Ameliorative Effects of Jamun Seed and Orange Peel Extracts on Serum Calcium and Phosphate Levels of Cadmium Challenged Male Wistar Rats

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Abstract: The aim of the present study was to investigate the changes in the serum calcium and phosphorous induced by cadmium and to evaluate the protective role of jamun (Syzygium cumini) seed and orange (Citrus sinensis) peel extracts. Male wistar rats were divided into six groups and following treatments were given as - Group A: Control; Group B: Cadmium (Cd); Group C: Cadmium and jamun seed extract (Cd+ JSE); Group D: Cadmium + orange peel extract (Cd + OPE); Group E: Orange peel extract (OPE); Group F: Jamun seed extract (JSE). The Cadmium dose was 10 mg/kg b wt whereas orange peel and jamun seed extract dose was 200 mg/kg b wt/day. Blood samples were collected on day 7 and day 14 after these treatments and analyzed for serum calcium and phosphate.

Rats from Group B (cadmium challenged) showed decrease in serum calcium level after 7 day as compared to control (Group A). Serum calcium level decreased further after 14 day after cadmium treatment. There was a significant increase in serum calcium level in Group C at 7 days and 14 days as compared to Group B (Cd treated). In group D the serum calcium level exhibited an insignificant increase on day 7, however, the levels were significant after 14 days treatment as compared to group B. No significant change was noticed in serum calcium level when rats were treated with orange peel extract and jamun seed extract after 7 day and 14 day.

Rats from Group B showed progressive decrease from 7 day to 14 day in serum phosphate level as compared to control (Group A). There was no significant change in serum phosphate level of group C and D at 7 day treatment as compared to Group B (Cd treated), however, the phosphate levels were significantly increased on day 14 in group C and D as compared to group B. No significant change was noticed in serum phosphate level when rats were treated with orange peel extract and jamun seed extract after 7 day and 14 day.

Keywords: Cadmium, Phosphate, Syzygium cumini, Citrus sinensis, Antioxidant, Hypocalcemia, Hypophosphatemia


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**Introduction**

Heavy metals like cadmium (Cd) and arsenic are widespread environmental toxicants responsible for a number of adverse health effects in humans and other organisms (Guo et al., 2016; Srivastava and Srivastav, 2017; Srivastava et al., 2023). Cadmium is a major threat to human health around the world and associated with pollution and anthropogenic activity (Lordan and Zabetakis, 2022). Environmental and occupational cadmium exposure may be related to various types of cancer, including breast, lung, prostate, nasopharynx, pancreas and kidney carcinomas (Genchi Giuseppe et al., 2020). Cadmium, a highly toxic environmental pollutant, is reported to induce toxicity and apoptosis of multiple organs and cells (Chen et al., 2015). Exposure to cadmium is contributing to dysfunction of the internal organs and development of chronic diseases (Markiewicz-Górka et al., 2019). Food contamination with cadmium may take place at various phases, such as agronomic conditions, food production, processing and consumer preparation for consumption. The presence of cadmium in food vary and is influenced by the geographic location, cadmium bioavailability from soil, crop genetics, and agronomic practices used and post harvest operations (Heather et al., 2020). Cadmium is a non essential transition metal that has been introduced by various anthropogenic activities into the biosphere. In the past decades, environmental pollution of Cd had a significant health risk and there have been extensive studies on its toxicity (Sandbichler and Höckner, 2016). Environmental diseases related to exposure to cadmium develop mainly as result of industrial sewage pollution and/or contaminated food (Yamanobe et al., 2015). As a normal cellular metabolic by-product, reactive oxygen species (ROS) are produced by living cells. Cells will accumulate a number of ROS when exposed to excessive stress, and the living beings are then gradually developing several response mechanisms in order to counteract to that exposure as well as use it for signal molecules. In the feedback mechanism, which involves a number of physiological processes such as apoptosis, necrosis and autophagocytosis, ROS molecules would be an inducer of oxidative stress (He et al., 2017).

The most important prooxidants, whose excessive accumulation leads to oxidative stress and molecular damage, are reactive oxygen species (ROS) together with reactive nitrogen species (RNS). Their physiological functions are determined by the concentration of ROS. When present at low concentration and high concentration, ROS involved in signaling processes essential for normal cellular functions and leads to DNA, lipid, protein damage and apoptosis, respectively (Macvanin et al., 2023).

Phytochemicals with antioxidant activity have been reported to be present in fruits, vegetables, spices and herbs, which scavenge free radicals (Mossa et al., 2015; Srivastava et al., 2018; Ahmed et al., 2019; Bashandy et al., 2019). Jamun (Syzygium cumini) belongs to family Myrtaceae. Antioxidant compound called flavonoids, phenols and anthocyanins are present in jamun fruits and seeds (Raza et al., 2017; Srivastava et al., 2018; Simon et al., 2018). Antidiabetic, antimalarial, antibiotic, free radical scavenging, antiulcerogenic and antifertility properties are present in jamun seeds (Singh et al., 2012; Kumari et al., 2017; Chagas et al., 2018; Srivastava et al., 2018, 2021). Jagetia (2018) have reported reduction of free radicals and activation of different enzymes like catalase, glutathione peroxidase, glutathione-transferase by jamun. It increases synthesis of glutathione and depletes lipid peroxidation.

