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Cypermethrin-Induced Alterations in Serum Calcium and Phosphate of Rats: Protective Role of Jamun Seed and Orange Peel Extracts

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Abstract

The present study investigated alterations in serum calcium and phosphate levels induced by cypermethrin (trade name Basathrin) exposure to rats and aimed to evaluate protecting role of jamun (*Syzygium cumini*) seed (JSE) and orange (*Citrus sinensis)* peel (OPE) extracts.

Wistar rats were treated as - Group A: Control; Group B: cypermethrin (CY); Group C: cypermethrin and jamun seed extract (CY+JSE); Group D: cypermethrin and orange peel extract (CY+OPE); Group E: orange peel extract (OPE); Group F: jamun seed extract (JSE). Cypermethrin dose was 25 mg/ kg body wt/day whereas orange peel and jamun seed extract dose was 200 mg/kg body wt/day. Serum calcium and phosphate were analyzed after 15 days and 30 days following the treatment.

Serum calcium of rat treated with cypermethrin decreased after 15 and 30 days. In-group C, serum calcium decreased on day 15 and 30. In-group D serum calcium decreased at day 15 but on day 30 level increased. Calcium levels in-group C increased on day 15 and 30 as compared to group B. Moreover, levels in-group D is not significant on day 15 and 30.

In cypermethrin exposed rats, serum phosphate declined from day 15 to 30. In-group C, serum phosphate decreased at day15, which continued till day 30. Serum phosphate in-group D treated rats decreased on day 15 and 30. In groups E and F, there is no change in serum phosphate of rats on day 15; however, on day 30 levels decreased.

It can be concluded that cypermethrin treatment (25 mg/ kg body wt/day) caused alterations in the serum calcium and phosphate of the rats. The changes in these electrolytes could be protected by supplementation of extracts of jamun seed and orange peel at 200 mg/kg body wt/day. It is suggested that the cypermethrin exposed organisms should be given dietary supplement of these botanical extracts, which would reverse the toxic symptoms.

Keywords: Cyprmethrin; Serum calcium; Serum phosphate; Jamun seed; Orange peel

1. Introduction

Pests have always been a nuisance, and they damage crops in the field as well in stores. For the increased yield of crops, human beings use pesticides for the noxious arthropods and pests (Tripathi and Srivastav, 2010). Pesticides, being important for controlling injurious pests, also cause hazards to non-target organisms including humans (Bhusan *et al*., 2013; Chrustek *et al*., 2018; Tewari *et al*., 2018; Mahat *et al*., 2020). Pyrethroids have potent insecticidal properties and are potentially non-toxic to most non-target species, especially mammals. Cypermethrin is a non-systemic, light stable synthetic pyrethroid which is used mostly as residual treatment for the control of flies, ectoparasite infestation of animals, mosquitoes, cockroaches and for the control of range of insects on crops (Nair *et al*., 2011; Sharma *et al*., 2018; Mahat *et al*., 2020). The widespread use of cypermethrin caused several health hazards to non-target animals (including humans) such as toxicological alterations in liver and kidney (Grewal *et al*., 2010; Mossa *et al*., 2015; Bhusan *et al*., 2013; Das *et al*., 2017; Hamid *et al*., 2017; Srivastava *et al*., 2018), hematological (Saxena and Saxena, 2010; Das *et al*., 2017), genotoxic and neurotoxic effects (Sharma *et al*., 2014; Mhadhbi *et al*., 2020), generation of ROS (reactive oxygen species) (Yousef *et al*., 2019) and reproductive toxicity (Grewal *et al*., 2010; Das *et al*., 2017; Simon *et al*., 2018; Sharma *et al*., 2018; Singh *et al*., 2020; Zhang *et al*., 2020).

Phytonutrients/phytochemicals have been reported to be present in fruits, vegetables, spices and herbs. These phytochemicals have antioxidant properties as they scavenge free radicals (Mossa *et al*., 2015; Attia *et al*., 2017 a, b; Srivastava and Srivastav, 2017; Srivastava *et al*., 2018; Ahmed *et al*., 2019; Bashandy *et al*., 2019). *Syzygium cumini* (Jamun) has antidiabetic, antibacterial, antimalarial, free radical scavenging property, anti-

