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Protective Effects of Secoisolariciresinol Diglucoside on Arsenic-induced Renal Damage and Oxidative Stress in Rats

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Abstract

The naturally occurring metalArsenic (AS) poses a hazard to human health as itcan generate oxygen free radicals and oxidative stress.The build-up of free radicals may cause chronic renalinsufficiency and abrupt renal failure. The significant lignan in flaxseed, secoisolariciresinol diglucoside (SDG), provides various health benefits. The current research investigates the preventive benefits of SDG against Arsenic trioxide (As_2O_3)-induced kidney damage.

Four groups of healthy Wistar rats were equally distributed and received daily injections for 5 days as follows: Group1 got injections (IP) of saline as a control; Group2 got subcutaneous (SC) injections of SDG at 10mg/kg/day; Group-3 got intraperitoneal (IP) injections of Arsenic trioxide (As_2O_3) at 20 mg/kg/day, and Group-4 got 20 mg/kg/day As_2O_3 IP followed by 10 mg/kg/day SDG subcutaneously one hour later. The impact of As_2O_3 on the kidney was measured using a variety of indicators including the Greatest Distributable, ROS levels in renal tissues, and malondialdehyde (MDA), as well as two renal function markers the blood urea nitrogen (BUN) and serum creatinine (CREA). Besides the histological examinations, the antioxidant molecule glutathione was evaluated, and so were the functions of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px).

Arsenic trioxide (As_2O_3) enhanced MDA generation, oxygen free radicals, and As_2O_3levels in kidney tissue while decreasing SODand GSH-Px activity, and the ratio of reduced glutathione to oxidized glutathione. Moreover, serum As_2O_3 elevated BUN and serum CREA activity. As_2O_3 induced kidney damage, according to histopathological alterations. Interestingly, SDG treatment reduced ROS generation in serum and kidney and restored antioxidant enzyme levels. In addition, the SDG-treated rats showed significant improvement in all nephrotoxic features.

According to the results of this investigation, SDG exhibited an exceptional restorative impact on Arsenic trioxide-mediated kidney cytotoxicity.

Keywords: Secoisolariciresinol diglucoside, Renal toxicity, Antioxidant enzyme, Heavy metal

1. Introduction

Humans and animals are exposed to environmental hazardous metals and compounds like mercury (Hg), lead (Pb), cadmium (Cd), uranium (U), and arsenic (As), which affect the structure and function of several organs. Natural sources of As include air, soil, and water, but it can also be created artificially. There are different types of As which may or may not contain carbon (organic and inorganic, respectively)(Hilal and Ismail, 2008; Sall *et al.*, 2020). The most frequent inorganic types of As that enter the human body from contaminated drinking water are trivalent arsenite (AsIII) and pentavalent arsenate (AsV)(Health and Services, 1999; Tchounwou *et al.*, 2019). When drinking As-contaminated water regularly, poisoning develops in all body regions. The kidneys, which are essential for As biotransformation and exclusion, are the most often targeted organ for As deposit. Excessive and continuous exposure to As increases the incidence of kidney cancer in animals (Sotomayor *et al.*, 2020).

Epithelial cells of the kidney have very high reabsorptive power. Because of their anatomical position, they are the prime target for filtered toxicants and hence are highly receptive to As toxicity(Chang and Singh, 2019). Studies carried out on rats treated with arsenate showed an enlargement of mitochondria and reduced respiratory functions(Zhao *et al.*, 2018). The multifactorial mechanisms in metal-induced toxicity involve the metal-induced production of ROS (Hu *et al.*, 2020).

Arsenic metal is a well-studied pro-oxidant. Metabolic abnormalities caused by oxidative stress include amino

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acid synthesis, methionine cycle (transmethylation), membrane phospholipid degradation, and purine metabolism, contributing to cell toxicity and death. Reduced tricarboxylic acid cycle (TCA cycle), disrupted carbohydrate metabolism, oxidative pentose pathway, and the hexose monophosphate shunt (HMP-shunt) pathway alteration or gluconeogenesis may be responsible for damaging the Brush Border Membrane (BBM) as well as the mitochondria inkidney's proximal tubules(Wang *et al.*, 2017; Al-Groom, 2022). Several researchers have shown that chelating compounds may be utilized to minimize As-induced toxicity(Flora *et al.*, 2007; Bjørklund *et al.*, 2020; Nurchi *et al.*, 2020). However, The role of different antioxidants against As poisoning has been studied widely. These studies have established the fact that the dispensation of antioxidants contributes to the prevention of symptoms of As poisoning and diminution of the absorption of As in tissues (Bjørklund *et al.*, 2020).