Citrus (Citrus sinensis) is an important fruit variety that has a high yield of phytochemicals such as flavanones, polyphenols, anthocyanins and hydroxycinnamic acids, hespiridin, naringin. Abundant flavanones and polymethoxylated flavones are exclusively found in peel of citrus fruits. The antioxidative potential of the orange peel has been reported (Srivastava et al., 2021). Hesperidin has been reported to increase the levels of superoxide dismutase (SOD), glutathione
S-transferase (GST), and total glutathione (GSH) in rats after chemical induction of oxidative stress (Jeon et al., 2002). Arafa et al. (2009) reported that naringin in citrus peel increased superoxide dismutase, catalase (CAT) and vitamin E levels in New Zealand white rabbits with high cholesterol levels.

The aim of this study was to investigate alterations in serum calcium and inorganic phosphate levels induced by exposure of cadmium in male Wistar rats and to evaluate the protective role of jamun (Syzygium cumini) seed and orange (Citrus sinensis) peel extracts against the toxicological effects caused by exposure to cadmium.

Materials and Methods

Experimental design:

Male wistar rats were purchased from Asia Scientific Emporium, Varanasi, India and were acclimatized for 15 days in polypropylene cages. They were fed on standard food and water ad libitum during the experiment.

The acclimatized rats were divided into 6 groups (A-F) each group containing 10 rats. The rats were treated as follow at 8:00 am everyday throughout the experiment:

Group A: Control
Group B: Cadmium treatment (Cd)
Group C: Cadmium + Jamun seed extract (Cd+JSE)
Group D: Cadmium + Orange peel extract (CD+OPE)
Group E: Orange peel extract (OPE)
Group F: Jamun seed extract (JSE)

The cadmium dose was 10 mg/kg b wt/day whereas orange peel and jamun seed extract dose was 200 mg/kg b wt/day. Five rats from each group were sacrificed 24 h after the last dose on 7th and 14th day after the initiation of the experiment. Rats were fasted overnight before sacrifice. The experimental protocol and handling of the animals was approved by the Research Degree Committee of DDU Gorakhpur University, Gorakhpur, India.

Extract preparation:

The orange peels and jamun seeds were purchased locally. The orange peels and jamun seeds were washed with water and dried in hot air oven at 40°C. After drying they were crushed and powdered. Powdered orange peels and jamun peels were mixed with 90% ethanol in 1:20 ratio (w:v) and kept at orbital shaker for 48 h. Then the samples were filtered through Whatman no. 1 filter paper. The filtrates were dried in oven at 40°C and stored at -20°C. For use the dried residues of orange peel and jamun seed were dissolved with ethanol to provide required dose to be given to rats.

Blood analysis:

The rats were slightly anesthetized with ether and blood was taken by cardiac puncture using a 3 ml syringe equipped with 23 gauge needles. The blood samples were allowed to clot at room temperature for a period of 30 min. Sera were separated by centrifugation (at 3,000 rpm) and kept at -20°C. Analysis of serum calcium and serum inorganic phosphate was performed by using Beacon kit (Beacon Diagnostic Private Ltd). Each sample was analyzed in duplicate.

Statistical analysis:

Data are presented as the Mean±SE of five specimens. To determine the significance level a Student t - test was performed. In all studies all the treated groups are compared with the control group. Analysis of Variance (ANOVA) was used to establish the significant differences between different treatment groups.

Results

The serum calcium level of rat treated with cadmium (Group B) exhibited a decrease after 7 day (P < 0.0001) and day 14 (P < 0.0001) as compared with control (Group A)(Fig. 1). In cadmium and jamun seed extract treated rats (Group C), the serum calcium increased on 7 day (P < 0.0197) and on 14 day (P < 0.0001) as
Fig. 1: Serum calcium levels (mg/100 ml) of Wistar rat: control (group A); cadmium (group B), cadmium + jamun seed extract (group C), cadmium + orange peel extract (group D), orange peel extract (group E) and jamun seed extract (group F) extract. All values indicate mean ± S.E. of five specimens.

compared to Group B. In cadmium and orange peel extract treated rats (Group D), the serum calcium level insignificantly increased on 7 day as compared to Group B. However, on day 14 the calcium level showed a significant increase (P< 0.0009) as compared to Group B (Fig. 1). This indicates that orange peel and jamun seed are effective in recovering the calcium levels which was decreased by treatment with cadmium. In orange peel extract (Group E) and Jamun seed extract (Group F) there was no change in serum calcium level on 7 and 14 day. Analysis of Variance (ANOVA) indicated that treatment was significant (7 day - F = 8.346, P< 0.0001; 14 day - F = 12.294, P < 0.0001).

