

Antioxidant Activity and Biovariables Improvement via Pomegranate Flower Methanolic Extract in Albino Rats Dosed by CdSNPs

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ABSTRACT



Cadmium (Cd), is a heavy metal that is toxic to both animals as well as humans Pomegranate flowers are an ancient herb used in conventional Chinese medicine. Pomegranate flower extract is advantageous. Pomegranate flowers have been collected from various locations, in the lab, a nano-cadmium sulfide solution was also made, and the nanoparticles (NPs) were viewed under an electronic microscope while their physical characteristics were investigated. The experiment included 5 groups of white rats (1 positive control), (1 negative control), and (3 groups for the treatment trial). The rats were dosed with the toxic substance of cadmium sulfide at regular intervals, then after 30 days the rats were dosed with pomegranate flower methanolic extract. Blood was collected from around the heart of all mice in the experiment, study (ALP, ALT, AST), (urea and creatinine), and (GPX, Catalase, and MDA) as an indicator of the therapeutic role of the alcoholic extract of pomegranate flowers. The present research's findings demonstrated that rats exposed to pomegranate flower methanolic extract improved in all required variables. Our results confirm the therapeutic role of the pomegranate plant and suggest that supplementation may be beneficial and treat many pathological problems, including kidney and liver problems.

Keywords: Antioxidant activity, Cadmium sulfide toxicity, Methanolic Pomegranate flower extract, Liver and kidney function, Oxidative stress

INTRODUCTION

Antioxidant-rich medicinal plants were utilized for treating and preventing several human illnesses; such natural remedies are safe for long-term usage because they have few or no adverse effects when put to comparison with allopathic medications. Despite being consumed in greater amounts, bioactive ingredients in natural products could achieve a similar potency to synthetic drugs [1].

Numerous research has demonstrated the positive effects of herbal medicines in lowering toxicity; as a result, such compounds may be suitable options for supplements or alternative therapies. The Punicaceae family, which includes pomegranate (i.e., Punica granatum), grows in a variety of climates and nations, including Turkey, Iran, China, the United States, South Africa, and India. According to studies, the major segments of pomegranate seeds, fruits, leaves, and peel—are used to cure conditions like inflammation, diabetes, pain, and cardiovascular disease [2]. Pomegranates and their active components have been shown in various studies to have strong anti-hepatic effects through the modulation of specific enzymes including ALT, AST, and ALP [3,4]. Ellagic acid, punicalagin, anthocyanins, tannins, polyphenols, lipoic acid, and vitamins C and E are among the primary components [5]. Oxidative stress is a major element in the pathogenesis of several diseases, which mclude cardiac and liver disorders. Pomegranate or its active constituents have been found in many studies to lower oxidative stress or lipid peroxidation [6].

One of the units of length is the milli-micron, which is shortened to nano. The length of ten hydrogen atoms arranged in a row is equal to one billionth of a meter, or a nanometer [7]. Cadmium oxide, selenide, telluride, sulfide, and other nanoparticles are examples of Cd. These Cd-containing NPs started to pose new challenges in their production and industrial use. They might use a variety of routes to reach secondary major interaction targets once they have entered the human body. Due to their high blood supply and capability of concentrating the toxins, the kidneys are especially susceptible to them [8]. According to a comparison of their toxicity, NPs have a higher potential for toxicity compared to microbeads [9,10]. This impact was associated with their vast surface area, small size, and high reactivity. These traits allow them to generate a higher amount of reactive oxygen species (ROS) and considerable toxicity [11]. The National Toxicology Program classifies Cd as a toxic, unnecessary transition metal and a human carcinogen [12]. Treatment with subchronic cadmium doses altered glucose metabolism [13] and had deleterious impacts on biochemical as well as neurobehavioral parameters [14], also harmed the kidneys and liver [15]. A significant reduction in the amount of GSH in cells was caused by medium concentrations of both micro- and nano-sized Cd particles. The toxicity of CdS might have resulted from the production of ROS, implying that expanding an NP's area could potentially increase its toxicity risk [16].

MATERIAL AND METHODS

Plant Collection

The flowers of the pomegranate plant were collected from different regions of Anbar province and the pomegranate trees at the University of Anbar, the flowers were collected in special bags and were transferred to the laboratories of the Center for Desert Studies at the University of Anbar and were classified according to special sources available within the center.

