ISSN 2449-8955 European Journal of Biological Research *Research Article*

DOI: http://dx.doi.org/10.5281/zenodo.3940650

Jamun seed and orange peel extracts protects effects of microcystin LR on serum calcium and phosphate of rats

Babita Deep Srivastava[1], Manish Srivastava[2], Sunil Kumar Srivastav[1], Makoto Urata[3,4], Nobuo Suzuki[4], Ajai Kumar Srivastav[1]*

[1] Department of Zoology, DDU Gorakhpur University, Gorakhpur 273009, India

[2] Department of Chemistry, Digvijai Nath P.G. College, Gorakhpur, India

[3] Institute of Noto Satoumi Education Research, Noto-cho, Ishikawa 927-0553, Japan

[4] Noto Marine Laboratory, Institute of Nature and Environmental Technology, Division of Marine Environmental Studies, Kanazawa University, Noto-cho, Ishikawa 927-0553, Japan

*Correspondence: Mobile +91 9336400846; E-mail: ajaiksrivastav@hotmail.com

Received: 09 June 2020; **Revised submission:** 27 June 2020; **Accepted:** 07 July 2020

http://www.journals.tmkarpinski.com/index.php/ejbr

ABSTRACT: Wistar rats were treated as Group A: Control; Group B: microcystin LR (MCLR); Group C: microcystin LR and jamun seed extract (MCLR+JSE); Group D: microcystin LR and orange peel extract (MCLR+OPE); Group E: orange peel extract (OPE); Group F: jamun seed extract (JSE). MCLR dose was 10 µg/kg body wt/day whereas OPE and JSE dose was 200 mg/kg body wt/day. Serum calcium and phosphate were analyzed on 15 and 30 day. Serum calcium of rat exposed for 15 day to MCLR; MCLR+JSE and MCLR+OPE decreased. Although there is little increase in levels of group C and group D but treatment with OPE and JSE is not able to completely restore decreased calcium levels caused by MCLR. After 30 day calcium decreased after MCLR; MCLR+JSE and MCLR+OPE treatment. Levels in group E and F remain unaltered. Levels in group C and D exhibit elevation as compared to group B which indicates that treatment with OPE and JSE recovered calcium. Serum phosphate decreased after 15 day in MCLR; MCLR+JSE and MCLR+OPE treated rats. Phosphate levels of group C (compared with group F) and group D (compared with group E) show decrease. After 30 day exposure to MCLR; MCLR+JSE and MCLR+OPE phosphate levels decreased. Levels of group C and D when compared with group B are increased. Phosphate levels of group C (compared with group F) and D (compared with group E) are decreased. This indicates that OPE and JSE treatment provoked some recovery of phosphate levels.

Keywords: Cyanobacteria; Microcystin; Serum calcium; Serum phosphate; Jamun seed; Orange peel.

1. INTRODUCTION

Cyanobacteria which are found in freshwater, eutrophic and municipal water supplies [1] produce hepatotoxins, neurotoxins and lipopolysaccharide endotoxins [2]. Cyanobacteria, after ingestion adversely affects domestic and aquatic animals as well as humans [2-4].

Microcystis aeruginosa is commonly observed in cyanobacterial blooms [4] which produce a cyclic hepatotoxin - microcystin LR [2, 3, 5]. Microcystin accumulates in organisms' body such as shrimp [6-8],

snails [6, 8], bivalves [9], frogs [8] and human liver [10]. Microcystin LR has been reported to produce adverse effects on liver [2, 5], kidney [4, 11-13], heart [4], germ cell apoptosis [14] and human respiratory system [15].

It has been reported that several plants possess antioxidant properties. Seeds of Jamun (*Syzygium cumini*) contains gallic acid, quercetin, jambosine, ellagic acid, 1-galloylglucose, corilagin, 3,6-hexahydroxy diphenoylglucose, β-sitoterol,3-galloylglucose, 4,6-hexahydroxy diphenoylglucose, etc. [2]. Jamun has been attributed to produce some biological activities namely - hypoglycemic, hepatoprotective, cardioprotective, anti-inflammatory, antineoplastic, hypolipidemic, antibacterial and antiallergic [2, 16, 17]. Orange (*Citrus sinensis*) peel contains flavonoids, hespiridin, alkaloids, limnoids, carotenoids, acridone vitamin C and B complex, essential oils and minerals [2, 18, 19] and possess antioxidant, antibacterial, larvicidal, antifungal, anti-inflammatory and antihypertensive activity [20-25].