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ulcerogenic and anti-fertility activities (Nair *et al*., 2013; Kumari *et al*., 2017; Srivastava and Srivastav, 2017; Chagas *et al*., 2018; Srivastava *et al*., 2018). These properties of jamun have been attributed to antioxidant compounds present in jamun namely flavonoids, phenolic acids and anthocyanins (Raza *et al*., 2007; Srivastava and Srivastav, 2017; Srivastava *et al*., 2018). Orange (*Citrus sinensis*) also possess vitamin C, flavonoids, acridone alkaloids, carotenoids, limonoids etc. (Hegazy and Ibrahim, 2012; Srivastava and Srivastav, 2017; Srivastava *et al*., 2018; Ahmed *et al*., 2019). Bashandy *et al*. (2019) reported that *Citrus* peel contain hespiridin which has anti-inflammatory, antioxidant, anti-cancer and anti-lipedemic activities. Naringen and naringenin have been found in *Citrus* peels which have antimicrobial, antidiabetic and toxicity protecting activities (Ahmed *et al*., 2019).

Calcium, particularly its ionic form, plays a vital role in several physiological processes of vertebrates – hormone synthesis and release, neuronal excitability, blood clotting, cell adhesion, permeability of cell membranes to ions, muscle contraction, reproduction, etc. (Srivastav *et al*., 2008). These physiological processes are severely affected if there is a minor change in ionic calcium. Phosphate is required for intermediary metabolism (phosphorylated intermediates), genetic information (DNA and RNA), enzyme/protein components (phosphohistidine, phosphoserine), phospholipids and membrane structure (Norman and Litwack, 1987).

There exists no report regarding the protective effects of jamun seed extract and orange peel extract on the blood parameters (calcium and phosphate) in vertebrates. Therefore, the present study was aimed to investigate the changes in serum calcium and phosphate levels induced by cypermethrin exposure to male rats and to evaluate the possible protecting role of jamun (*Syzygium cumini*) seed and orange (*Citrus sinensis)* peel extracts.

2. Materials and methods

Male Wistar rats (115-130 g) were housed in polypropylene cages and acclimatized for 2 weeks in the laboratory under natural photoperiod (Light -11:46 to 12:08 hour) and provided standard laboratory feed and water *ad libitum.* Animal care and sacrifice were carried out according to the guidelines provided by Ethics Committee of the University.

The animals were randomly divided into six groups -- A, B, C, D, E, and F, each consisting of 20 animals (5 rats per cage). During experiment, rats were maintained under natural photoperiod (Light -11:46 to 12:08 hour) and on the standard laboratory feed and water *ad libitum.* Dose of cypermethrin used in this study has been selected considering the doses used earlier by other investigators—(i) 40-120 mg/kg b wt (Nair *et al.*, 2011), (ii) 30 mg/ kg b wt (Hamid *et al.*, 2017 and (iii) 21.2-85 mg/kg b wt (Madu, 2015). The dose of jamun seed extract used in this study has been selected on the basis of doses used by earlier workers – (i) 250 mg/kg b wt (Behera *et al*., 2014), (ii) 200-800 mg/kg b wt (Vihan and Brashier, 2017) and (iii) 200 and 400 mg/kg b wt (Kumar and Thakur, 2018). Dose of orange peel extract used in this study has been selected considering the doses used earlier by other investigators—(i) 125, 250 and 500 mg/kg b wt (Muhtadi *et* al., 2015), (ii) 100, 200 and 400 mg/kg b wt (Selmi *et al.*, 2017) and 200 mg/kg b wt (Bashandy *et al*., 2019). Following treatments were given daily to these groups at 08:00 each day throughout the experiment:

- Group A: Control
- Group B: CY-treated: Rats received daily cypermethrin (25 mg/ kg body wt)
- Group C: CY+JSE: These rats were given daily cypermethrin (25 mg/ kg body wt) and jamun seed extract (200 mg/kg body wt) simultaneously
- Group D: CY+OPE: These rats were given daily cypermethrin (25 mg/ kg body wt) and orange peel extract (200 mg/kg body wt) simultaneously
- Group E: OPE: Rats received daily orange peel extract (200 mg/kg body wt)
- Group F: JSE: Rats received daily jamun seed extract (200 mg/kg body wt)

Cypermethrin (trade name Basathrin) used in the present study was manufactured by BASF India Limited, Mumbai, India. Every day fresh cypermethrin dose was prepared. Jamun (*Syzygium cumini*) seeds were obtained from M/S SVM Naturals, Tamilnadu, India. *Citrus sinensis* fruits were obtained locally and peels were separated. Seeds and peels were thoroughly washed with water and dried at 40 °C. The dried materials were powdered and mixed with ethanol (90%) in 1:20 ratio (w/v) and kept on an orbital shaker for 48 h. The solution was filtered with Whatman grade No.1 filter paper and filtrates were dried at 40 °C. The dried residue was weighed and kept at -20 °C for further use. For experiment, the residues were reconstituted with ethanol to provide desired dose to be given to rats (Srivastava *et al*., 2018).