Plant Extraction

Punica granatum flowers were extracted in solvent methanolic by using the Soxhlet [17]. In this procedure, 500 g of the sample was placed in pure thimble cellulose. Extraction was conducted for 72 hours. A rotary vacuum evaporator has been utilized in order to concentrate the extract, and the dried extract has been kept in a sterile container at a temperature of 4 Celsius.

Cadmium Sulfide Nanoparticle

Utilize commercially available nano-CdS powder with a particle size of less than 100 nm. An ultrasonic probe was used to dissolve the powder in distilled deionized water in order to prevent molecule aggregation and preserve the material's nature after the application of additional organic solvents [8].

Animals

In this study, 25 mature male albino rats (Sprague Dawley, *Rattus norvegicus*) were utilized. The animals were aged 12–14 weeks and weighed between 200 and 250 grams. They were housed in the Animal House Unit of the Department of Life Sciences at the College of Education for Pure Sciences, University of Anbar. The rats were maintained under standard laboratory conditions, including a controlled temperature of 24 ± 1 °C, a 12-hour light/dark cycle, and unrestricted access to food and water.

Experimental Design

As seen below, the experimental animals have been divided into five groups, each of which had five randomly selected animals of similar weights. First group: as indicated below, the experimental animals were split up into five groups [18], each of which contained five animals chosen at random and of comparable weight.

First Group: The animals in this group were administered a normal saline solution at a dose of 0.5 mkg as a control.

Second Group: This group received the toxic substance cadmium sulfide (CdS). The toxic material was administered to the animals at a dose of 4 cc mixed with their food.

Third Group: The animals in this group were exposed to CdS toxicity for two weeks (4 cc), followed by two weeks of treatment with the methanolic extract of pomegranate flowers at a concentration of 0.025% (5 cc).

Fourth Group: The animals in this group were exposed to CdS toxicity for two weeks (4 cc), followed by two weeks of treatment with the methanolic extract of pomegranate flowers at a concentration of 0.05% (5 cc).

Fifth Group: The animals in this group were exposed to CdS toxicity for two weeks (4 cc), followed by two weeks of treatment with the methanolic extract of pomegranate flowers at a concentration of 0.1% (5 cc).

Collection of Blood Samples

Seven milliliters of whole blood were taken from the cardiac cavity of experimental rats after they had been given chloroform anesthesia. The collected whole blood was poured into a plastic tube and left for half an hour to clot. For separating serum from blood, the plastic tube was put in a centrifuge and spun for 10 min at 1000 rpm. With the use of a Pasteur pipette, serum was separated from blood and transferred into a plain, dry, and clean bottle.

The Biochemical Parameters

Serum levels of biochemical markers of liver function alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) have been measured with the use of an autoanalyzer (Abbott, ci-8,200, Jiangsu, China).

Biochemical markers of renal function: the levels of creatinine and urea in serum have been measured by diagnostic laboratory tests (POCh) and spectrophotometry (SEMCO S/E-UV spectrometer). The clearance of creatinine was computed.

Antioxidant Assessment

The determination of glutathione peroxidase (GPX) activity was based on its ability to promote glutathione oxidation using cumene hydroperoxide. In the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH), oxidized glutathione was rapidly reduced back to its original form, while NADPH was simultaneously oxidized to NADP+. To assess GPX activity in hemolysates, $10 \ \mu$ L of the samples were mixed with 500 μ L of reagent R1 and 20 μ L of cumene reagent R2. Following the manufacturer's instructions, absorbance was measured at 340 nm, and GPX activity was calculated using the Ransel® kit (Randox Laboratories, Antrim, U.K.). Enzyme activity was expressed in units per milliliter (U/mL).

Using [19] spectrophotometric technique, catalase activity was measured. $10 \,\mu\text{L}$ of the sample incubated for ten minutes with $100 \mu\text{mol/mL}$ of H₂O₂ in 0.05mmol/L of Tris-HCl buffer (pH = 7). A quick addition of $50\mu\text{L}$ of 4% ammonium molybdate resulted in stopping the process. At 410nm, the yellow combination of H₂O₂ and ammonium molybdate has been detected. The amount of the enzyme that is needed in order to break down one μ mol H₂O₂ per minute has been referred to as one catalase activity unit.