As the best plant source of omega-3 fatty acid ALA, flaxseed (Linum usitatissimum) has been well known. It also contains minerals such as manganese, which is an essential mineral for bone and blood health. Flaxseed contains several lignans, among whichSDGis the major one (Al-Mamoori *et al.*, 2019; Ebrahimi *et al.*, 2021; Al-Mamoori and Aburjai, 2022). In vivo and in vitro studies revealed the antioxidant activity of the SDG in flaxseed (Kezimana *et al.*, 2018). Flaxseed therapy has been shown to slow the progression of atherosclerosis because of its lignan concentration and the antioxidant impact of SDG (Parikh and Pierce, 2019; Bujok *et al.*, 2021; AlRamadneh *et al.*, 2022).When rats with streptozotocin-induced diabetes were given SDG, there was a considerable reduction in glucose and lipid concentrationsand an improvement in insulin levelswhichreduces the chance of developing diabetes (Prasad *et al.*, 2000; Draganescu *et al.*, 2021). SDG is effective against inflammation, lowering reactive oxygen species levels and decreasing apoptosis. Furthermore, SDG has been shown to defend against heavy metal-induced kidney impairment (Aqeel *et al.*, 2019; Aqeel *et al.*, 2020; Zhang *et al.*, 2020; Aqeel *et al.*, 2021). Nevertheless, no studies have been done to test whether SDG can prevent As -induced nephrotoxicity. As a result, the current research looked at the antioxidant status, several biochemical indicators, and histological alterations in rats with As-induced nephrotoxicity to determine whether flaxseed lignan-SDG might protect them.

2. Materials and methods

2.1. Chemicals

Chemicals and reagents utilized in this investigation were purchased from Sigma-Aldrich (Burlington, MA, USA), except the following: Sodium arsenite from Indiamart (Bengaluru, India), Reference SDG from Teco Diagnostics (Anaheim, CA 92807, USA), and Blood urea nitrogen (BUN) and creatinine (CREA) from StressMarq Biosciences (Cadboro Bay Village, Canada).

2.2. Isolation of SDG from flaxseed

As already mentioned, SDG was extracted and refined using HPLC(Aqeel *et al.*, 2019). Lignans were extracted from purified defatted flaxseed flour (200gm) using 50ml of methanol. The mixed components were incubated at 65°C for 4 hours in a circulating water bath, then centrifuged at 3000 rpm for 30 minutes to separate the sediment. The supernatant was acidified with 2M H_2SO_4 at pH 3.0 before being submitted to HPLC analysis using a Water Alliance e26925 model (Waters Corporation, USA) with an RP-C18 column (Sun fire) with a particle size of 5m, photodiode array detectors (Waters model 2996), and chromatograms recorded at 280 nm. Five percent (v/v) acetonitrile in 0.01 M phosphate buffer at pH2.8 (solvent A), and acetonitrile were used in the mobile phase (solvent B). All linear-gradient profiles were performed for 32 minutes at 1ml/min, and the temperature of the column was reset to 25°C. Running 0.5mg/ml of standard SDG produced a linear curve.

2.3. Experimental design

Animal experiments followed the guidelines approved by the institution (Approval No. In vivo/IAEC/012/2020). The rodents utilized were 24 male Wistar albino rats weighing 150 - 200 grammes. They were kept in a controlled environment with a temperature of 24^o C, 12-hour light-dark cycles, and a humidity of 40 %. A regular pellet diet was provided to the rats (Hindustan Lever, Bangalore, India). Following a week of acclimation, the rats were separated into 4 groups: Group 1 (Control) received daily IP injections of 0.5 ml saline (0.9 % NaCl); Group 2 got SC injections of 10 mg/kg/day SDG in 0.5 ml saline, and Group 3 got IP injections of 20 mg/kg/dayAs_2O_3 in 0.5 ml saline. Since our objective was to study the acute effects of As exposure, sodium arsenate was injected in 4 doses, over a short period of time We used a smaller dose of sodium arsenate in our study as compared to the standard value of sodium arsenate LD50 (FRANKE and Moxon, 1936), the amount utilized in prior studies (Hood *et al.*, 1987; Hood *et al.*, 1988).

Animals in Group 4 were given As_2O_3 + SDG.The rats in this group were given As_2O_3 IP injections of 20 mg/kg/day in 0.5 ml saline and SC injections of 10 mg/kg/day SDG in 0.5 ml saline 1 hour later. All animals in each group received their daily medications at a similar time. During the trial, hazardous signs, body mass, and feed consumption were followed. All animals were euthanized 24 hours after completing the experimental phase on the 5th day. Blood and urine samples were collected. The kidneys were extracted and homogenized in 15% (w/v) 0.1 M Tris–HCl, pH 7.5 using a Potter–Elvehjem homogenizer(Remi Motors, Mumbai, India). To eliminate debris from the cells, the homogenized tissues were spunfor 15 min at 3000 rpm at 5°C. The supernatantswere aliquoted and kept at -20 °Cfor later use.