In vertebrates calcium plays a vital role in various physiological processes and a minor change in its level severely affects these [26]. Therefore, the blood calcium levels are maintained strictly. Hence, the present study evaluated the protective effects of extracts of jamun (*Syzygium cumini*) seeds and orange (*Citrus sinensis*) peels against microcystin LR (MCLR) induced alterations in serum calcium and phosphate of male rats. Prior to this study no report exists regarding protective effects of extracts of seed of *Syzygium cumini* (JSE) and peels of *Citrus sinensis* (OPE) on serum calcium and phosphate induced by microcystin LR.

2. MATERIALS AND METHODS

Male Wistar rats (70-90 g) were housed in polypropylene cages under laboratory conditions and acclimatized for 2 weeks prior to experimentation. The rats were maintained on the standard laboratory feed and water *ad libitum* throughout the acclimation and experimental periods. Animal handling and sacrifice were carried out following the guidelines provided by Ethics Committee of the University (No. F.Sc.9008/D/S/3-4-14).

After acclimatization rats were separated into six groups - A, B, C, D, E, and F, each consisting of 20 animals. Following treatments were orally given daily to these groups at 08:00 each day throughout the experiment:

Group A: Control: No treatment was given

Group B: Microcystin-treated rats (MCLR): given microcystin (10 µg/kg body wt)

Group C: Microcystin + jamun seed extract (MCLR+JSE): received microcystin (10 µg/kg body wt) and jamun seed extract (200 mg/kg body wt) simultaneously

Group D: Microcystin + orange peel extract (MCLR+OPE): given microcystin (10 µg/kg body wt) and orange peel extract (200 mg/kg body wt) simultaneously

Group E: Orange peel extract (OPE): rats received orange peel extract (200 mg/kg body wt)

Group F: Jamun seed extract (JSE): given jamun seed extract (200 mg/kg body wt).

Rats (10 from each group) from all the groups were sacrificed 24 h after last dose on 15th and 30th day after initiation of the experiment under light ether anesthesia animals were fasted overnight before sacrifice.

Purified microcystin LR was purchased from Enzo Life Sciences, Inc. and dissolved in 0.9% NaCl for use in experiment. Details regarding the preparation of jamun seed and orange peel extracts have been given by Srivastava et al. [27].

Blood samples were collected (under slight ether anesthesia) by cardiac puncture by using 3 ml syringes and 23 gauge needles and were allowed to clot at room temperature. Sera were separated by centrifugation (at 3000 rpm) and kept at -20ºC until analyzed for serum calcium (Calcium kit, Sigma-Aldrich)

and inorganic phosphate (Pointe Scientific, USA). All determinations were carried out in duplicates for each sample.

Each data represents mean ± S.E. of five specimens. Student's t test was used to determine statistical significance. In all studies, the experimental group was compared to its specific time control group. Analysis of variance (ANOVA) was used to observe the significant differences between different exposure periods and different treatments.

3. RESULTS

Serum calcium levels of rat exposed for 15 day to microcystin LR (MCLR; group B; P<0.0001); microcystin LR and jamun seed extract (MCLR+JSE; group C; P<0.0001) and microcystin LR and orange peel extract (MCLR+OPE; group D; P<0.0005) exhibit a decrease (Fig. 1) as compared to the calcium levels of control rats (group A). The calcium levels remain unaffected in rats treated only with orange peel extract (OPE; group E) and jamun seed extract (JSE; group F). The calcium levels of group B (MCLR) is not significant when compared with group C (MCLR+JSE) and group D (MCLR+OPE) which clearly indicates that although there is a little increase in levels of group C and group D (as compared to group B) but the treatment with OPE and JSE is not able to completely restore the decrease in calcium levels caused by microcystin treatment. This derives support from the significant decrease in levels when group C was compared with group F (P<0.015) and group D was compared with group E (P<0.014). Analysis of variance (ANOVA) indicates that the treatments are significant (F=8.626; P< 0.0001).

