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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 06

Res. Jr. of Agril. Sci. (2022) 13: 1842–1848

CARAS

Res. Jr. of Agril. Sci. (Nov-Dec 2022)
13(6): 1842–1848

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

www.rjas.org

Full Length Research Article

Ameliorative Effect of *Punica granatum* Peel Against Dimethoate Toxicity on Protein Profile and Histopathology of Liver and Kidney of Aged Mice

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Received: 24 Sep 2022 | Revised accepted: 20 Nov 2022 | Published online: 15 Dec 2022

A B S T R A C T

Antioxidants prevents damage caused by various Reactive Oxygen Species (ROS). *P. granatum* is an ancient fruit and possess the lots of therapeutical significance due to the presence of various phytochemical constituents like tannins, alkaloids, glycosides, volatile oils, flavonoids and resins, gums. Therefore, the present work was carried out to check hepatoprotective and renoprotective potency of methanolic extract of *Punica granatum* peel. To evaluate ameliorative effect, male albino mice (*Mus musculus* L.) were divided into four groups viz, a) Control group-received 0.5 ml d/w orally for 15 days. b) Toxicated group- received dimethoate 15mg/kg body weight orally /day for 15 days. c) PPME group-received 200mg/kg body weight of pomegranate peel methanolic extract for 15 days. d) Treated group-received dimethoate for 15 days and then after Pomegranate peel extract 200mg/kg body weight for 15 days. The histological study showed hepatocellular hypertrophy, karyomegaly and increased number of Kuffer cells in liver and glomerular shrinkage (Glomeruloaclerosis), tubular dilatation, hypertrophy, Tubular atrophy in aged Kidney as compared to control. While treated group showed recovery in liver and kidney tissue. Thus, above results elucidate the protective effect of pomegranate peel against aging in liver and kidney of aged mice.

***Key words*:** Oxidative stress, Dimethoate, Histopathology, Protein, *Punica granatum peel* extract

Aging accelerates continuous decline in metabolic activity and all physiological functions of tissue and impaired regeneration, it causes functional limitations [1-3] imbalanced homeostasis, increased susceptibility, vulnerability for chronic diseases, disability and mortality [4]. The important pathway and mechanism behind the aging process is oxidative stress and lipid peroxidation resulted from the accumulation of cellular oxidative free radicals [5]. The cellular deterioration is the main effect of aging process [6]. Due to cellular damage and stress, aged cells willingly undergo senescence and exhibits age-associated pathological changes through its morphologically appearances and secretary activities [7].

The liver is a complex metabolic organ which is essential for maintaining whole body homeostasis by regulation of energy metabolism, xenobiotic clearance, and molecular biosynthesis [8] so age-related changes in liver function give to systemic susceptibility to age-related diseases. The anatomical changes during liver aging mostly include decline in the liver volume and blood flow of the liver. According to studies, the liver volume decreases by 20–40% as one gets older [9-11]. In rodent and human hepatocytes, the number of dense bodies increases with aging [12-13]. The aging process is strongly connected to a number of degenerative changes in the liver, including a decrease in hepatic structure and cell function [14].

The kidney is one of the most target organs of experimental animals attacked by organopesticides [15-16]. Kidney aging is a normal physiological process linked with various molecular, morphologic and functional changes in the kidney tissues. The anatomical changes during kidney aging mostly include decline in the kidney volume due to the decrease of the renal cortical volume and increase of renal medullary volume [17-19]. Microscopic histological changes of kidney show development of arteriosclerosis of renal vessels. It includes hypertrophy, nephropathy and vascular hyalinosis, glomerulosclerosis, wrinkling glomerulus, adhesion of glomeruli with Bowman's capsule and decrease in number of functional glomeruli [20-22]. These structural changes in the kidney affects the rate of glomerular filtration, urinary concentration and hormonal production [23-24].

The pesticides are one of the most probably harmful chemicals released in the environment in an unplanned manner. The misuse of pesticide may be harmful to humans, animals and environment. Dimethoate is insecticide generally used in

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agricultural land [25] (Sharma et al. 2005). Dimethoate (IUPAC name O,O-diethyl S-methyl carbamoyl methyl phosphorodithioate) is a broadly used systemic and contact organophosphate insecticide in as well as indoor to control houseflies [26] [Rome, 1999]. Toxicity of dimethoate results in deleterious effects on many organs and systems in human and other mammals particularly the reproductive system, immune system, nervous system, sex hormones and liver [27-28], kidney [29]. The primary mechanism of action of Organophophosphate pesticides (OP) is based acetylcholinesterase (Ache) activity [30]. Few studies have been made on the histopathological effects of dimethoate in animals [31-32].

Oxidative stress usually alters the cellular antioxidant status and determines the susceptibility to oxidative damage. Natural antioxidants from fruits and vegetables are described to provide protection that slows down the process of oxidative damage caused by various reactive oxygen species (ROS) [33-35]. Recently pomegranate is considered as functional food having variety of healthy effects [36]. Different parts of pomegranate contain bioactive compounds such as phenolics, anthocyanins, vitamins and minerals [37-38]. This richness ascribed to them a antioxidant activity and protective effects against major diseases [39]. Finally, more studies need to be designed to confirm all these positive properties of pomegranate on human or animal health. In view of these facts the purpose of our study was to evaluate the ameliorative effect of *Punica granatum* peel against dimethoate toxicity on histopathology of liver and kidney of aged mice.

