The Pharma Innovation Journal 2021; 10(7): 1437-1443

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(7): 1437-1443

www.thepharmajournal.com
Received: 01-04-2021
Accepted: 07-06-2021

Dr. Harshal J Shete
M.V.Sc. Scholar, Department of Veterinary Pathology, College of Veterinary & Animal Sciences, Parbhani, Maharashtra, India

Dr. Santosh D Moregaonkar
Professor and Head, Department of Veterinary Pathology, College of Veterinary & Animal Sciences, Parbhani, Maharashtra, India

Dr. Govind R Gangane
Associate Professor, Department of Veterinary Pathology, College of Veterinary & Animal Sciences, Parbhani, Maharashtra, India

Dr. Anand N Choudhari
M.V.Sc. Scholar, Department of Veterinary Pathology, College of Veterinary & Animal Sciences, Parbhani, Maharashtra, India

Dr. Akshay R Chaure
M.V.Sc. Scholar, Department of Veterinary Pathology, College of Veterinary & Animal Sciences, Parbhani, Maharashtra, India

Dr. Pooja B Kachave
M.V.Sc. Scholar, Department of Veterinary Pathology, College of Veterinary & Animal Sciences, Parbhani, Maharashtra, India

Corresponding Author:
Dr. Harshal J Shete
M.V.Sc. Scholar, Department of Veterinary Pathology, College of Veterinary & Animal Sciences, Parbhani, Maharashtra, India

Pathological changes in Profenofos Toxicated Gramapriya birds and its ameliorations by *Tephrosia purpurea*

Dr. Harshal J Shete, Dr. Santosh D Moregaonkar, Dr. Govind R Gangane, Dr. Anand N Choudhari, Dr. Akshay R Chaure and Dr. Pooja B Kachave

Abstract
The present experiment was planned to study the sub-acute oral toxicity of profenofos in Gramapriya birds and hepatoprotective effects of *T. purpurea* in toxicated birds. 100 healthy days old 'Gramapriya' chicks randomly distributed into four treatment groups, having 25 chicks each up to 28 days. The birds of control group I was given standard feed and *ad libitum* of water; groups II were intoxicated daily with a solution of Profenofos @ 1.6 mg/kg body weight through oral gavage; group III were fed with a *T. purpurea* leaves powder @ 0.1% of feed and group IV was treated with Profenofos @ 1.6 mg/kg body weight daily through oral gavage + *T. purpurea* leaves powder @ 0.1% of feed daily. Pathological studies including clinical signs, organ weights, gross lesion and histopathological examination in birds of all groups were examined at 0^{th} 14^{th} and 28^{th} day of study. All pathological examinations of birds from Group I and III showed non-significant changes throughout the experiment. On 14^{th} and 28^{th} day of experiment, pathological studies and histopathological examination of the sections from birds of group II, showed significant alterations. Efficacy of *T. purpurea* leaves powder in group IV birds revealed the reduced necrotic changes in the sections of liver, kidney and intestine of birds in group IV as compared to group II birds.

Keywords: Subacute Profenofos toxicity, *Tephrosia purpurea*, Gramapriya birds and histopathology

1. Introduction
India's livestock sector (containing poultry) is one of the largest in the world with a holding of 11.6% of world livestock population. In India Livestock and poultry sectors play a multi-faceted role in socio-economic development of rural households, as these assets are more equitably distributed than land. Poultry industry has made considerable growth in both commercial and backyard poultry sector in India. The total Poultry population in the country is 851.81 million in 2019, which was increased by 16.8% over previous Census. However, the total Backyard Poultry population in the country is 317.07 million in 2019, which showed the increment of 45.8% over previous Census (20^{th} Livestock Census, 2019). Backyard poultry products have more price values than that of commercial poultry products, which is the best alternative for the rural farmers and provides them the socio-economic improvement also[20]. Also, the rural backyard poultry farming has the pivotal role in elevating the food and nutrition securities of the poorest households and farmers, which helps them to improve livelihood standards. It also is the source of the nutritional supplement in the form of animal protein for poor households in India [16].

In rural India, until now most of the chicken breeds reared were generally Desi type (non-descript), which had low egg and meat production capacity. In last decades their contribution to the total egg production was stagnant due to their low production capacity [17]. Gramapriya birds showed better performance in case of age at first egg laying, annual egg production and body weight under backyard system of rearing. Considering these points, Gramapriya birds can improve the livelihood and nutritional requirements of small-scale farmers in rural and tribal parts of India [22].

Toxicity of organo-phosphoros/organo-chlorine compounds through the feed and water and their residues in poultry products is commonly seen in India [1]. The uncontrolled use of pesticides consequences many serious problems including toxic residues in crops and cereals, which are further used as the source of feed to mammals and birds.

Profenofos is one of them extensively used as a crop protectant in the field, especially in and around Parbhani. Profenofos was readily used by farmers on the maize and soybean crops, which are further used as principal feed constituents in poultry feed.

In India it is observed that these is increase in use of herbal medicines for various disease conditions. There were large number of plants and herbal formulations have hepatoprotective activity. Among them *Tephrosia purpurea* was readily used as the hepatoprotective plant. Considering the current facts of increasing use of profenofos, losses being caused in poultry & a trend of using herbal medication; the present trial have been planned to observe pathological alterations in subacute Profenofos toxicity and its ameliorations by *Tephrosia purpurea* leaves powder in Gramapriya birds.