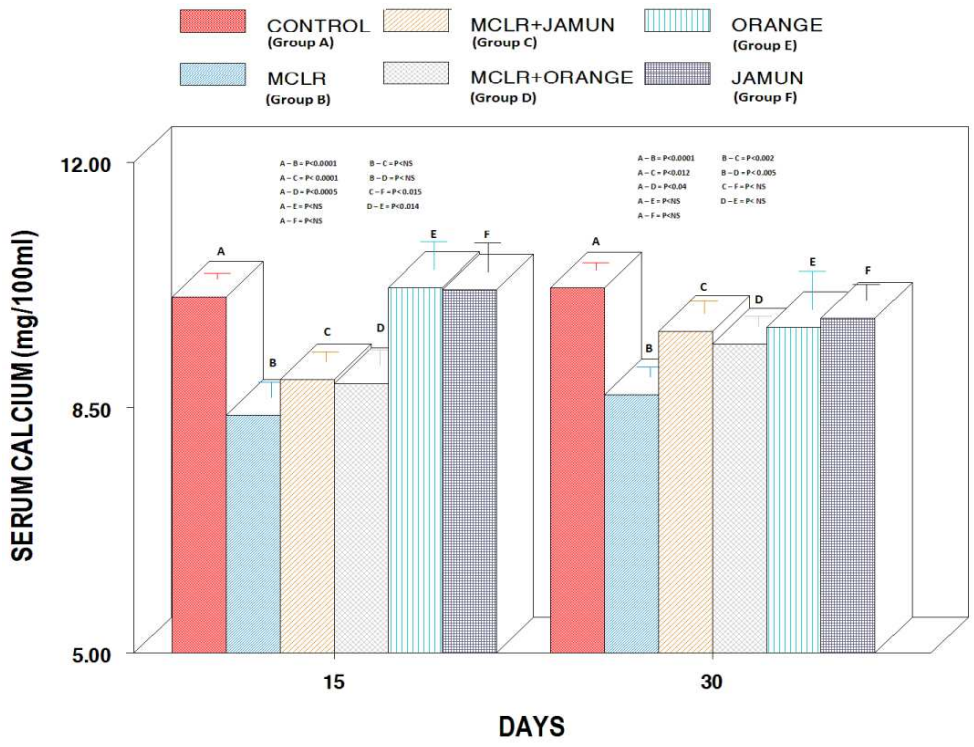

Figure 1. Serum calcium levels (mg/100 ml) of Wistar rat treated either with microcystin, microcystin+jamun seed extract, microcystin+orange peel extract, orange peel extract or jamun seed extract. All values indicate mean ± SE of five specimens.

After 30 day there is decrease in the serum calcium levels (Fig. 1) following treatment with MCLR (group B; P<0.0001; the value is slightly increased as compared to value at day 15); MCLR+JSE (group C;

P<0.012) and MCLR+OPE (group D; P<0.048). The levels in group E (OPE) and group F (JSE) remain unaltered. The levels in group C and group D exhibit a significant elevation as compared to group B which is indication that treatment with OPE and JSE is effective in recovering the decrease in calcium levels caused by the treatment with MCLR. ANOVA indicates that the treatment is significant (F= 2.874; P< 0.03).

The serum inorganic phosphate levels exhibit a decrease after 15 day in MCLR (group B; P<0.001); MCLR+JSE (group C; P<0.003) and MCLR+OPE (group D; P<0.009) treated rats (Fig. 2). Treatment with OPE (group E) and JSE (group F) could not provoke any alteration in phosphate levels. The phosphate levels of group C and group D when compared with group B are insignificant which is indicative of non-recovery of decreased phosphate levels provoked by MCLR. Phosphate levels of group C (when compared with group F) and group D (when compared with group E) show significant decrease. ANOVA indicates that all treatments are significant (F=6.131; P< 0.005).

After 30 day exposure to MCLR (group B; P<0.0002); MCLR+JSE (group C; P<0.0003) and MCLR+OPE (group D; P<0.001) the serum phosphate levels exhibit a decline (Fig. 2) as compared to control (group A). The levels of group E (OPE) and group F (JSE) remain unchanged. The levels of group C (P<0.02) and group D (P<0.01) when compared with group B are significantly increased. Phosphate levels of group C (compared with group F; P<0.015) and group D (when compared with group E; P<0.006) are significantly decreased. This indicates that although OPE and JSE treatment have provoked recovery of serum phosphate levels but could not be able to restore the normophosphatemia. ANOVA indicates significant differences between treatments (F=13.092; P<0.0001).