MATERIALS AND METHODS

Chemicals

All chemicals used for this experiment were obtained from sigma chemicals CO. USA, Dimethoate (Technical grade) purchased from SRL, Hyderabad.

Plant sample

The fresh pomegranate fruits, were purchased from local market, dried and powdered before extraction. An extract of the pomegranate peel prepared by mashing in a proportion of 1:2:2 (w peel/v water/v methanol) and kept for about 48 hours in refrigerator. After Mashing, the resulting extract was filtered and then the solvent was evaporated under reduced pressure at 40 – 50 °C. The extract was stored at 3 – 4 °C [40].

Animals

Healthy Swiss albino mice *Mus musculus* Linn. were used for present investigation. The mice were obtained from Rajarambapu College of Pharmacy, Kasegaon, Tal -Walwa; District Sangli - 415 404 (1290/PO/Re/S/09/CPCSEA,16th Mar.2019) Adult mice 35 to 40 ± 2 gm/BW were used for present investigation. They were fed with Amrut mice feed (Pranav Agro Industries, Pvt. Ltd, Sangli) and water *ad libitum*. All animals were housed in plastic cages with daily observations. Animals were maintained under controlled laboratory conditions 12h dark/light cycle,24-25°C temperature and 35-60 % relative humidity.

Experimental design

Mice were divided into Four groups (n = 6)

Group I: Control group

The adult and old mice were given oral administration of 0.5 ml of distilled water/ day/ animal for 15 days.

Group II: DM Toxicated group

The adult and old mice were given dimethoate 15mg/kg bw/ day/animal for 15 days by orally with oral gavage.

Group III: PPME group

The adult and old mice were received 200 mg/kg body weight of pomegranate peel methanolic extract for 15 days [41].

Group IV: Curative group

The adult and old mice were received dimethoate 15mg/kg bw/ day/ for 15 days and then followed by Pomegranate peel extract 200mg/kg body weight [41] for 15 days.

After completion of the treatment mice were dissected and liver and kidney were removed and fixed for histopathological studies using 10% neutral formalaline for 24 hrs. After routine processing, paraffin –embedded (58-60 °C) tissue samples were sectioned at 4-5µm thickness and stained with harries haematoxylin-eosin [42]. Finally stained sections were observed under light microscope and photographs were taken.

Body weight

Animals were examined daily throughout the experimental period for signs of toxicity. Body weight was recorded.

Statistical analysis

The obtained results are presented as means ± standard deviations (SD). Comparisons were made between control and treatment groups using ‘student t’ test. Probability values of $p < 0.05$ were regarded as statistically significant.

RESULTS AND DISCUSSION

I) *Body weight*

(Graph 1-2) showed that the administration of dimethoate induced a significant decrease ($p < 0.05$) in the body weight comparing with the control groups. Whereas combination of DM and Peel was significantly **(**$p < 0.05$) significantly able to increase the body weight comparing with dimethoate group in both adult and aged mice.

II) *Protein profile*

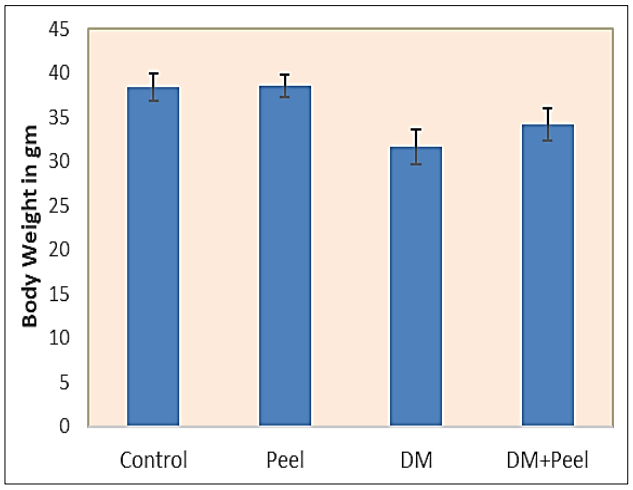
Total protein content in the liver and kidney of control and exposed groups are presented in the (Graph 3-4) respectively. At the end of 15-day dimethoate treatment period, liver protein level was significantly increased and kidney protein level was significantly decreased compared to control groups. Whereas group treated with pomegranate peel alone or pomegranate peel supplemented to dimethoate treated group caused significant improved $p < 0.05$ in total protein of both liver and kidney of adult mice.

Data represented in (Graph 5-6) showed that dimethoate treated group high increased significantly $p < 0.05$ total protein in the liver and high significantly decreased $p < 0.05$ total protein in kidney compared to control group. Whereas pomegranate peel group alone or pomegranate peel supplemented to dimethoate treated group caused significant enhanced $p < 0.05$ in total protein of both liver and kidney of aged mice.