In cadmium (Group B) exposed rat the serum phosphate levels gradually decreased from 7 day (P < 0.0006) to 14 day (P< 0.0001) as compared to control (Group A) (Fig. 2). In cadmium and jamun seed extract (Group C), the serum phosphate level exhibited insignificant increase at day 7, however, on 14 day there was a significant increase (P< 0.0467) as compared to the level of Group B. In Group D serum phosphate levels insignificantly increased on day 7, whereas the levels were noticed significantly increased (P< 0.0146) on 14 day as compared to Group B. In orange peel extract (Group E) and jamun seed extract (Group F) exposed rats there was no change in serum phosphate levels as compared to control rats (Group A). Analysis of Variance (ANOVA) indicated that treatment was significant (7 day - F= 5.642, P< 0.0009; 14 day - F = 16.037, P< 0.0001).

Discussion

In present study, Cd treated rats exhibited hypocalcemia. This is in agreement with the report of Tripathi and Srivastav (2011b) who have also noticed hypocalcemia in rats after exposure to
The present study is in agreement with the reports of earlier workers who have noticed hypocalcemia after exposure to different toxicants in -- (i) rats – cypermetrin (Srivastava et al., 2021); microcystin LR (Srivastava et al., 2020), chlorpyrifos (Tripathi et al., 2013), diazinon (Rangoonwala et al., 2005), mipcin (Rangoonwala et al., 2007), cypermetrin (Rangoonwala et al., 2008), heroin (Barai et al., 2009); (ii) in chickens: gamma benzene hexachloride and quinalphos (Agarwal et al., 2009); and (iii) in frogs: chlorpyrifos (Srivastav et al., 2018) and cadmium (Srivastav et al., 2019). In toxicant exposed fish hypocalcemia has also been reported--deltamethrin (Srivastav et al., 1997b, 2010); aldrin (Singh et al., 1996); malachite green (Srivastava et al., 1995); formothion (Singh et al., 1997); cadmium (Rai and Srivastav, 2003, Chowdhary et al., 2004, Suzuki et al., 2004), cypermethrin (Mishra et al., 2010), herbal pesticides (Kumar et al., 2011 a, b; Prasad et al., 2011 a,b) and dimethoate (Pandey and Das, 2015). Treatment with nickel to Cyprinus carpio caused no significant changes in serum calcium levels (Bozorgzahed et al., 2023).

In the present study rats exposed to cadmium exhibited hypophosphatemia. In past, other investigators have also reported hypophosphatemia after toxicant exposure to rats-microcystin LR (Moreno et al., 2003; Srivastava et al., 2020), cypermetrin (Srivastava et al., 2021); cadmium (Tripathi and Srivastav, 2011b), chlorpyrifos (Tripathi et al., 2013), in chickens-gamma benzene hexachloride and quinalphos (Agarwal et al., 2009). After treatment with chlorpyrifos hypophosphatemia was recorded in...
frog (Srivastav et al., 2018). Hypophosphatemia was noticed in fish after exposure to chlorpyrifos (Srivastav et al., 1997 a), deltamethrin (Srivastav et al., 1997 b), cypermethrin (Mishra et al., 2001), cadmium (Rai and Srivastav, 2003), Nerium indicum leaf extract (Prasad et al., 2011 a), Euphorbia royleana (Prasad et al., 2011 b), azadirachtin (Kumar et al. 2011 a), Euphorbia tirucalli (Kumar et al., 2011b), lead (Srivastav et al., 2013) and microcystin LR (Prakash et al., 2015, 2016). In contrast, no alteration was noticed in blood phosphate of rats treated with heptachlor (Rangoonwala et al., 2004), diazinon (Rangoonwala et al., 2005) and mipcin (Rangoonwala et al., 2007). In heroin administered rats, Barai et al. (2009) noticed hyperphosphatemia.

The hypocalcemia induced by cadmium in rats may due to the degeneration of kidney in cadmium exposed rats (our unpublished data). In mammals various toxicants caused degeneration of kidney (Chmielnicka et al., 1989; Prozialeck et al., 2009; Tripathi and Srivastav, 2010, 2011 a). Increased efflux of calcium may occur due to kidney damage by toxicants (Chmielnicka et al., 1989; Prozialeck et al., 2009) which can be attributed to the observed hypocalcemia in present study. Few investigators have attributed toxicant induced hypocalcemia to the degenerative changes in the renal tubules in fishes (Koyama and Itazawa, 1977; Roch and Maly, 1979; Larsson et al., 1981; Haux and Larsson, 1984). Hypocalcemia in mipcin (Rangoonwala et al., 2007) and heroin (Barai et al., 2009) treated rats has been attributed to the degeneration of parathyroid glands.

Conclusion

It is concluded that cadmium exposure adversely affects the serum calcium and phosphate levels of the rats. The fluctuation observed in these parameters after cadmium exposure could be protected by the supplements obtained from extracts of jamun seed and orange peel. It is suggested that living beings after their exposure to cadmium should intake supplements of these given extracts which would help in the reduction of the cadmium toxicity.

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References