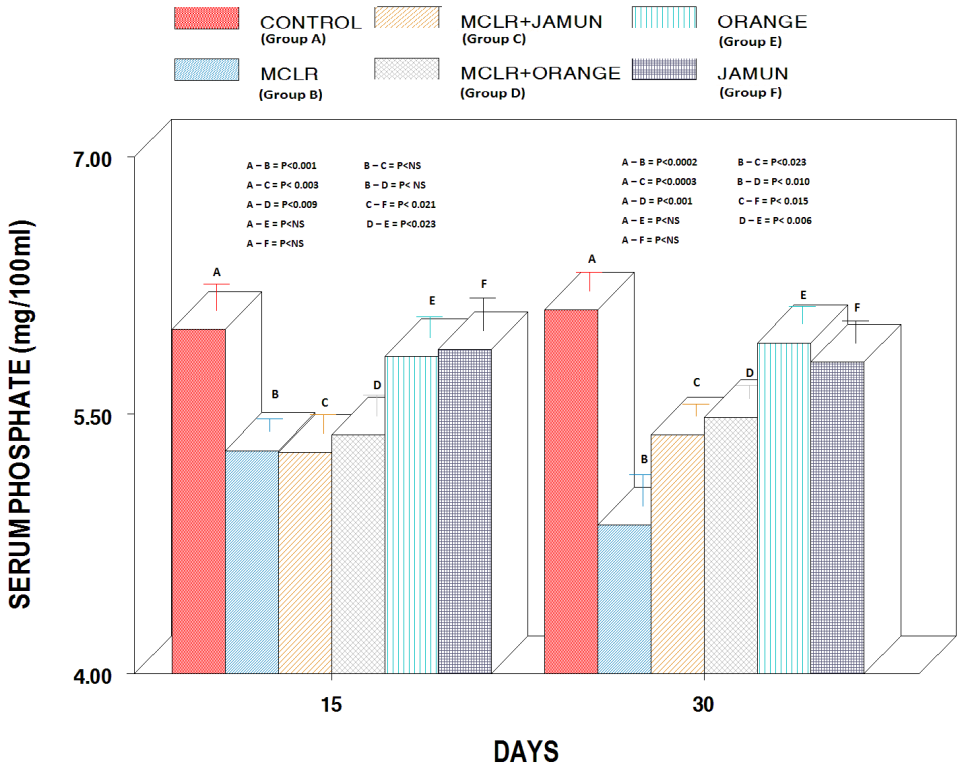

Figure 2. Serum phosphate levels (mg/100 ml) of Wistar rat treated either with microcystin, microcystin+jamun seed extract, microcystin+orange peel extract, orange peel extract or jamun seed extract. All values indicate mean ± SE of five specimens.

4. DISCUSSION

In the foregoing study MCLR treated rats exhibited hypocalcemia. This is in agreement with the observations of other workers who have also noticed hypocalcemia in microcystin LR exposed rats [28] and fish [29, 30]. In contrast, Hooser et al. [31] have not noticed any change in blood calcium levels of rats within 24 h after intraperitoneal injection of lethal dose of MCLR (160 µg/kg bw). The observed hypocalcemia in the present study derives support from the reports of other researchers who have also reported hypocalcemia after administration of toxicants in rats after treatment with heptachlor [32]; diazinon [33]; mipcin [34]; heroin [35]; cadmium [36] and chlorpyrifos [37]. Andjelkovic et al. [38] have noticed a reduction in serum calcium levels after cadmium exposure to rats, however, the decrease in levels was not significant.

Several investigators have noticed hypocalcemia in fish after treatment with cypermethrin [39]; deltamethrin [40]; cadmium [41-43]; and botanical pesticides [44-47]. In chickens gamma benzene hexachloride and quinalphos provoked hypocalcemia [48].