Rats (from each group, under light ether anesthesia) were sacrificed 24 h after last dose on 15^{th} and 30^{th} day following the start of the experiment. Animals were fasted overnight before sacrifice. Blood samples (n=5 from each group at each interval) were collected by cardiac puncture and allowed to clot at room temperature. Sera were separated and kept at -20 ºC until analyzed for serum calcium (Calcium kit, Sigma-Aldrich) and inorganic phosphate (Pointe Scientific, USA). Analysis was performed in duplicates for each sample.

Data are presented as mean ± S.E. of five specimens. For multiple group comparisons, Two-way analysis of variance (ANOVA) was used. Differences between groups were determined by the *post hoc* Duncan test.

3. Results

Serum calcium level of cypermethrin (group B) treated rat exhibits a decrease after 15 ($P < 0.0001$) and 30 day ($P < 0.0001$) (Fig. 1). In group C (cypermethrin and JSE), the serum calcium level decreased on 15 day ($P < 0.0001$) and on 30 day ($P < 0.014$) as compared to group A. However, the levels at day 30 in group C are slightly increased as compared to value of group C at day 15. In group D (CY+OPE), the serum calcium level shows a decrease ($P < 0.0006$) at 15 day as compared to group A but on 30 day the level increases (not significant as compared to group A). The calcium levels in group C is increased on day 15 ($P < 0.008$) and day 30 ($P<0.002$) as compared to group B. Moreover, the levels in group D are not significant on day 15 and day 30 as compared to group B This indicates that

orange peel extract is not effective in recovering the decrease in calcium levels caused by cypermethrin. In group E (OPE) and group F (JSE), there is no change in serum calcium levels on 15 and 30 day. Analysis of Variance (ANOVA) indicates that the treatment is significant (15 day -- F=12.004, P< 0.0001; 30 day – F=2.658, P< 0.041).

Serum phosphate levels of cypermethrin (group B) exposed rats decrease progressively from 15 day (P< 0.0008) to 30 day (P< 0.0001) (Fig. 2). In group C, the serum phosphate level displays a decline at 15 day (not significant) which continued till 30 day (P< 0.0004). Serum phosphate levels in group D treated rats show decreased value on day 15 (P< 0.009) and day 30 (P< 0.033). In group E and group F, there is no change in serum phosphate levels of rats on day 15 as compared to control (group A); however, on day 30 the levels show significant decrease (group E (P< 0.04) as compared to group A. ANOVA indicates that the treatment is significant (15 day -- F=4.014, P< 0.006; 30 day – F=10.125, P< 0.0001).

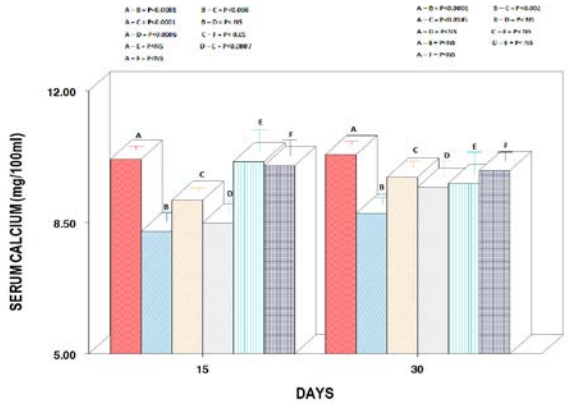

Figure 1: Serum calcium levels (mg/100 ml) of Wistar rat. Control (, Group A), CY (, Group B), CY+JSE (, Group C), CY+OPE (, Group D), OPE (, Group E) or JSE (, Group F). All values indicate mean ± SE of five specimens.

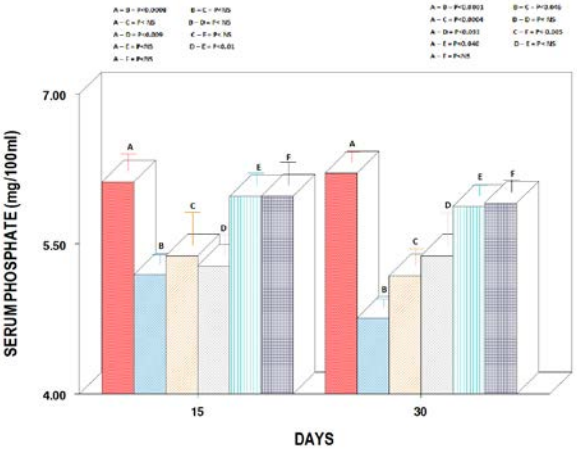

Figure 2: Serum phosphate levels (mg/100 ml) of Wistar rat. Control (, Group A), CY (, Group B), CY+JSE (, Group C), CY+OPE (, Group D), OPE (, Group E) or JSE (, Group F). All values indicate mean ± SE of five specimens.