2.4. Serum analysis

Jugular vein blood was collected using vacuum blood collection tubescontaining the anticoagulant heparin sodium. Blood urea nitrogen (BUN) and creatinine (CREA) were measured by kits from StressMarq Biosciences after the samples were spun at 3,000 rpm for 10 minutes.

2.5. Biochemical examination

Renal tissue homogenates were used to analyze the levels of GSH, and GSSH(Puri and Meister, 1983) along with the antioxidant enzymes activity of CAT, SOD, and GSH-Pxbased on methods described bySani,Radák, and

Avissar, respectively(Avissar *et al.*, 1994; Radák *et al.*, 1996; Sani *et al.*, 2006).

2.6. ROS & MDA level measurement in kidney tissue

The procedure of Weiss et al. was used to create a single-cell solution of 100gm kidney tissue (Weiss *et al.*, 2008).The amount of ROS produced inside the cells was measured using the oxidation-sensitive fluorometric probe dichloro-dihydro-fluorescein diacetate (DCFH-DA)as described previously (Aranda *et al.*, 2013).

Deng et al. approach was used to quantify MDA in kidney tissue (Deng et al., 2012). 0.1 ml liver homogenate, 0.1 mol/L phosphate buffer, and 0.1 mol/L FECl2. The samples were boiled for 20 minutes with 1.0 ml of 10% TCA and 0.67 %TBA. Finally, the samples were chilled on ice, centrifuged at 3,000 rpm, and the amount of MDA generated in the supernatants was measured spectroscopically at 535 nm and reported as nmol/mg of protein.

2.7. Urine analysis to determine As species

Urine samples from rats were obtained (from 8 a.m. to 8 a.m.) and kept at -20°C before being analyzed. After spinning the urine samples for 30 minutes at 12,000 rpm, the supernatants were passed through 0.5-m PTFE unlaminated membrane filters. Analysis of urine was conducted with High-Performance Liquid Chromatography–hydride generation–atomic fluorescence spectrometry, as previously described, to determine total As, arsenate (V), arsenite (III), and monomethylated metabolites (MMA and DMA, respectively) (Yu et al., 2013). There was a concentration difference between AsandAs -related compounds in urine samples.

2.8. Estimationof the level of Asin kidneys

A total of 0.5g of kidney tissue was digested for 2 days at 130^{o}C with HNO_3–$HClO_4$ solution. The samples were diluted with de-ionized water after the HNO_3 had evaporated. The level of

Asin kidney samples was determined using an Atomic Fluorescence Spectrometer (Perkin-Elmer 3100) and expressed as ng/g(Cui *et al.*, 2004).

2.9. Histopathological analysis

For histopathological studies, left kidneys were removed from rats of all groups and were incubated in 10% formalin solution for 30h at room temperature. 5-6µm thick sections of the kidneys were prepared, fixed, and paraffin-embedded.The sections were then stained with Hematoxylin and Eosin (H&E) and were examined usingBX-FM; ZEISS microscopes (Jena, Germany).

2.10. Statistical analysis

The statistics were presented as mean ± standard error mean (SEM). A one-way ANOVA was used to determine the statistical differences among the subjects. Statistical significance was calculated using Tukey's post hoc test ($p<0.05$). The statistical program used for analysis was GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. High-Performance Liquid Chromatography analysis of SDG

High-Performance liquid chromatogram indicated that SDG was the dominant lignan (2.0 mg/g)of flaxseed extract with the highest absorption at 280nm (Figure 1). SDG had a 34.714-retention time.

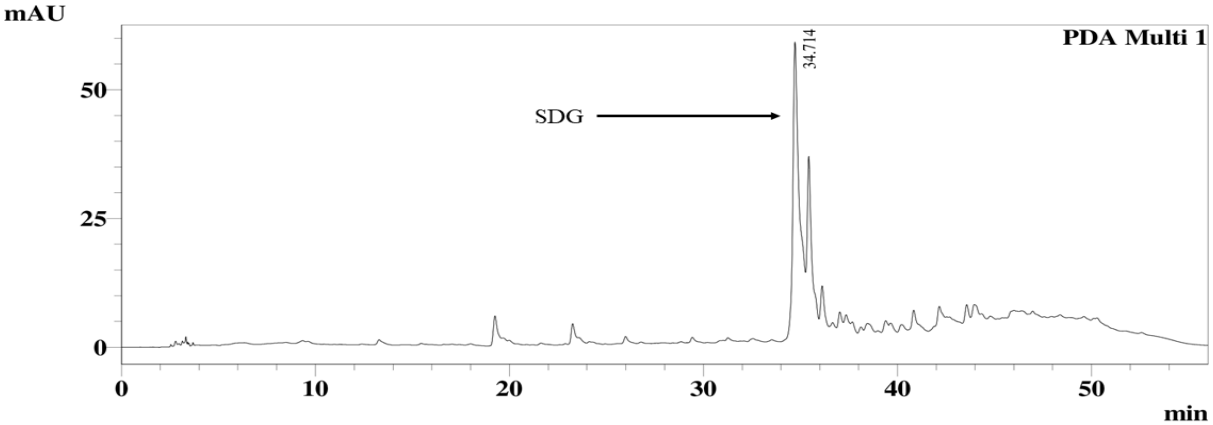

Figure 1. High-Performance Liquid Chromatography chromatograms of compounds in Flaxseed extract with SDG (Rt 34.714).