Malondialdehyde (MDA) levels in the samples were measured using the thiobarbituric acid reaction method described by [20]. Thiobarbituric acid reactive substances were quantified by measuring absorbance at 532 nm and comparing it to a standard curve of MDA equivalents generated by the acid-catalyzed hydrolysis of 1, 1, 3, 3 - tetramethoxypropane. To measure MDA levels, a working solution containing 15% trichloroacetic acid, 0.25 N hydrochloric acid, and 0.375% thiobarbituric acid was prepared. For each sample, 250 μ L of serum was combined with 500 μ L of the working solution, and the mixture was incubated in boiling water for 10 minutes. After cooling,

the samples were centrifuged at 3000 rpm for 10 minutes. Following centrifugation, 200 μ L of each supernatant was transferred to microplates, and the optical density was measured at 535 nm. MDA concentrations are expressed as μ mol/L.

Statistical Analysis

Effects of several elements on research parameters have been detected with the use of the Statistical Analysis System [21] program. In order to make a meaningful comparison between the means in this research, the LSD test (Analysis of Variation, or ANOVA) has been utilized.

RESULTS AND DISCUSSION

Table 1 (Catalase, GPX) shows that there is no significant difference between the control group (0.258) and the groups that have been treated with cadmium sulfide (Catalase 0.274 and GPX 2.041). However, there is a significant reduction in levels of Catalase and GPX in groups that have been treated with pomegranate flower extract (Catalase 0.501, 0.423, and 0.430) in comparison with the group injected with cadmium sulfide. This suggests that the primary cause of Cd toxicity is the production of oxidative stress [22], which could be the result of ROS being produced in excess [23]. Regarding the improvement noted in the pomegranate extract-treated groups, it is possible that the treatment raised GSH levels, which could aid in shielding tissue from oxidative damage. GSH, glutathione reductase, and glutathione peroxidase activities regulate the propagation of free-radical reactions that result in lipid peroxidation within the GSH redox cycle [24]. The presence of many phenolic components (punicalin, ellagic acid, and punicalagin) instead of just one pure polyphenol is what gives pomegranates their superior antioxidant action [25]. In the case when examined utilizing the phosphomolybdenum method, the pomegranate methanolic fraction (at 80g/mL concentration) had shown the maximum total antioxidant activity (5067,70mol ascorbic acid equivalents [AAE]/g) and a high quantity of ellagitannins [26]. Pomegranate has been considered one of the good sources of natural antioxidants because of its excellent effectiveness in scavenging superoxide as well as hydroxyl anion radicals [27]. Fomegranate fruit carpellary membrane extract showed strong antioxidant activity in DPPH radical scavenging, superoxide radical scavenging, and lipid peroxidation inhibitory tests [28]. Ellagic acid and gallic acid, two potent free radical scavengers included in PoP, reduce lipid peroxidation and aid in the restoration of hepatic enzyme function (peroxidase, catalase, and superoxide dismutase) [29].

As for the level of malondialdehyde (MDA), table 1 shows us an increase in malondialdehyde in the group exposed to cadmium sulfide (56.87) compared to the control group (29.10), while an improvement appears in the groups that were dosed with pomegranate flower extract (37.07, 35.75 and 40.71) respectively compared with the group that has been injected with cadmium sulfide. To rise in the group that was injected with CDS malondialdehyde indicates an increase in free radicals in the body of rats, and this causes an increase in oxidative stress to the cell A condition that arises from an imbalance between antioxidants and radicals Free oxygen in living tissues, where it becomes the cell Weak to the invasion of free radicals, it attacks the two nost important components, the two main components of the cell are protein and DNA the carrier of the cell's genetic code, through which it undergoes changes and formation Mutations that lead to cell cancer [30]. The groups who received pomegranate fruit is thought to be polyphenols. Both in vitro and in vivo, they exhibit antioxidant activity. Dietary polyphenols have antioxidant properties that include oxidative stability, enzyme regulation to disrupt cell signaling, and reactive species scavenging. Pomegranates are also a significant source of soluble polyphenols, which include punicalagin, quercetin, ellagic acid, and gallic acid [31].