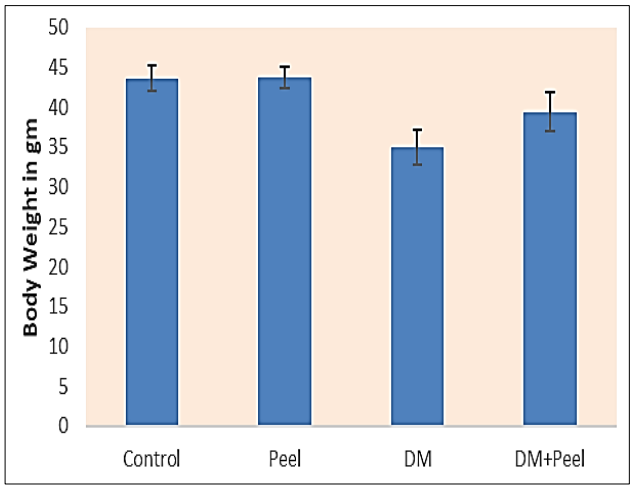
Histopathological study of liver

Histopathological examination of the liver sections in the control mice (Fig I(A) and II(A) showed a normal histological structure. The central vein located in the center of the lobule

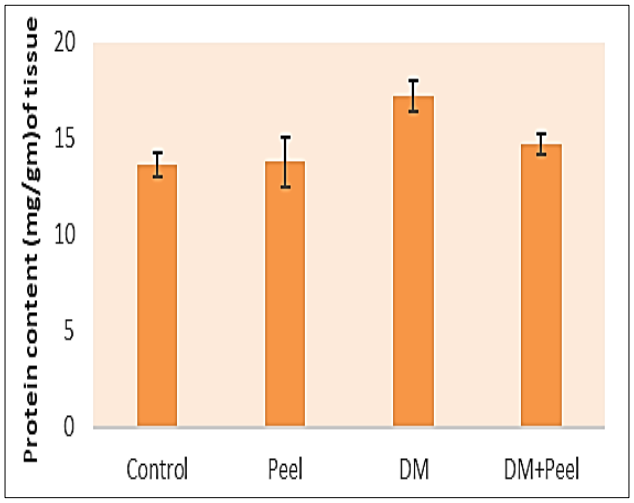
and it surrounded by hepatic cells. The distinct nuclei and hepatic sinusoids are also observed. The sections from treated mice showed changes in structure when compared with control mice (Fig I(B) and II(B). These changes include the liver congestion and Vasodilation, Lymphatic infiltration and cell pycnosis dead nuclei, congestion, necrosis and hemorrhage, Parenchyamal cells shows degeneration in nuclei and vacuolization and enlargement of hepatic sinusoids. Moreover, an increase in number of Kuffer cells was observed. The curative group (Fig I(C) and II(C) showed recovered structure of liver with normal central vein and hepatic cells as compared with normal structure of the liver.



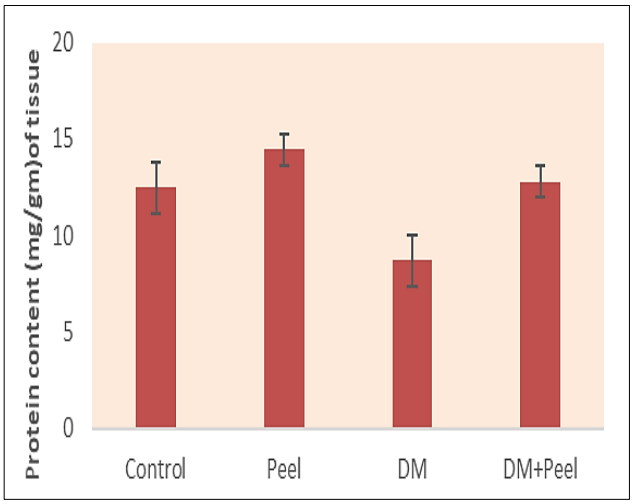
Graph 1 Effect of pomegranate peel extract on body weight (gm) of dimethoate induced adult mice



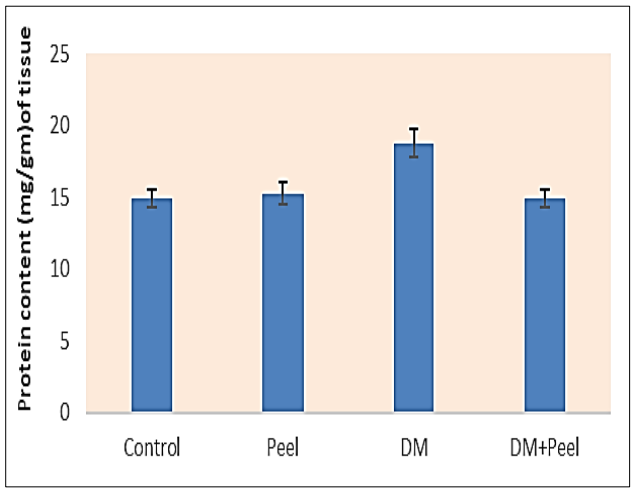
Graph 2 Effect of pomegranate peel extract on body weight (gm) of dimethoate induced aged mice



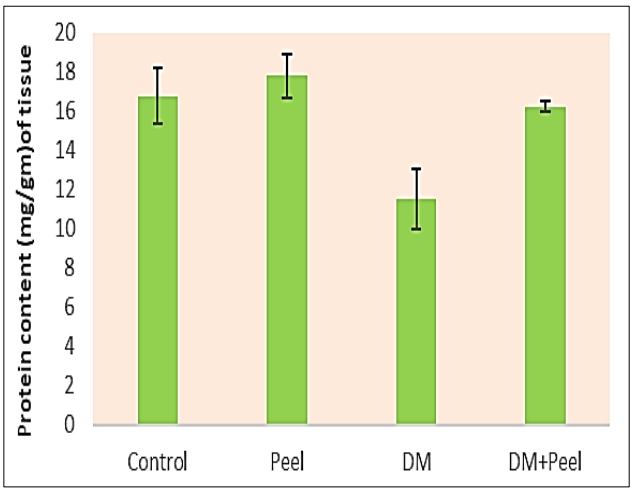
Graph 3 Effect of pomegranate peel extract on protein content (mg/gm of tissue) of dimethoate induced in liver of adult mice