3.2. SDG attenuatedAs2O3-induced renal damage

The two most essential indications for determining renal impairment are BUN and CREA. As a result, serum BUN and CREA concentrations were measured to see whether SDG might reduce As_2O_3-induced kidney tissue damage. The levels of BUN and CREA in the As_2O_3 treated animals were substantially higher compared to the controls ($P<0.05$). The values of BUN and CREA were not affected in animals receiving SDG alone. In the group that took As_2O_3followed by SDG, however, BUN and CREA levels were lowered to near-normal levels (Figure 2).

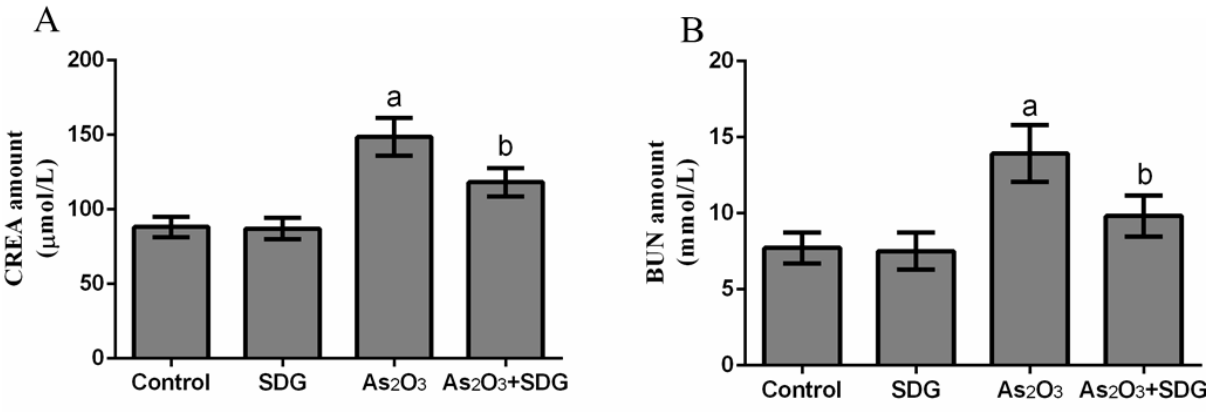

Figure 2. Impacts of SDG on the level of blood serum creatinine (CRET) and level of blood urea nitrogen (BUN) of rats that received As trioxides (As_2O_3), a considerable change at p<0.05 when compared to the control group. b Significant change at P<0.05 from the As_2O_3group.

3.3. SDGrestored the production of antioxidant enzymes in kidneys of rats withAs2O3-induced toxicity

The batch of rats given As_2O_3 showed a considerable drop (P<0.05) in CAT, SOD, and GSH-Px levels, whereas the saline-treated group showed no such changes (Table1). As_2O_3 considerably reduced the activity of SOD and GSH-Px (24.18 4.53U per mg protein and 93.82 6.29 mol/min/mg protein, respectively) in the As_2O_3-treated group relative to the control group (52.775.18U per mg protein and 139.159.42mol/min/mg of protein, respectively). Furthermore, compared to the control group (233.1713.39 mol/min/mg of protein), the activity of CAT was significantly lowered (151.1712.77mol/min/mg protein). However, in the As_2O_3-SDGtreated group, concentrations of the measured enzymes were considerably higher (P<0.05).

Table1. Impact of SDG on antioxidant enzymes in As_2O_3-treated kidney homogenate

Parameters*	Group A	Group B	Group C	Group D
SOD (µmol/min /mg protein)	52.77±5.18	55.14±6.03	24.18±4.53[a]	40.33±5.13[b]
GSH-PX (µmol/min /mg protein)	233.17±13.34	227.55±15.77	151.17±12.77[a]	212.37±17.18[b]
CAT(µmol/min /mg protein)	139.15±9.61	142.88±8.93	93.82±6.29[a]	125.14±12.22[b]

Values are mean ± SEM. [a] Significant change at p<0.05 from group A. [b]Significant change at P<0.05 from group C. (Group A: Control group; Group B: SDG; Group C: As_2O_3Group; Group D: As_2O_3 + SDG).

3.4. SDG reducedoxidative stress induced by As2O3 in kidneys

Kidney tissues exposed to As_2O_3 showed significantly higher levels of ROS and MDA than the controls (P<0.05) (Figure 3). Yet, following the exposure to As_2O_3, treatment of renal tissues with SDG dramatically lowered ROS and MDA to levels comparable to those of the control group (Figure 3).