Exposure of microcystin LR to rats caused hypophosphatemia. Hypophosphatemia has been noticed in rats [28] and in fish *Heteropneustes fossilis* [29] after exposure to microcystin LR. Hooser et al. [31] have reported that in microcystin LR exposed rats the blood phosphorus varied inconsistently. In chicken treated with gamma benzene hexachloride and quinalphos hypophosphatemia has been noticed by Agarwal et al. [48]. Several investigators have reported occurrence of hypophosphatemia after exposure of various toxicants to fish - chlorpyrifos [49], deltamethrin [50], cypermethrin [51], cadmium [41], lead [52], azadirachtin [44], *Nerium indicum* leaf extract [46], *Euphorbia royleana* [47] and *Euphorbia tirucalli* [45]. However, serum phosphate level remained unaffected in rats treated with heptachlor [32], diazinon [33] and mipcin [34]. In contrast, hyperphosphatemia has been noticed in heroin administered rats [35].

In the past, few investigators have noticed degeneration in the kidney after exposure of toxicants to mammals [53, 54]. Moreover, toxicants also induced a decrease in the intestinal calcium absorption [55]. Toxicant induced kidney degeneration may result into increased urinary output [53]. In the present study, degeneration in kidney has been noticed in MCLR treated rats. Hence, the observed hypocalcemia and hypophosphatemia in rats exposed to MCLR could be attributed to the degeneration in kidney tubules thus affecting increased efflux of these electrolytes and/or decreased intestinal absorption of these electrolytes. Degenerative changes in the renal tubules have also been suggested to be the main causes of hypocalcemic responses in toxicant treated fishes [56]. Lead-induced ionoregulatory toxicity in rainbow trout, particularly the disturbance of Ca^{2+} homeostasis has been suggested as not exclusively a branchial phenomenon, but is in part a result of disruption of ionoregulatory mechanisms at the kidney [57]. In rats treated with mipcin [34] and heroin [35], the observed hypocalcemia has been attributed to the degenerating changes in the parathyroid glands. Heroin-induced hyperphosphatemia has been suggested by Barai et al. [35] due to degenerating changes in parathyroid cells.

5. CONCLUSION

Based on the findings of this study, we can conclude that exposure to microcystin adversely affected the serum calcium and phosphate of the rats. The disturbances in these vital electrolytes could be protected by supplementation of extracts of jamun seed and orange peel. It is advisable that the organisms, particularly the aquatic ones, exposed to microcystin should be given dietary supplement of these botanical extracts which would ease the toxic symptoms.

Authors' Contributions: All authors contributed equally to this work. All authors read and approved the final manuscript.

Conflict of Interest: The authors have no conflict of interest to declare.

Acknowledgments: Ajai Kumar Srivastav is thankful to University Grants Commission, New Delhi, India for providing financial assistance for this study. This work was also partly supported by the cooperative research program of the Institute of Nature and Environmental Technology, Kanazawa University, Japan (Accept No. 20007).