Graph 4 Effect of pomegranate peel extract on protein content (mg/gm of tissue) of dimethoate induced in kidney of adult mice

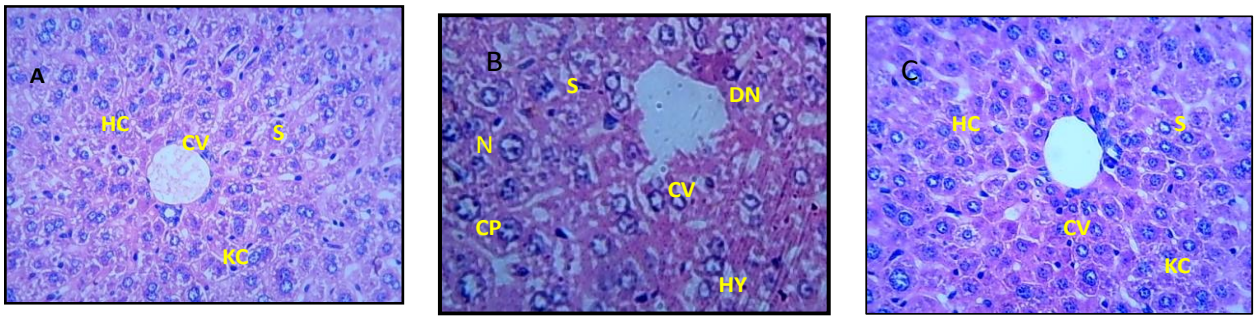


Graph 5 Effect of pomegranate peel extract on protein content (mg/gm of tissue) of dimethoate induced in liver of aged mice



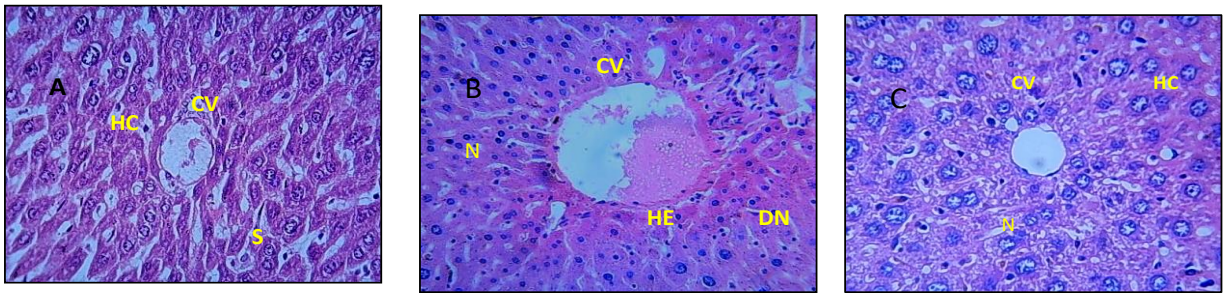
Graph 6 Effect of pomegranate peel extract on protein content (mg/gm of tissue) of dimethoate induced in kidney of aged mice

III) *Histopathology*



Adult Control Group-Liver Toxicated group Curtive Group (Peel extract)

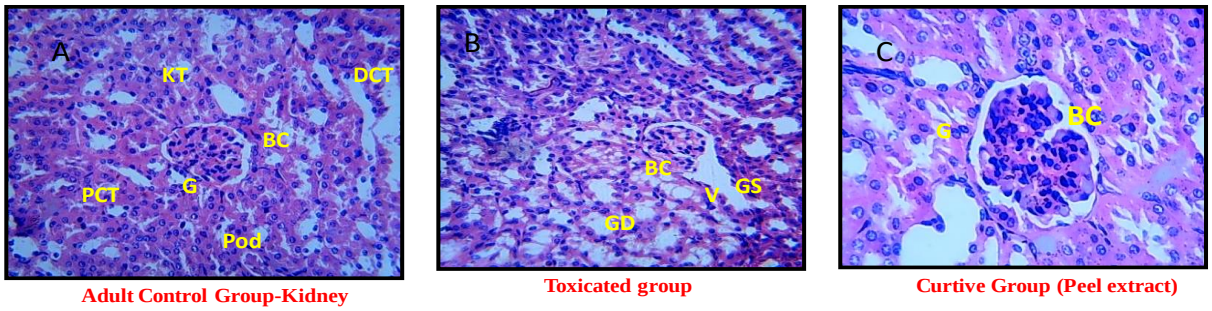
Figure I: Photomicrograph Of the Liver Sections Of adult Mice Treated With 15 mg/Kg B.W Of Dimethoate (H&E Stain X400). A] Control group ,B]DM Toxicated group -Central vein(CV), hepatic cells (HC),Sinusoids (S) , Kupffer Cells (KC), ,hypertrophy (HY),Necrosis(N), Dead nuclei (DN),Cell pycnosis(CP) C] Curative Group-Central vein(CN),Hepatic cells (HC).