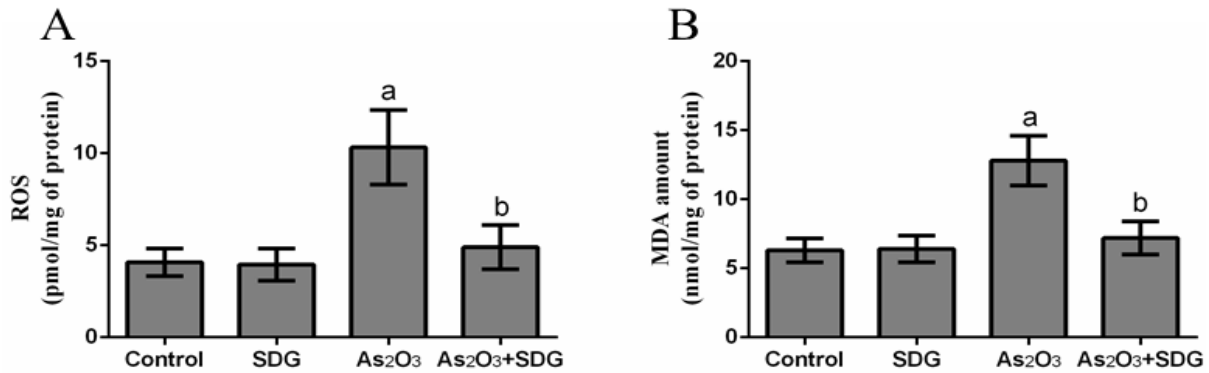

Figure 3. Impacts of SDG on MDA and ROS level in rats kidneys that received Astrioxides (As_2O_3), [a] Considerable change at p<0.05 when compared to the control group. [b] Significant change at P<0.05 from the As_2O_3 group.

3.5. SDG increasedGSH and GSH/GSSG ratioin kidney tissues

In addition to ROS and electrophiles, GSH protects condensed protein situations against oxidative stress. Thiol GSH plays a dominant role in the human antioxidant defence system. Thus, the GSH concentration and GSH/GSSG ratio in rat kidneys were determined. As per the study's findings, As_2O_3 treatment lowered GSH levels and GSH/GSSG ratios. On the other hand, SDG therapy raised GSH levels and the GSH/GSSG ratio in As_2O_3-treated rats, (Figures4 a &4b).

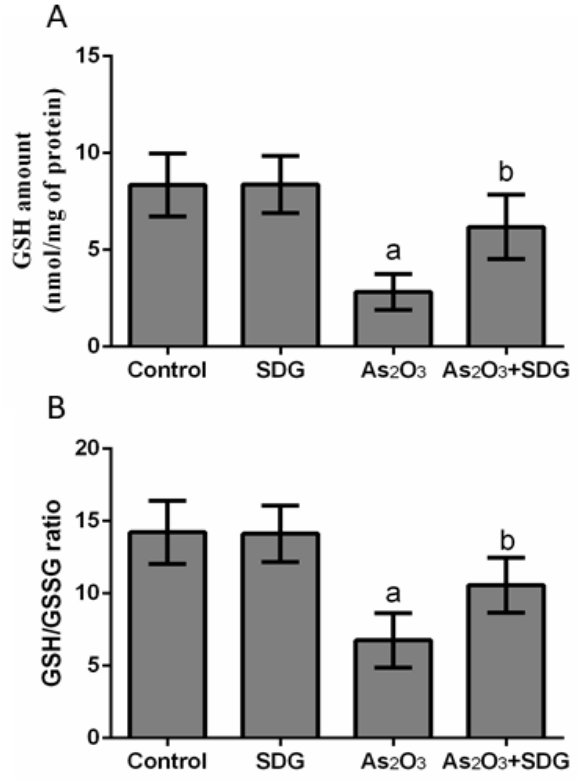

Figure 4. Effect of SDG on GSH level and the ratios of GSH/GSSG levels in rats kidneys that received As trioxides (As_2O_3), [a] Considerable change at p<0.05 compared to the control group. [b] Considerable change at P<0.05 from the As_2O_3group.

3.6. SDGreduced the concentration of Asin the kidneys

The kidneys' ability to retain As was examined to see whether SDG offered any protection against As_2O_3-induced renal damage. For the As_2O_3 group, the As levels in the kidney were significantly greater than those in the controls (Figure 5). Significantly lower As concentration in the kidney (P<0.05) was achieved after injection of As_2O_3 and treatment with SDG (Figure. 5).