REFERENCES

1. Filipic M, Zegura B, Sedmak B, Horvat-Znidarsic I, Milutinovic A, Suput D. Subchronic exposure of rats to sublethal dose of microcystin-YR induces DNA damage in multiple organs. Radiol Oncol. 2007; 41:15-22.
2. Srivastava BD, Srivastav AK. Hepatoprotective effects of extracts of *Syzygium cumini* seeds and *Citrus sinensis* peels against microcystin LR-induced toxicity in rat. Int J Zool Invest. 2017; 3: 95-105.
3. Benson JM, Hutt JA, Rein K, Boggs SE, Barr EB, Fleming LE. The toxicity of microcystin LR in mice following 7 days of inhalation exposure. Toxicon. 2005; 45: 691-698.
4. Shahi N, Monalisa S, Mallika SK, Sarmaa D, Dasa P. The microcystins-induced DNA damage in the liver and the heart of zebrafish, *Danio rerio*. Toxicol Environ Chem. 2012; 94: 340-349.
5. Kim B, Cho JW, Kwon HN, Blank I, Borisova I, Ejaz S, Chekarova I, Kwon J, Lim CW. Hepatotoxicity induced by microcystin-LR in rat. J Toxicol Pub Hlth. 2006; 22: 375-380.
6. Xie I, Xie P, Guo L, Miyabara Y, Park H. Organ distribution and bioaccumulation of microcystins in freshwater fish and different trophic levels from the eutrophic lake Chaohu, China. Environ Toxicol. 2005; 20: 293-300.
7. Zhang D, Den X, Xie P, Yang Q, Chen J, Dai M. Determination of microcystin-LR and its metabolites in snail (*Bellamya aeruginosa*), shrimp (*Macrobrachium nipponensis*) and silver carp (*Hypophthalmichthys molitrix*) from Lake Taihu, china. Chemosphere. 2009; 76: 974-981.
8. Papadimitriou T, Kagalou I, Stalikas C, Pilidis G, Leonardos ID. Assessment of microcystin distribution and biomagnification in tissues of aquatic food web compartments from a shallow lake and evaluation of potential risks to public health. Ecotoxicol. 2012; 21: 1155-1166.
9. Amorim A, Vasconcelos V. Dynamics of microcystins in the mussel *Mytilus galloprovinvialis*. Toxicon. 1999; 37: 1041-1052.
10. Greer B, Meneely JP, Elliott CT. Uptake and accumulation of microcystin-LR based on exposure through drinking water: An animal model assessing the human health risk. Sci Rep. 2018; 8: 4913.
11. Dias E, Mariana A, Elsa A, Paulo P, Batoréu MCC, Jordan P, Silva MJ. Comparative study of the cytotoxic effect of microcistin-LR and purified extracts from *Microcystis aeruginosa* on a kidney cell line. Toxicon. 2009; 53: 487-495.
12. Xiping Y, Shuaishuai X, Feiyu H, Cong W, Shuilin Z, Hai F, Jian G, Jihua C, Xiangling,F, Fei Y. Effects of chronic exposure to microcystin-LR on kidney in mice. Int J Environ Res Public Hlth. 2019; 16: 5030.
13. Wang Z, Li G, Wu Q, Liu C, Shen J, Yan W. Microcystin-LR exposure induced nephrotoxicity by triggering apoptosis in female zebrafish. Chemosphere. 2019; 214: 598-605.

14. Zhao S, Liu Y, Wang F, Xu, D, Xie P. N-acetylcysteine protects against microcystin-LR-induced endoplasmic reticulum stress and germ cell apoptosis in zebrafish testes. Chemosphere. 2018; 204: 463-473.
15. Brózman O, Kubickova B, Pavel B, Petra L. Microcystin-LR does not alter cell survival and intracellular signaling in human bronchial epithelial cells. Toxins. 2020; 12: 165.
16. Raza A, Butt MS, Ul-Haq I, Suleria HAR. Jamun (*Syzygium cumini*) seed and fruit extract attenuate hyperglycemia in diabetic rats. Asian Pac J Trop Biomed. 2017; 7: 750-754.
17. Chagas VT, Coelho RMRS, Gaspar RS, Silva SA, Mastrogiovanni M, Mendoca CJ, et al. Protective effects of a polyphenol-rich extract from *Syzygium cumini* (L.) skeels leaf on oxidative stress-induced diabetic rats. Oxidative Med Cellular Long. 2018; 2018: ID 5386079.
18. Muhtadi H, Azizah T, Suhendi A, Yen KH. Antidiabetic and antihypercholesterolemic activities of *Citrus sinensis* peel: in vivo study. Nat J Physiol Pharm Pharmacol. 2015; 5: 382-385.
19. Ashraf H, Butt MS, Iqbal MJ, Suleria HAR. *Citrus* peel extract and powder attenuate hypercholesterolemia and hyperglycemia using rodent experimental modeling. Asian Pacif J Trop Biomed. 2017; 7: 870-880.
20. Gorinstein S, Martin-Belloso O, Park Y, Haruenkit R, Ciz M. Comparison of some biochemical characteristics of different citrus fruits. Food Chem. 2001; 74: 309-315.
21. Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak J Pharmac Sci. 1985; 22: 277-281.
22. Giri J, Sathidevi T, Dushyanth, N. Effects of jamun seed extract on alloxan induced diabetes in rats. J Diabetic Assoc India. 2009; 25: 115-119.
23. Ramful D, Bahorum T, Bourdom E, Tarmus E, Aruoma OI. Bioactive phenolics and antioxidant propensity of flavedo extracts of Mauritian citrus fruits: Potential prophylactic ingredients for functional foods application. Toxicology. 2010; 278: 75-87.
24. Hegazy AE, Ibrahim MI. Antioxidant activities of orange peel extract. World Appl Sci J. 2012; 18: 684-688.
25. Parashar S, Sharma H, Garg M. Antimicrobial and antioxidant activities of fruits and vegetable peels: A review. J Pharmacol Phytochem. 2014; 3: 160-164.
26. Srivastav AK, Yadav S, Srivastav SK, Suzuki N. The ultimobranchial gland in poikilotherms: Morphological and functional aspects. In: Experimental endocrinology and reproductive biology. Haldar C, Singaravel M, Pandi-Perumal SR, Cardinali DP, eds. Science Publishers, U.S.A. 2008: 269-296.
27. Srivastava BD, Srivastava M, Srivastav SK, Suzuki N, Srivastav AK. Cypermethrin-induced liver histopathology in rat: Protective role of jamun seed and orange peel extracts. Iranian J Toxicol. 2018; 12: 25-30.
28. Moreno IM, Mate A, Repetto G, Vázquez CM, Cameán AM. Influence of microcystin-LR on the activity of membrane enzymes in rat intestinal mucosa. J Physiol Biochem. 2003; 59: 293-300.
29. Prakash C, Kumar A, Prasad M, Srivastav SK, Srivastav AK. Microcystin-LR attenuates blood calcium and phosphate levels in stinging catfish *Heteropneustes fossilis*. Int J Pharm Bio Sci. 2015; 6: 1147-1153.
30. Prakash C, Prasad M, Kumar A, Mishra D, Chaudhary A, Srivastav SK. Protective effect of ZnCl2 on toxicity produced by microcystin-LR on serum calcium and phosphate levels of freshwater catfish *Heteropneustes fossilis*. Int J Pure App Biosci. 2016; 4: 111-117.