Aged Control Group-Liver Toxicated group Curative Group (Peel extract)

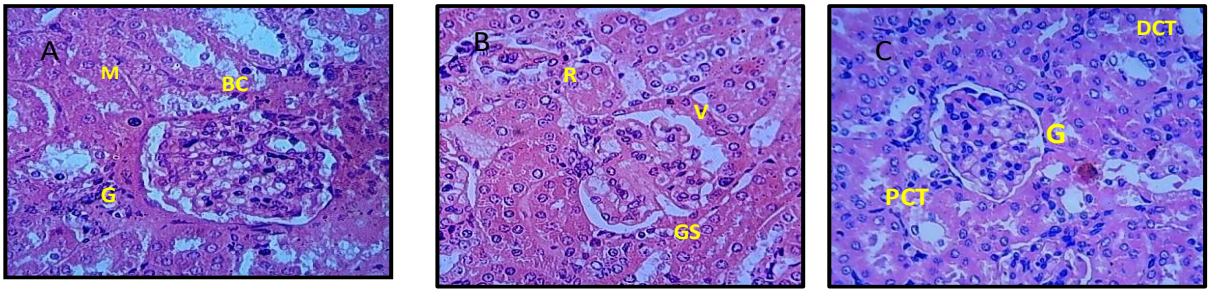
Figure II: Photomicrograph Of the Liver Sections Of aged Mice Treated With 15 mg/Kg B.W Of Dimethoate (H&E Stain X400). A] Control group ,B]DM Toxicated group -central vein(CV), hepatic cells (HC),Sinusoids (S) , Kupffer Cells (KC), ,hypertrophy (HY),Necrosis(N), Dead nuclei (DN),,Liver hemorrhage(HE ,Ccurative Group-Distinct lining of CV, Distinct nuclei(N)

Fig. I(A) and II(A): T.S. of liver of control adult and aged mice H.E. staining X 400 showing normal structure of liver
Fig. I(B) and II(B): T.S. of liver of toxicated adult and aged mice H.E. staining X 400 showing disturbed structure of liver
Fig. I(C), II(C): T.S. of liver of Pomegranate peel extract treated adult and aged mice H.E. staining X 400 showing recovered structure of liver



Adult Control Group-Kidney Toxicated group Curtive Group (Peel extract)

Figure III: Photomicrograph Of The Kidney Sections Of adult Mice Treated With 15 mg/Kg B.W Of Dimethoate (H&E Stain X400). A] Control group B] DM Toxicated group - Glomerular Degeneration(GD), Tubular Degeneration (TD) Hemorrhage(H) , Glomerular Shrinkage(GS), Vacuolation in the glomerulus. Hypoplsia in cells of Bowman's capsule, Rupture(R), ,Normal kidney tubules(KT),Podocytes(POD),Medullary rays(M),Distal convoluted tubule (DCT), Proximal convoluted tubule (PCT), C] Curative Group -Intact Bowmans capsule,Glomerulus



Aged Control Group-Kidney Toxicated group Curtive Group (Peel extract)

Figure IV: Photomicrograph Of The Kidney Sections Of adult Mice Treated With 15 mg/Kg B.W Of Dimethoate (H&E Stain X400). A] Control group B] DM Toxicated group - Glomerular Degeneration(GD), Tubular Degeneration (TD) Hemorrhage(H) , Glomerular Shrinkage(GS), Vacuolation in the glomerulus. Hypoplsia in cells of Bowman's capsule, Rupture(R), ,Normal kidney tubules(KT),Podocytes(POD),Medullary rays(M),Distal convoluted tubule (DCT), Proximal convoluted tubule (PCT), C]Curative Group-Intact Bowmans capsule,Glomerulus.

Fig. III (A) and IV (A): T.S. of Kidney of control adult and aged mice H.E. staining X 400 showing normal structure of kidney
Fig. III (B) and IV (B): T.S. of Kidney of toxicated adult and aged mice H.E. staining X 400 showing disturbed structure of kidney
Fig III (C), IV (C): T.S. of Kidney of Pomegranate peel extract treated adult and aged mice H.E. staining X 400 showing recovered structure of kidney

Histopathological study of kidney

Histopathological examination of the Kidney sections in the control mice (Fig I(C) and II(C) showed a normal histological structure, normal renal tubules, renal corpuscles, proximal convoluted tubules ,distal convoluted tubules, the Glomerulus, Bowman's capsule, urinary space, podocytes, medulalry rays .The treated Kidney section with dimethoate (Fig III (B) and IV (B) showed Glomerular, Bowman's capsule with swollen cells and hypoplasia, glomerular shrinkage, Vacuolization Compressed blood vessels and hemorrhage ,tubular degeneration and Tubular Widened Lumen, Cell rupture and swollen proximal convoluted tubule. The curative group (Fig III (C) and IV (C) showed recovered structure of kidney with normal Glomerular structure, PCT, DCT, intact Bowman's capsule as compared with normal structure of the kidney.