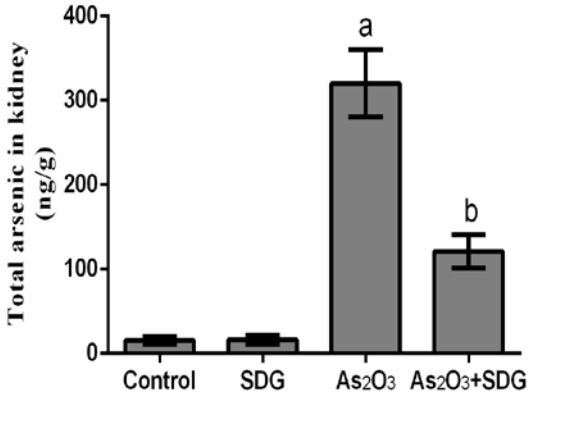

Figure 5. Effect of SDG on the total As accumulation in rat kidneys that received As trioxides (As_2O_3), [a] Significant change at p<0.05 compared to the control group. [b] Significant change at P<0.05 from the As_2O_3 group

3.7. SDG enhancedAsexcretion in the urine

We looked at the amounts of As excretion in the urine of rats to see whether SDG had any effect. The As levels in the urine of the As_2O_3 + SDG-treated rats rose substantially more than in the As_2O_3 alone-treated individuals (Table 2). Rats administered As_2O_3 + SDG had significantly lower urinary iAs(III) concentrations than rats given As_2O_3 alone (P<0.05).

Table2. Effects of SDG on the urine sample of rats received As_2O_3

Parameters*	Group A	Group B	Group C	Group D
Urine total arsenic (µg/L)	5.19±1.19	6.22±1.58	30.22±6.37[a]	62.75±12.89[b]
Permillage of iAs(III) in total arsenic (%)	7.63±2.48	7.97±2.77	48.47±9.79[a]	57.94±12.81[b]
Permillage of as(V) in total arsenic (%)	38.14±9.61	35.46±6.48	5.94±1.76[a]	14.33±4.29[b]
Permillage of MMA in total arsenic (%)	3.48±1.08	2.91±0.93	18.83±3.19[a]	29.91±4.67[b]
Permillage of DMA in total arsenic (%)	10.43±2.09	9.86±2.93	55.84±8.81[a]	82.73±13.60[b]

Values are mean ± SEM. [a] Significant change at p<0.05 from group A. [b]Significant change at P<0.05 from group C. (Group A: Control group; Group B: SDG; Group C: As2O3Group; Group D: As_2O_3 + SDG).

3.8. Histopathological observation

The magnitude of kidney injury caused by As was assessed through histopathological examination. SDG-treated kidney tubules appeared normal in all control groups (Fig. 6a) and the SDG-treated group (Fig. 6b). In the group that did not receive SDG, As_2O_3-induced changes in the kidney tissues' histopathology including epithelial damage, modified tubular cell organization, necrosis and interstitial fibrosis, infiltration of inflammatory cells, and hyperemia of the glomerular capillaries, dilation, as well as a decline and desertion of the Bowman's capsule (Figure.6c). Figure 6d shows that As_2O_3-induced necrosis, interstitial fibrosis, and epithelial degeneration were significantly reduced by SDG therapy.