31. Hooser SB, Beasley VR, Lovell RA, Carmichael WW, Haschek WM. Toxicity of microcystin-LR , a cyclic hepatopeptide hepatotoxin from *Microcystis aeruginosa* to rats and mice. Vet Pathol. 1989; 26: 246-252.
32. Rangoonwala SP, Suryawanshi SA, Pandey AK. Responses of serum calcium and inorganic phosphate levels, parathyroid gland and C cells of I to heptachlor administration. J Environ Biol. 2004; 25: 75-480.
33. Rangoonwala SP, Kazim M, Pandey AK. Effects of diazinin on serum calcium and inorganic phosphate levels as well as ultrastructures of parathyroid and calcitonin cells of *Rattus norvegicus*. J Environ Biol. 2005; 26: 217-221.
34. Rangoonwala SP, Suryawanshi SA, Pandey AK. Responses of serum calcium and inorganic phosphate levels as well as parathyroid gland and calcitonin producing C cells of *Rattus norvegicus* to mipcin administration. J Environ Biol. 2007; 28: 475-481.
35. Barai SR, Suryawanshi SA, Pandey AK. Responses of parathyroid gland, C cells, and plasma calcium and inorganic phosphate levels in rat to sub-lethal heroin administration. J Environ Biol. 2009; 30: 917-922.
36. Tripathi S, Srivastav AK. Alterations in the serum electrolytes, calcitonin cells and parathyroid gland of Wistar rat in response to administration of cadmium. Proc. Intern. Con. Environ. Pollution Remediation Ottawa, Ontario, Canada, 17-19 August 2011, Paper No. 126. 2011.
37. Tripathi S, Suzuki N, Srivastav AK. Response of serum minerals (calcium, phosphate and magnesium) and endocrine glands (calcitonin cells and parathyroid gland) of Wistar rat after chlorpyrifos administration. Microscopy Res Techn. 2013; 76: 673-678.
38. Andjelkovic M, Buha Djordjevic A, Antonijevic E, Antonijevic B, Stanic M, Kotur-Stevuljevic J, et al. Cyanobacterial toxins and human health. Rev Med Microbiol. 1994; 5: 256-264.
39. Mishra D, Tripathi S, Srivastav SK, Suzuki N, Srivastav Ajai K. Corpuscles of *Stannius* of a teleost *Heteropneustes fossilis* following intoxication with a pyrethroid (cypermethrin). North-Western J Zool. 2010; 6: 203-208.
40. Srivastav AK, Srivastava SK, Mishra D, Srivastav SK. Deltamethrin-induced alterations in serum calcium and prolactin cells of a freshwater teleost, *Heteropneustes fossilis*. Toxicol Environ Chem. 2010; 92: 1857-1864.
41. Rai R, Srivastav AK. Effects of cadmium on the plasma electrolytes of a freshwater fish *Heteropneustes fossilis*. J Ecophysiol Occup Hlth. 2003; 3: 63-70.
42. Chowdhury MJ, Pane FF, Wood CM. Physiological effects of dietary cadmium acclimation and waterborne cadmium challenge in rainbow trout: respiratory, ionoregulatory, and stress parameters. Comp Biochem Physiol C. 2004; 139: 163-173.
43. Suzuki N, Yamamoto M, Watanabe K, Kambegawa A, Hattori A. Both mercury and cadmium directly influence calcium homeostasis resulting from the suppression of scale bone cells: the scale is a good model for the evaluation of heavy metals in bone metabolism. J Bone Mineral Metabol. 2004; 22: 439-446.
44. Kumar A, Prasad M, Mishra D, Srivastav SK, Srivastav AK. Botanical pesticide, azadirachtin attenuates blood electrolytes of a freshwater fish *Heteropneustes fossilis*. Pest Biochem Physiol. 2011; 99: 170-173.
45. Kumar A, Prasad M, Mishra D, Srivastav SK, Srivastav AK. Effects of *Euphorbia tirucalli* latex on blood electrolytes (calcium and phosphate) of a freshwater air-breathing catfish *Heteropneustes fossilis*. Toxicol Environ Chem. 2011; 93: 585-592.