From several years the excessive use of various pesticides on agriculture land and for household pest causes drastic effect on many non-target species like man [43-45]. The present study was performed to investigate biochemical and histopathological effect of commonly used organophosphorus pesticide, dimethoate on adult and aged albino mice. The results showed mild to severe effects on the target organs and some notable results were found in the study. The biochemical results of the current study showed that liver total protein levels of adult and aged mice were significantly increased in both exposed groups compared to those of control animals. These liver protein levels may have been the result of increases in detoxification enzyme (xenobiotic-metabolizing enzyme) levels, as a protective response against toxic chemicals in both groups [46-47]. The kidney total protein level of adult and aged mice was significantly decreased in both exposed groups compared to those of control animals. The histology of liver sections showed more severely affected by dimethoate. The changes noted includes degeneration of nuclei, cell rupture, Congestion, hemorrhage, lymphatic infiltration, nuclear death, increase in Kuffer cell number, enlargement of hepatic sinusoids, parenchymatous cells showing vacuolation, nuclear death or pycnosis. The liver is most target organ in xenobiotic metabolism. Livers from mice treated with dimethoate showed hepatocytic nuclear death or cell pycnosis. Similar findings were reported on fish toxicities by carbofuran by Singh *et al.* [48] showed that liver cytoplasmolysis, nuclear pycnosis and necrosis leading to disintegration of hepatocytes. Khogali *et al*. [49], reported that Dimethoate-induced vacuolation, blood congestion, hepatic pycnosis, and high lymphatic infiltration around the central vein. These results are agreed with many authors; Sharma *et al.* [50] reported that an exposure of technical grade dimethoate caused portal inflammation, focal hepatocyte necrosis and centrizonal congestion in the liver of rats. Another investigation on the histological changes in the liver were done by Abd Rabou [51] which showed hypertrophy in hepatic cells and fatty change amyloid – like structure and distintension of sinusoids; whereas kidney showed Bowman's capsule with swollen cells and hypoplasia, glomerular shrinkage, odema, Vacuolization Compressed blood vessels and hemorrhage, tubular degeneration and Tubular Widened Lumen, Cell rupture and swollen proximal convoluted tubule [49]. Sakr and Al-Amoudi [52] reported that tubular degeneration, glomerular atrophy, leucocytic infiltrations and congestion of renal blood vessels during deltamethrin intoxication in kidneys of male wistar rats [53]. The mechanism of liver and kidney destruction is because of the generating oxidative stress which involves the secretion of cytokines, mainly tumor necrosis factor TNF-α, interleukin IL-1, and IFN-c [54].

Supplementation with antioxidants effectively suppressed the oxidative damage induced by organophosphate pesticides [55-59]. Pomegranate is recently described as nature's power fruit, is a plant used in folkloric medicine for the treatment of various diseases [60]. *Punica granatum* has obtained widespread popularity as a functional food and nutraceutical source is gaining tremendous attention due to its powerful antioxidant properties [36]. Pomegranate extracts have been shown to exhibit 6–8-fold greater effect than grape, grapefruit and orange juice [61]. Previous studies revealed the ability of pomegranate peel extract [62] to suppress lipid peroxidation.

Histomorphometry findings of liver and kidney tissue of curative group of current study (Fig I-IV) showed that administration of pomegranate peel extract with dimethoate perceived to abort dimethoate-induced histopathological changes in hepatic and renal tissues, this magnificent pomegranate peel extract protection against dimethoate histopathological alteration could attribute the molecules of the active ingredients in the pomegranate extract as these ingredients could contribute to preventing oxidative stress, degeneration, apoptosis and inflammatory mechanisms in both adult and aged mice. Thus, some positive attributes of pomegranate peel extract in this study may be the ability to lower the oxidative stress. Excessive level of antioxidant in pomegranate could boost to quenching of some free radicals inside cells, as well as have the capability to protect kidney and liver tissue from oxidative stress damage. According to our results, pomegranate peel extract have shown abilities to preserve the activity of antioxidant enzymes and cure the damaged cells during dimethoate toxicity in both adult and aged mice.

CONCLUSION

In conclusion, we think that administration of a substance with powerful anti-oxidant activity such as pomegranate peel extract can protect humans against the oxidative stress and reduce consequently, the risk of hepatotoxicity, nephrotoxicity and cellular damage in rat liver and kidney after oral exposure to dimethoate. We think that pomegranate peel extract is an inexpensive, easily available, and easy to use alternative in the elimination or reduction of damaging side effects of organophosphate pesticide dimethoate on the kidney and liver.

Acknowledgement

The authors are grateful to the Rayat Institute of Research and Development, Satara (RIRD) and our Principal Dr. M. M. Rajmane for their continuous support and providing all necessary facilities. I must thanks to Rajarambapu College of Pharmacy Kasegaon for providing all laboratory facilities.

LITERATURE CITED

1. Kirkwood T, Austad S. 2000. Why do we age? *Nature* 408: 233-238.
2. Hamet P, Tremblay J. 2003. Genes of ageing. *Metabolism* 52(10)2: 5-9.
3. Schmucker DL. 2005. Age-related changes in liver structure and function: implications for disease? *Exp Gerontology* 40: 650-659.