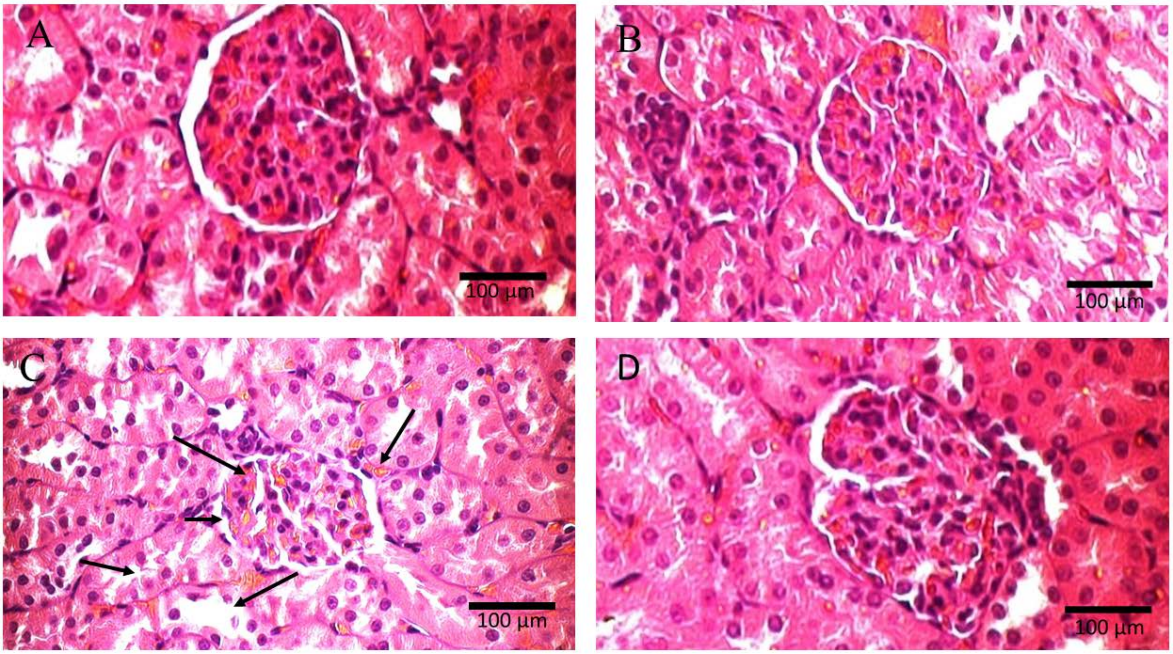

Figure 6.After five days of treatment with As trioxides (As_2O_3) and SDG, histology of rat kidneys. (a) Control group with normal morphology glomeruli and tubules. (b) Normal tubules and glomeruli in the SDG group. (c) As_2O_3treated rats' kidneys showing atrophied glomeruli with widened urinary space and de- generated tubules with a disturbed contour in the proximal tubule and distal tubule with some vacuoles inside it plus interstitial blood accumu- lationwith tubular necrosis and glomerular hyperemia. (d) Group As_2O_3 + SDG. In comparison to the control group, renal tubules and glomeruli histology were almost normal.

4. Discussion

Arsenic (As), an ecological and environmental toxin, is associated with an elevated chance of heart disorders and tumoursin humans. As compounds have adverse effects on many organs, including the kidneys(Rana *et al.*, 2018; Guo *et al.*, 2020). Due to its propensity to alter ROS configuration, As has a toxic impact on the human body(Guo *et al.*, 2020; Zaid Alkilani *et al.*, 2022). Various elements such as chelating agents were examined for their defensive impending in the toxicity induced by As. However, clinical applications have not been proved safe yet (Bjørklund *et al.*, 2020). The utilization of antioxidant dietary substances, which are naturally occurring to counteract the toxic effects of differentenvironmental agents and toxicants, together with metalloids/ metals, has been gaining importance lately (Pace *et al.*, 2017). SDG enriched with ALA, and phytoestrogenic lignans provide nutritious sources of bioactive components that are co-therapeutic and co-preventative in several disorders(Rizwan *et al.*, 2014). Ameliorate treatment comprises dietary SDG consumption against toxicity induced by lead acetate was investigated (Aqeel *et al.*, 2021). But an in-depth study of the effectiveness of SDGagainst As_2O_3 nephrotoxicity has not been reported yet.The current study suggests that lipid peroxidation, ROS, and oxidative stress add to the nephrotoxicity caused by As_2O_3. Prior treatment with SDG would help decrease the oxidative stress induced by As_2O_3 and reduce its damaging effect on kidneys. According to our results, SDG lowered renal As retention and elevated the concentration of distinct species of As and the quantity of methylated As excreted in the urine, which might explain its nephroprotective benefits in rats poisoned with As. Similar As properties related to environmental contact with arsenicals including renal injury,GSH-Px, CAT, SOD activities, GSH concentration, and the GSH/GSSG ratio were all reduced by As_2O_3 , which caused renal tubule and glomerulus damage. This is in accordance with previous studies where long exposure to As damaged endothelial cell membrane lipids and proteins and nucleic acids, resulting in kidney tubule and glomerular dysfunction (Rizwan *et al.*, 2014).

Arsenic is mainly eliminated by the kidney. Consequently, renal tissues are the chief targets for toxicity due to the accumulation of As.As a result of the nephrotoxic effects of As, it was concluded that oxidative stress played a significant part in As -induced kidney injury (Emadi and Gore, 2010). It is during the cytoplasmic and mitochondrial metabolism of As that the free radicals of hydrogen peroxide (HO), superoxide anion (O_2-), nitric oxide, hydroxyl radicals, and dimethylarginine oxidation are made (Shi *et al.*, 2004; Robles-Osorio *et al.*, 2015). An additional effect of arsenicals is their ability to slow down the thioredoxin reductase enzyme as well as the antioxidant glutathione synthase (Miodragović *et al.*, 2019).

The formation of free radicals overwhelms the body's antioxidant defense system (comprising antioxidants and antioxidant enzymes), and interrupts the antioxidant or pro-oxidative balance in kidney tissue, leading to OS-induced kidney damage, as evidenced by the increase in ROS and GSH reduction, antioxidant enzyme activity and enhancement of lipid peroxidation in renal tissue. It is possible that cellular energy metabolism is inhibited by As (Fu and Xi, 2020).