4. Discussion

Cypermethrin treatment to rats provoked hypocalcemia. This is in agreement with the studies of earlier researchers who have observed hypocalcemia in rats after exposure to toxicants — mipcin (Rangoonwala *et al.*, 2007), microcystin LR (Moreno *et al.*, 2003), diazinon (Rangoonwala *et al.*, 2005), heroin (Barai *et al.*, 2009), heptachlor (Rangoonwala *et al.*, 2004), cadmium (Tripathi and Srivastav, 2011) and chlorpyrifos (Tripathi *et al.* 2013). Agarwal *et al.* (2009) have reported hypocalcemia from chicken after exposure to gamma benzene hexachloride and quinalphos. Toxicants-induced hypocalcemia has also been reported from amphibian – chlorpyrifos (Srivastav *et al.*, 2018) and fish-- cadmium (Rai and Srivastav, 2003; Chowdhury *et al.*, 2004), deltamethrin (Srivastav *et al.*, 1997 b, 2010), cypermethrin (Mishra *et al.*, 2010), malachite green (Srivastava *et al.*, 1995), aldrin (Singh *et al.*, 1996), formothion (Singh *et al.*, 1997), botanical pesticides (Prasad *et al.*, 2011 a, b; Kumar *et al.*, 2011 a, b), microcystin LR (Prakash *et al.*, 2015, 2016) and combination of dimethoate, chlorpyrifos and malathion (Rani *et al.*, 2017). Ghelichpour and Mirghaed (2019) have noticed an increase in plasma calcium levels after lufenuron and flonicamid exposure to common carp after 24 h, however, with elongation of exposure the levels decrease. Andjelkovic *et al.* (2019) have recorded an insignificant decrease in serum calcium levels after exposure of cadmium to rats. In the present study, the calcium levels in group C are increased on day 15 and day 30 as compared to group B. This indicates that jamun seed extract is effective in recovering the calcium levels which were decreased by treatment with cypermethrin. Moreover, the levels in group D are not significant on day 15 and day 30 as compared to group B. This indicates that orange peel extract is not effective in recovering the decrease in calcium levels caused by cypermethrin.

Rats exposed to cypermethrin exhibited hypophosphatemia. Contradictory reports have been given by other investigators regarding the effects of toxicants on phosphate levels of rats – hypophosphatemia (microcystin – Moreno *et al.*, 2003; cadmium --Tripathi and Srivastav, 2011 ; Andjelkovic *et al.*, 2019; chlorpyrifos --Tripathi *et al.*, 2013), hyperphosphatemia (heroin – Barai *et al.*, 2009), intermittent effect (microcystin LR –Hooser *et al.*, 1989) and no effect (mipcin –Rangoonwala *et al.*, 2007; diazinon-- Rangoonwala *et al.*, 2005; heptachlor-- Rangoonwala *et al.*, 2004). Several workers have noticed hypophosphatemia in fish after treatment with toxicants such as -- pyrethroids (deltamethrin, Srivastav *et al.*, 1997 b; cypermethrin, Mishra *et al.*, 2001), cadmium (Rai and Srivastav, 2003), organophosphate (chlorpyrifos, Srivastav *et al.*, 1997 a), botanical pesticides (Kumar *et al.*, 2011 a, b; Prasad *et al.*, 2011 a, b) and microcystin LR (Prakash *et al.*, 2015, 2016). Ghelichpour and Mirghaed (2019) have recorded an initial increase in plasma phosphate levels after 24 h exposure of common carp to pesticide – lufenuron and flonicamid. Later, these authors noticed a decrease in phosphate levels after elongation of exposure. The observed decrease in blood electrolytes of cypermethrin exposed rats could be attributed to the degeneration of kidney tubules (our unpublished work)

which might have caused decreased reabsorption of these electrolytes.

5. Conclusion

It can be concluded that cypermethrin treatment at 25 mg/kg body wt/day caused alterations in the serum calcium and phosphate of the rats. The changes in these electrolytes could be protected by supplementation of extracts of jamun seed and orange peel at 200 mg/kg body wt/day. It is suggested that the cypermethrin exposed organisms should be given dietary supplement of these botanical extracts which would reverse the toxic symptoms.

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