4. Seals DR, Justice JN, La Rocca TJ. 2016. Physiological geroscience: targeting function to increase health span and achieve optimal longevity. *Jr. Physiology* 594(8): 2001-2024.
5. Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, Dhama K. 2014. Oxidative stress, prooxidants, and antioxidants: the interplay. *Biomed. Res. Int.* 2014: 761264.
6. Vellai T. 2009. Autophagy genes and ageing. *Cell Death Differ* 16: 94-102.
7. Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM. 2011. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* 479: 232-236.
8. Rui L. 2011. Energy metabolism in the liver. *Compr. Physiology* 4(1): 177-197.
9. Schmucker DL. 2005. Age-related changes in liver structure and function: Implications for disease? *Exp. Gerontology* 40(8/9): 650-659.
10. Wynne HA, Cope LH, Mutch E. 1989. The effect of age upon liver volume and apparent liver blood flow in healthy man. *Hepatology* 9: 297-301.
11. Iber FL, Murphy PA, Connor ES. 1994. Age-related changes in the gastrointestinal system. Effects on drug therapy. *Drugs Aging* 5: 34-48.
12. Schmucker DL, Woodhouse KW, Wang RK, Wynne H, James OF, McManus M, Kremers P. 1990. Effects of age and gender on in vitro properties of human liver microsomal monooxygenases. *Clin. Pharmacol. Ther*. 48: 365-374.
13. Schmucker DL, Sachs H. 2002. Quantifying dense bodies and lipofuscin as a function of aging: a morphologist' perspective. *Arch. Gerontol. Geriatr*. 34: 249-261.
14. Hunt NJ, Kang SWS, Lockwood GP, Le Couteur DG, Cogger VC. 2019. Hallmarks of aging in the liver. *Computational and Structural Biotechnology Journal* 17: 1151-1161.
15. Sivapiriya V, Jayanthisakthisekaran J, Venkatraman S. 2006. Effects of dimethoate (O,O-dimethyl S-methyl carbamoyl methyl phophorodithioate) and ethanol in antioxidant status of liver and kidney of experimental mice. *Pest Biochem. Physiology* 85: 115-112.
16. Mansour SA, Mossa AH. 2011. Adverse effects of exposure to low doses of chlorpyrifos in lactatingrats. *Toxicol. Ind. Health* 27: 213-224.
17. Wang X, Vrtiska TJ, Avula RT, Walters LR, Chakkera HA, Kremers WK, Lerman LO, Rule AD. 2014. Age, kidney function, and risk factors associate differently with cortical and medullary volumes of the kidney. *Kidney International* 85(3): 677-685.
18. Denic A, Glassock RJ, Rule AD. 2016. Structural and functional changes with the aging kidney. *Advances in Chronic Kidney Disease* 23(1): 19-28.
19. Fang Y, Gong AY, Haller ST, Dworkin LD, Liu Z, Gong R. 2020. The ageing kidney: Molecular mechanisms and clinical implications. *Ageing Research Reviews*. pp 101151.
20. Zhou XJ, Rakheja D, Yu X, Saxena R, Vaziri ND, Silva FG. 2008. The aging kidney. *Kidney International* 74(6): 710-720.
21. Rule AD, Amer H, Cornell LD, Taler SJ, Cosio FG, Kremers WK, Textor SC, Stegall MD. 2010. The association between age and nephrosclerosis on renal biopsy among healthy adults. *Annals of Internal Medicine* 152(9): 561-567.
22. Denic A, Glassock RJ, Rule AD. 2016. Structural and functional changes with the aging kidney. *Adv. Chronic Kidney Dis*. 23(1): 19-28.
23. Esposito C, Dal Canton A. 2010. Functional changes in the aging kidney. *Journal of Nephrology* 15: 41-45.
24. Wiggins JE. 2012. Aging in the glomerulus. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences* 67(12): 1358-1364.
25. Sharma Y, Bashir S, Irshad M, Nag TC, Dogra TD. 2005. Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. *Toxicology* 215(3): 173-181.
26. Anonymous. 1999. Manual on the development and use of FAO specifications for plant protection products, 5th Edition. FAO plant production and protection paper, 149, FAO.
27. Gomes J, Dawodu AH, Lloyd O, Revitt DM, Anilal SV. 1999. Hepatic injury and disturbed amino acid metabolism in mice following prolonged exposure to organophosphorus pesticides. *Hum. Exp. Toxicology* 18: 33-37.
28. Sayim F. 2007. Dimethoate-induced biochemical and histopathological changes in the liver of rat. *Experimental and Toxicologic Pathology* 59: 237-243.
29. Khogali FA, Sheikh JB, Rahman SA, Rahman AA, Daghestani. 2005. Histopathological and hematological effects of dimethoate 40EC on some organs of albino mice. *Journal King Saud University* 18(2): 73-87.
30. De-Bleecker J, Van-Den-Neucker K, Colradyn F. 1993. Intermediate syndrome in organophosphorus poisoning: a prospective study. *Critical Care Medicine* 21(11): 1706-1711.
31. Thangavel P, Baby Skaikila I, Ramaswamy M. 1994. Synergistic effect of *Dimecroncuman* L. on the histology of liver and brain of *Sarotherodon mossambicus*. *Jecobio* 6(3): 173-178.
32. Persis VT, Kalaiarasi JMV. 2001. Histopathological responses of mystusvittatusto chronic sublethal and acute lethal toxicity of an organophosphate pesticide. *Jr. Expt. Zoo. India* 4(1): 103-108.
33. Jacob RA, Burri BJ. 1996. Oxidative damage and defense. *Am. Jr. Clin. Nutr*. 63: 985S-990S.
34. Ross JA, Kasum CM. 2002. Dietary flavonoids: Bioavailability, metabolic effects, and safety. *Annu. Rev. Nutrition* 22: 19-34.
35. Soobrattee MA, Bahorun T, Aruoma OI. 2006. Chemopreventive actions of polyphenolic compounds in cancer. *Biofactors* 27: 19-35.
36. Johanningsmeier SD, Harris GK. 2011. Pomegranate as a functional food and nutraceutical source. *Annu. Rev. Food Science and Technology* 2: 181-201.
37. Pérez-Vicente A, Gil-Izquierdo A, García-Viguera C. 2002. In vitro gastrointestinal digestion study of pomegranate juice phenolic compounds, anthocyanins,and vitamin C. *Jr. Agric. Food Chemistry* 50(8): 2308-1312.
38. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA. 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Jr. Agric. Food Chemistry* 48(10): 4581-4589.