Arsenate is a phosphate analoguethat can restore phosphate in cellular respiration and glycolytic pathways(Nurchi *et al.*, 2020). Research states that arsenite can interfere with the citric acid cycle and cellular ATP

production by reacting with proteins that include sulfuryl groups. This results in decreased intake of glucose, fatty acid oxidation, glucose production, and the movement of pyruvate dehydrogenase, which results in a decreased ability to generate ATP from citric acid (Bergquist *et al.*, 2009). Chromatin swapping, chromosomal abnormalities, and DNA hypo- and hypermethylation are all consequences of As exposure(Navasumrit *et al.*, 2019). The glomerular filtration membrane is damaged as demonstrated by renal histological abnormalities and higher than usual levels of BUN and CREA due to mitochondrial depolarization, ATP reduction, and angiogenic endothelium mortality. Antioxidant enzyme activity, GSH levels, and GSH/GSSG ratio were significantly restored following dual treatment with SDG and As.

As shown by histological studies, the repair of structural alterations in the renal tissues gave further proof of SDG protective action. Nephron-protecting properties may be attributed to SDG capabilityto inhibit the activity of radical's species and enhance the antioxidant defence system. Antioxidants, such as coenzyme Q and SDG, remove free radicals and reduce the complicated oxidative chain complex that produces reactive oxygen species. Previous studies have shown that O_2^- radicals produced in the mitochondria stimulate many antioxidant enzymes, including SOD, glutathione reductase, and CAT (Karapehlivan *et al.*, 2014). In a previous study, SDG suppressed NF-kappaB, JNK, c-Jun/AP-1, caspase activation, and lipid peroxidation, which resulted in apoptosis (Kutuk *et al.*, 2006). SDGs have also been linked to reduced inflammation, increased cellular respiration, and elevated mitochondrial biogenesis (Tennen *et al.*, 2012).The SDG was found to improve renal microcirculation and guard the tubular epithelium. The antioxidant capacity of plasma, the amount of lipid peroxidation, and the impact of Fenton reaction products on lipid peroxidation were all increased when SDG was administered(Cadenas and Barja, 1999). In a reliability investigation, SDG protected the kidneys against oxidative stress generated by As_2O_3 and DNA destruction (Olson, 2021).

Arsenic accumulation, which is linked to kidney impairment, was observed to be influenced by SDG therapy. To make iAs, pentavalent iAs are reduced to trivalent iAs, which are oxidatively methylated to MMA,then reduced to trivalent MMA, and finally methylated to DMA, which is eliminated in the urine. Since As is not completely methylated, some MMA and iAs are also eliminated in the urine with DMA(Hayakawa *et al.*, 2005). By sticking to protein fragments, iAs, MMA, and DMA may all exist in the kidney simultaneously(Thomas *et al.*, 2001; Kuo *et al.*, 2017). Because of this, biological measures of methylation and exposure capacity such as concentrations of As in kidneys and levels of MMA, As (III), As(V), and DMA in urine are considered appropriate (Chiou et al., 1997, Chanda et al., 2020). DMA and MMA, the primary methylation products, are rapidly eliminated from the body and do not have the same affinity for macromolecules as iAs and are not well absorbed by cells. Due to its ability to enhance exocytosis, Asmethylation may be considered a detoxification method. The SDG co-administration led to levels of low tissues, augmented levels of different species of As, and a high percentage of arsenical methylationin urine, showing that the treatment of SDG assuaged - nephrotoxicity induced by As by improving the capacity of methylation and assistingthe excretion of As.

The mechanisms mediated by SDG that impactthe metabolism of As are: 1. a stimulatory role by GSH is first played in pathways of methylation as a reducing agent or as anundeviatingassociation with arsenicals(Davison *et al.*, 2003; Zhao *et al.*, 2019). SDG provides methylation of Asby avoiding the GSH depletion induced by ROS. 2. P-glycoproteins and other multidrug-resistant proteins with ATP-binding transporters move glutathione-conjugated arsenicals between extracellular space and intracellular space(Zhou *et al.*, 2021). SDG may enhance arsenic metabolism by enhancing mitochondrial biogenesis and avoiding ATP depletion, both of which are caused by As, and therefore provide enough energy for As secretion. 3. SDG affects the activity and expression of enzymes that metabolize medicines, according to Patel et al.(Patel *et al.*, 2012). Increased As regulation (oxidation state) methyltransferases may have decreased DNA breakage due to their role in the methylation of the toxic metal Asas well as the efflux system(De la Rosa *et al.*, 2017; Lin *et al.*, 2018).

5. Conclusion

SDG can prevent renal damage caused by As_2O_3 by alleviating oxidative stress and increasing As methylation metabolism. Hence, it is opined that SDG might be effective in the cure and healing and could serve as an alternative therapy to prevent arsenical toxicity. Nevertheless, in-depth studies are mandated to prove and propose dietary SDG as a therapeutic agent for arsenAsic nephrotoxicity.

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Ethical approval

Animal experiments followed the guidelines approved by the institution (Approval No. In vivo/IAEC/012/2020). The experimental protocol was approved by *Invivo* Biosciences.Bangalore. India.

Competing Interests Declaration

The study's authors claim that they have no financial or personal links that might have impacted the findings provided in this study.

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