulation after cellular damage has occurred²⁷. Our result demonstrated that Jaggery (400 and 800 mg/kg) showed significant recoument in these enzymes activity thus revealing that Jaggery preserved the structural integrity of cells from toxic effect of SNPs like as other extracts such as *Warburgia salutaris*²⁸, *Syzygium cumini*²⁹ and Propolis³⁰ which ultimately maintained the integrity of plasma membrane and checked the leakage of transaminases (AST and ALT), LDH and SALP. We have observed the elevated level of serum urea, uric acid and creatinine and declined level of albumin which indicated kidney and liver damage. It may be due to dysfunctional and dystrophic changes in the liver and kidney. Due to severe renal impairment, urea, uric acid and creatinine excretion falls and the serum concentration rise rapidly, which is in agreement with other epidemiological as well

as experimental studies³¹. The decreases in the serum albumin values may be due to liver injury including degenerative and necrotic changes as previously reported³². In our experiment Jaggery helped to cope with alterations in cellular structure of kidney and thus, effective in renal protection, thereby improved functional status of kidney³³.

The generation of intracellular reactive oxygen species (ROS) caused oxidative damage followed by the production of lipid peroxidation and the inhibition of antioxidant activities. It was reported that SNPs showed stable surface radicals and sustained release of ROS triggers extensive cellular damage. ROS generated by the SNPs surface can induce cell membrane damage via lipid peroxidation that may subsequently lead to increased cellular permeability³⁴. The depletion of antioxidant defences and/or rise in free radical production deteriorates the prooxidant balance, leading to oxidative stress-induced cell death. Thiobarbituric acid reactive substances (TBARS) were one of the main LPO products; its elevated levels could reflect the degree of LPO injury in liver, kidney and lung tissues³⁵. The increase in TBARS level in SNPs treated rats indicates enhanced peroxidation leading to a failure of the antioxidant defence mechanism to prevent formation of excess free radicals³⁶. Treatment with Jaggery prevented significantly LPO either directly or through GSH by scavenging the free radicals. These results indicate the potential electron

donating ability of Jaggery. These findings are in support with results of earlier investigations³³. Damage to the cellular membrane from LPO also leads to decreases in the activity of enzymes bound to the endoplasmic reticulum enzyme G-6-Pase. Our study has shown that subchronic exposure to SNPs in rats significantly decreased G-6-pase activity in liver, which might be due to the membrane's fragility and the permeability of the organs. Therapy with Jaggery at both the doses significantly restored the metabolic enzyme activities, which indicates improved physiological functions in liver tissue. Glutathione is one of most abundant tripeptide antioxidants which protect biomolecules from free radical-mediated damage. Decreased GSH levels in SNPs administered rats might be due to either reduced GSH synthesis or increased utilization of glutathione in the detoxification process against SNPs toxicity. Treatment with Jaggery (400 and 800 mg/kg) restored the GSH levels in liver, kidney and lung. Jaggery at two different doses reversed SNPs induced chain of events possibly via better maintaining the redox equilibrium within the body¹² and may be due to an initial reduction in tissues peroxidative activities, thereby leading to restoration of GSH content. It is known that SOD and CAT constitute a mutually supportive team of antioxidant enzymes, which provides a defence system against ROS. In this study the activity of SOD and CAT decreased significantly in SNPs treated rats. The decline in these enzymes levels can be explained by the fact that excessive superoxide anions may inactivate SOD. Hence, inactivation of the H₂O₂ scavenging enzyme CAT³⁰. Treatment with Jaggery effectively prevented the decrease in SOD and CAT activities because Jaggery might be well absorbed in liver, kidney and lung and could suppress SNPs induced ROS and maintained cellular antioxidants pool²⁹.

It is widely accepted that SiO₂-induced cytotoxicity is due to the disruption of phagolysosomal membrane integrity. After phagocytosis of SiO₂, reactive particle surfaces may interact with phagolysosomal membranes leading to the release of

lysosomal enzymes into the cytosol and cell death³⁷. Lysosomes contribute to caspase activation and apoptosis in an in vitro model of silica-induced apoptosis. SNPs were shown to induce lysosomal permeability and apoptosis in a response that required an acidic lysosomal environment, lysosomal cathepsin D activity and lysosomal acidic sphingomyelinase activity³⁸. This might be the possible cause of reduction of ALPase in liver, kidney and lung after silica exposure. Treatment with Jaggery might help in improvement of membrane integrity and thus, maintained lysosomal enzymes. The silanol (SiOH) group present on the surface of silica particle forms H-bond with oxygen and nitrogen group found in the biological cell membranes which may subsequently lead to oxidative stress, chronic inflammation, loss of membrane structure, lysosomal leakage and cellular damage. Thus present data suggest that exposure of SNPs leads to cellular morphological modifications and oxidative stress as indicated by elevation of intracellular ROS and TBARS and depletion of GSH. The therapeutic protective potential of Jaggery can be attributed to the presence of -OH and -H groups of its polyphenolic components. It is analyzed that these groups are involved in loss of lipid peroxy radicals in the cellular membrane and it may directly combine with free radicals and hinder the formation of these radicals. It may help to maintain the redox equilibrium within the body to minimize the molecular and cellular oxidative damage caused by SNPs. An important ingredient of Jaggery is vitamin C; a non-enzymatic antioxidant can mobilize and up regulate antioxidant capacity of cell to neutralize excessive free radicals¹¹. Our study verifies for the first time, the therapeutic effects of Jaggery against SNPs exposure as evidenced by blood and tissue biochemical parameters and histological studies. Thus, looking in to a variety of biological properties of Jaggery, this study advocates the protective value of Jaggery against SNPs induced toxicity. To the best of our knowledge detailed scientific investigation on Jaggery as counter measure to SNPs induced toxicity has not been done yet.

CONCLUSION

It can be concluded that the aqueous extract of Jaggery possesses protective activity against silica nanoparticles intoxication in hepatic renal and pulmonary tissues. This may be due to the stabilizing effect on the membrane of cells by the antioxidant effect of the Jaggery, which consequently helps to maintain the structural and functional integrity of the cells.

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CONFLICT OF INTEREST

The authors declare that they share no conflict of interest regarding this study and manuscript.

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