	Mean ± SE		
Group	Catalase U/ml	GPX µmol/L	MDAµmol/L
Control	0.258 ± 0.02 b	$1.762\pm0.06~b$	$29.10\pm4.72~b$
CDS	0.274 ± 0.05 b	$2.041 \pm 0.27 \text{ b}$	56.87 ± 7.91 a
0.025 group	0.501 ± 0.02 a	4.24 ± 0.74 a	$37.07\pm5.68~b$
0.05 group	0.423 ± 0.03 a	5.61 ± 0.39 a	$35.75 \pm 5.49 \text{ b}$
0.10 group	0.430 ± 0.07 a	5.56 ± 0.65 a	40.71 ± 4.37 ab
LSD value	0.136 **	1.554 **	18.191 *
P-value	0.0098	0.0004	0.0494
Means with different superscript	letters within the same column are sign	nificantly different (P \leq 0.050; **P \leq 0.0	010).
	^{0.5}	Control	
	F	CDS	
Y	- 0.6	0.025 group	
	<u>s</u> 0.50	0 423 0 430 0.05 group	

0.1 group

Table 1 Comparison between different groups in Anti-Oxidant

Fig. 1 Comparison between various groups in Catalase

Percent of sam

0.0

0.285

Catalase



Fig. 5 Comparison amongst various groups in the MDA

According to statistical analysis, the group that received CDS injections had higher levels of AST, ALT, and ALP (242.67, 71.67, and 382.33) than the control group (123.33, 27.33, and 110.33), but the group that received pomegranate flower extract after receiving cadmium sulfide injections showed improvement. The liver cytosol's release of these enzymes into the bloodstream causes elevated ALT and AST activity. According to earlier reports, hepatic enzymes such as AST, ALT, and ALP leaked into the bloodstream as a result of lysosomal instability brought on by Cd toxicity [32]. Because of their high polyphenolic content, which has potent free radical scavenging properties, they concluded that treating pomegranate flowers with methanolic extract improves their antioxidant defense status against the toxicity caused by CDS [33].

Table 2 Comparison betw	veen different groups of Liver enzymes	N	
	Mean ± SE		
Group	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	$27.33 \pm 0.88 c$	123.33 ± 8.66 c	$110.33 \pm 15.43 \text{ d}$
CDS	71.67 ± 6.88 a	242.67 ± 29.40 a	382.33 ± 24.76 a
0.025 group	48.33 ± 4.09 b	$188.33 \pm 4.09 \text{ b}$	228.33 ± 9.93 b
0.05 group	$26.00 \pm 3.78 \text{ c}$	$147.00 \pm 1.00 \text{ bc}$	$184.00 \pm 12.22 \text{ bc}$
0.10 group	$38,67 \pm 3.48$ bc	$158.00 \pm 1.73 \text{ bc}$	164.67 ± 22.78 cd
LSD value	13.476 ***	43.678 **	**
P-value	0.0001	0.0012	0.0001

Means with different superscript letters within the same column are significantly different ($P \le 0.050$; ** $P \le 0.010$).



Fig. 4 Comparison amongst various groups in ALT



Results(Table 2) have indicated that while there has not been a significant change in creatinine levels, the group treated with nanocadmium sulfide had a higher percentage of urea (63.67) than the control group (30.00). These effects have been due to the unique physicochemical properties of CdSNPs, their increased capacity to produce ROS, the induction of oxidative stress, and the impairment of renal structure and function [34]. However, the groups who received pomegranate flower extract showed improvement (31.00, 23.67, and 23.67). Pomegranate extract's high vitamin C and phenolic component concentration, which function as potent antioxidants and free radical scavengers, might be responsible for its protective impact [35]. These compounds could shield the kidneys from irreversible cellular harm brought on by substances like CDS and stop the advancement of oxidative kidney disease [36]. The increase in antioxidant enzyme activity in the recommended pomegranate groups [37,38] might be the cause of this effect. This, in turn, increases the kidneys' capacity to remove harmful free radicals like lipid peroxides as well as hydrogen peroxide [1].