46. Prasad M, Kumar A, Mishra D, Srivastav SK, Srivastav AK. Alterations in blood electrolytes of a freshwater catfish *Heteropneustes fossilis* in response to treatment with a botanical pesticide, *Nerium indicum* leaf extract. Fish Physiol Biochem. 2011; 37: 505-510.
47. Prasad M, Kumar A, Mishra D, Srivastav SK, Srivastav AK. Blood electrolytes of a freshwater catfish *Heteropneustes fossilis* in response to treatment with a botanical pesticide, *Euphorbia royleana* latex. Integrative Zool. 2011; 6: 150-156.
48. Agarwal S, Batra M, Chauhan S. Effects of gamma benzene hexachloride and quinalphos on serum calcium and phosphorus levels in experimentally fed broiler chickens. J Immunol Immunopathol. 2009; 11: 57-59.
49. Srivastav AK, Srivastava SK, Srivastava AK. Response of serum calcium and inorganic phosphate of freshwater catfish, *Heteropneustes fossilis*, to chlorpyrifos. Bull Environ Contam Toxicol. 1997; 58: 915-921.
50. Srivastav AK, Srivastava SK, Srivastav SK. Impact of deltamethrin on serum calcium and inorganic phosphate of freshwater catfish *Heteropneustes fossilis*. Bull Environ Contam Toxicol. 1997; 59: 841-846.
51. Mishra D, Srivastav S, Srivastav SK, Srivastav AK. Plasma calcium and inorganic phosphate levels of a freshwater catfish, *Heteropneustes fossilis* in response to cypermethrin treatment. J Ecophysiol Occup Hlth. 2001; 1: 131-138.
52. Srivastav AK, Rai R, Suzuki N, Mishra D, Srivastav SK. Effects of lead on the plasma electrolytes of a freshwater fish, *Heteropneustes fossilis*. Int Aquat Res. 2013; 5: 4.
53. Prozialeck WC, Edwards JR, Vaidya VS, Bonventre JV. Preclinical evaluation of novel urinary biomarkers of cadmium nephrotoxicity. Toxicol Appl Pharm. 2009; 238: 301-305.
54. Tripathi S, Srivastav AK. Cytoarchitectural alterations in kidney of Wistar rat after oral exposure to cadmium chloride. Tissue Cell. 2011; 43: 131-136.
55. Chertok RJ, Sasser LB, Callaham MF, Jarboe GE. Influence of cadmium on the intestinal uptake and absorption of calcium in the rat. J Nutr. 1981; 111: 631-638.
56. Haux C, Larsson A. Long term sublethal physiological effects on rainbow trout, *Salmo gairdneri*, during exposure to cadmium and after subsequent recovery. Aquat Toxicol. 1984; 5: 129-142.
57. Patel M, Rogers JT, Pane EF, Wood CM. Renal responses to acute lead waterborne exposure in the freshwater rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol. 2006; 30: 362-371.