39. Xu KZ-Y, Zhu C, Kim MS, Yamahara J, Li Y. 2009. Pomegranate flower ameliorates fatty liver in an animal model of type 2 diabetes and obesity. *Jr. Ethnopharmacology* 123(2): 280-2877.
40. Abdel Moneim AE. 2011. Antioxidant activities of *Punica granatum* (pomegranate) peel extract on brain of rats. *Jr. Med. Plant Research* 6(2): 195-199.
41. Parmar HS, Kar A. 2008. Medicinal values of fruit peels from *Citrus sinensis*, *Punica granatum*, and *Musa paradisiaca* with respect to alterations in tissue lipid peroxidation and serum concentration of glucose, insulin, and thyroid hormones. *Journal of Med. Food* 11: 376-381.
42. Gurr E. 1962. *Staining Animal Tissue: Practical and Theoretical*. London: Leonard Hill. pp 631.
43. WHO/PCS. 1996. Pesticides residues in foods. 1996 evaluations. Part II, Toxicological, Geneva. Publication no. WHO/PCS/97.1.1997, 1996.
44. Chantelli-Forti G, Paolini M, Hrelia P. 1993. Multiple end point procedure to evaluate risk from pesticides. *Environ Health Perspective* 101: 15-20.
45. Chaudhuri K, Selvaraj S, Pal AK. 1999. Studies on the genotoxicity of endosulfan in bacterial systems. *Mutat Research* 439: 63-67.
46. Sharma Y, Bashir S, Irshad M, Nagc TC, Dogra T. 2005. Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. *Toxicology* 215: 173-181.
47. Mutch E, Williams FM. 2005. Diazinon, chlorpyrifos and parathion are metabolised by multiple cytochromes P450 in human liver. *Toxicology* 224: 22-32.
48. Singh N, Srivastava AK. 1994. Formothion Induced haematological changes in the fresh water Indian catfish *Meteropneustes fossili. Jr. Ecotorico Environ. Moni.* 4(2): 137-140.
49. Khogali FA, Sheikh JB, Rahman SA, Rahim AA, Daghestan MH. 2005. Histopathological and hematological effects of dimethoate 40ec on some organs of albino mice. *Jr. King Saud University* 18(2): 73-87.
50. Sharma Y, Bashir S, Irshad M, Nagc TC, Dogra T. 2005. Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. *Toxicology* 215(2): 173-181.
51. Abdu Rabou AN. 1996. On the hazards of pesticide use and misuse in Khartoum state and some histopathological studies on the effects of carbamates on experimental animals. *M. Sc. Thesis*, in Environmental Studies. Sudan: University of Khartoum, 1996.
52. Sakr SA, Al-Amoudi WM. 2012. Effect of leave extract of *Ocimum basilicum* on deltamethrin induced nephrotoxicity and oxidative stress in albino rats. *Journal Applied Pharmaceutical Science* 2(5): 22-27.
53. Kerem M, Bedirli N, Gurbus N, Ekinci N, Bedirli A, Akkaya T, Sakrak, Pasaoglu H. 2007. Effects of acute fenthion toxicity on liver and kidney function and histology in rats. *Turk. Jr. Med. Sciences* 37: 281-288.
54. Sugano M, Yamato H, Hayashi T, Ochiai H, Kakuchi J, et al. (2006:) High-fat diet in low-dose-streptozotocin-treated heminephrectomized rats induces all features of human type 2 diabetic nephropathy: a new rat model of diabetic nephropathy. *Nutr. Metab. Cardiovasc Diseases* 16: 477-484.
55. Gupta RC. 2001. Depletion of energy metabolites following acetylcholinesterase in leptazol-induced status epilepticus and protection by antioxidants. *Neurotoxico.* 22: 271-282.
56. Karaöz E, Gultekin F, Akdogan M, Oncu M and Gokcimen A. 2002. Protective role of melatonin and a combination of vitamin C and vitamin E on lung toxicity induced by chlorpyrifos-ethyl in rats. *Exp. Toxicol. Pathology* 54: 97-108.
57. Oncu M, Gultekin F, Karaöz E, Altuntas I and Delibas N. 2002. Nephrotoxicity in rats induced by chlorpyrifos-ethyl and ameliorating effects of antioxidants. *Human Exp. Toxicology* 21: 223-230.
58. Camkayali I, Demirag K, Eris O, Ersoz B, Moral AR. 2005. The effect of N-acetylcysteine on oxidative stress in organophosphate poisoning model. *Adv. Ther.* 22: 107-116.
59. Elhalwagy MEA, Darwish NS, Zaher EM. 2008. Prophylactic effect of green tea polyphenol against liver and kidney injury induced by fenitrothion insecticides. *Pesti. Bio. Physio* 91: 81-89.
60. Ajaikumar KB, Asheef M, Babu BH, Padikkala J. 2005. The inhibition of gastric mucosal injury by *Punica granatum* L. (pomegranate) methanolic extract. *Jr. Ethnopharmacology* 96: 171-176.
61. Tzulker R, Glazer I, Bar-Ilan I. 2007. Anti-oxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. *Jr. Agri. Food Chemistry* 55(23): 9559-9570.
62. Singh RP, Murthy KNC, Jayaprakasha GK. 2002. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *Journal of Agricultural and Food Chemistry* 50: 81-86.