Table 3 Comparison among different groups in Kidney functions Mean ± SE Group Creatinine (mg/dl) Urea (mg/dl) Controls 30.00 ± 0.57 b 0.646 ± 0.04 CDS 63.67 ± 6.56 a 0.607 ± 0.23 $31.00\pm4.16~b$ 0.667 ± 0.28 0.025 group 0.05 group $23.67 \pm 1.33 \text{ b}$ 0.756 ± 0.06 23.67 ± 0.67 b 0.740 ± 0.04 0.10 group LSD value 11.185 ** 0.534 NS 0.0001 P-value 0.963

Means with different superscript letters within the same column are significantly different ($P \le 0.050$; ** $P \le 0.010$).



Fig. 7 Comparison among different groups in Urea



Fig. 8 Comparison between different groups in Creatinine

The current study highlights the protective effects of pomegranate flower methanolic extract against cadmium sulfide nanoparticle (CdSNP)-induced toxicity in rats, primarily through its antioxidant properties and its positive impact on liver and kidney function. These findings are consistent with a growing body of evidence supporting the therapeutic potential of pomegranate and its various components in mitigating the adverse effects of environmental toxins and other stressors.

Specifically, our results demonstrated that the extract significantly increased the levels of catalase and GPX, while simultaneously reducing lipid peroxidation, as indicated by decreased MDA levels. This observation aligns with previous research indicating that pomegranate extracts are rich in bioactive compounds, such as ellagic acid, punicalagin, and anthocyanins, known for their potent antioxidant activities [5]. These compounds can effectively scavenge free radicals and reduce oxidative stress, a key mechanism in Cd-induced toxicity [39,11]. The observed improvement in antioxidant status following pomegranate flower extract treatment suggests that the extract may enhance the activity of the GSH redox cycle, which plays a critical role in regulating free-radical reactions and preventing lipid peroxidation [24]. Furthermore, [40] found that pomegranate seed extract attenuated chemotherapy-induced liver damage in rabbits, reinforcing the potential of pomegranate components to protect against drug-induced or toxin-induced hepatic damage.

Moreover, the present study observed that the indicators of kidney function (urea and creativine) decreased significantly following exposure to pomegranate flower extract, suggesting its protective effects against cadmium-induced kidney damage. This result is in accordance with [41], who reported that pomegranate juice had a protective effect on kidney and liver tissues in lead acetate-treated rats. The ability of pomegranate to improve kidney function could be attributed to its antioxidant and anti-inflammatory properties, which help to alleviate the oxidative stress and inflammation associated with Cd toxicity. As the kidneys are particularly vulnerable to the accumulation of toxins due to their high blood supply [8], the protective effects of pomegranate flower extract on this organ hold significant implications.

Beyond its antioxidant and organ-protective effects, pomegranate has also demonstrated potential in other areas, such as combating multidrug-resistant (MDR) bacteria [42] and inhibiting cancer cell metastasis [43]. While these findings may seem unrelated to the current study, they highlight the diverse range of therapeutic benefits associated with pomegranate and its components. Furthermore, the inclusion of pomegranate seed oil in food products, such as cupcakes, has been shown to improve their nutritional profile and microbial stability [44], suggesting that pomegranate can be incorporated into the diet to promote overall health.

In conclusion, our results, in conjunction with the existing literature, strongly support the therapeutic potential of pomegranate flower methanolic extract in mitigating CdSNP-induced toxicity. The extract's antioxidant properties and protective effects on liver and kidney function make it a promising natural supplement for combating oxidative stress and heavy metal exposure. Future studies should focus on elucidating the specific mechanisms of action underlying these beneficial effects and on evaluating the efficacy of pomegranate flower extract in clinical settings.

CONCLUSION

Based on the results this study confirms the therapeutic potential of pomegranate flower methanolic extract in mitigating cadmium sulfideinduced toxicity in rats. The extract demonstrated significant antioxidant activity, evidenced by increased catalase and GPX levels and a reduction in lipid peroxidation. Furthermore, it effectively improved kidney function indicators, suggesting its potential in alleviating cadmium-related kidney damage. These findings support the use of pomegranate flower extract as a natural supplement to combat oxidative stress and protect against the harmful effects of heavy metal exposure on liver and kidney health. Further research is warranted to explore the specific mechanisms of action and to evaluate its efficacy in clinical settings.

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