Material and Methods

A total of 100 healthy day old 'Gramapriya chicks were procured from Government Central Hatchery; Padegaon, Aurangabad. All the chicks were vaccinated with Marek's disease vaccine on the day of hatching at hatchery and maintained till the end of trial. Birds were acclimatized to animal house for 7 days of period. The study was carried out for a period of 28 experimental days which were uniformly distributed into four groups of 25 birds in each. The group I was served as healthy control and was given standard feed and water ad libitum for 28 days. The birds of groups II were intoxicated daily with a solution of Profenofos @ 1.6 mg/kg body weight through oral gavage. The birds of group III were treated on plant control and fed with a *Tephrosia purpurea* leaves powder @ 0.1% of feed daily for 28 days. The group IV was treated with Profenofos @ 1.6 mg/kg body weight daily through oral gavage + *Tephrosia purpurea* leaves powder @ 0.1% of feed daily.

Profenofos was procured from the local market Parbhani and was given to the treatment group chicks through oral gavage (with water as a vehicle) @ 1.6 mg/kg Bwt. starting from 1st day (As LD_{50} dose of Profenofos in birds was 16 mg/kg Bwt.) until end of experiment[8]. The *Tephrosia purpurea* plant was acquired from the local areas of Parbhani and villages surrounding the Parbhani. Subsequently, the plant leaves were dried in room under controlled environment. Afterwards it was grinded by using electric grinder. The coarse powder will be prepared from the dried leaves of *Tephrosia purpurea*.

Clinical signs and symptoms: All the birds from each group were examined for clinical signs and symptoms related to profenofos toxicity throughout the experimental period.

Absolute organ weight (g): All the experimental birds from each group were sacrificed on 14th and 28th day of experimental trial. Organs like liver, kidneys, spleen and heart of experimental birds were separated carefully from the carcass and weighed and weights were expressed in grams (g).

Gross pathological examination: The experimental Gramapriya birds were sacrificed on 14th day and 28th day of experimental trial and critically examined by conducting systematic post mortem examination and gross lesions observed were recorded**.**

Histopathological examination: After recording the gross lesions the tissue pieces of suitable thickness of brain, heart, lungs, liver, kidneys, spleen, bursa and intestine were collected to evaluate microscopic toxic pathological alterations. The collected tissue samples were fixed and preserved in 10 percent neutral buffer formalin. After fixation the collected tissue pieces were processed as per the standard procedure. Paraffin embedded tissues were sectioned at 3-5µ thickness and stained with routine Haematoxylin and Eosin method [3].

Statistical Analysis

The data generated from haematological and biochemical parameters were statistically analysed by Completely Randomized Design (CRD) using WASP (Anonyms, 2018 WASP version 2.0 http: //www.ccari.res.in/wasp2.0/index.php.).

Result and Discussion

Clinical signs and symptoms

During experimental trial the birds in group I and group III were active, healthy and with normal physiological growth parameters. Throughout the experimental trial the birds of group II were showed mild clinical signs and symptoms, in which sluggishness, dullness, depression, decreased water and feed intake, reduced appetite and reduced growth were observed at last weeks of experimental trial. These clinical signs observed in birds of group II might be due to accumulation of acetylcholine at nerve endings which potentiates inhibition of acetylcholinesterase enzyme well known for its muscarinic, nicotinic and central nervous system effects [4]. The birds of group IV were showed mild clinical signs as compared to the toxin control group (group II).

Absolute organ weights (gm)

Mean (± SE) values of absolute weights (gm) of liver, kidney, heart, spleen, testis and ovary of birds in at different intervals of study are depicted in table 1.

At 14th and 28th day of experiment the mean absolute organ weights of birds in group I and group III were statistically at par and represent normal range as compared to other groups in experiment. Whereas the absolute liver and kidney weights of birds in group II were significantly decreased as compared to control groups. The mean absolute liver and kidney weights of birds in the group IV were significantly ($P<0.01$) improved than the liver and kidney weights of birds in group II, however this improvement was not up to the levels in healthy control group. Mean (± SE) values of absolute weights of heart, spleen, testis and ovary of birds at different intervals of study in different groups are depicted in table No. 1. At both the intervals of study (14th and 28th day) mean absolute weights of these organs were not comparable within all groups of experiments.

Table 1: Mean (± SE) Absolute organ weights of experimental birds at different intervals of study

Groups of birds		Absolute weights of Liver, Kidney, Heart, spleen, testis and ovary (Mean ±S. E, gm)											
		Weight of liver		Weights of kidneys		Weight of heart		Weight of spleen		Weight of testis		Weight of ovary	
		14th day	28th day	14th day	28th day	14th day	28th day	14th day	28th day	14th day	28th day	14th day	28th day
Group I		10.06[a] ±0.19	13.95[a] ±0.20	3.61 ±0.28	6.17[a] ±0.15	2.66 ±0.15	3.69 ±0.11	0.70 ±0.06	1.13 ±0.14	0.10 ±0.01	0.21 ±0.02	0.18 ±0.05	0.36 ±0.03
Group II		8.98[b] ±0.24	10.32[c] ±0.12	3.06 ±0.21	4.58[b] ±0.28	2.18 ±0.17	2.94 ±0.27	0.54 ±0.04	0.88 ±0.12	0.08 ±0.01	0.13 ±0.03	0.13 ±0.01	0.29 ±0.05
Group III		9.98[a] ±0.21	13.44[a] ±0.21	3.49 ±0.27	6.10[a] ±0.25	2.51 ±0.09	3.48 ±0.26	0.70 ±0.09	0.99 ±0.09	0.09 ±0.03	0.18 ±0.01	0.19 ±0.05	0.35 ±0.04
Group IV		9.09[b] ±0.27	11.00[b] ±0.26	3.37 ±0.24	4.80[b] ±0.27	2.30 ±0.11	3.35 ±0.15	0.62 ±0.02	0.91 ±0.10	0.09 ±0.01	0.15 ±0.03	0.14 ±0.01	0.31 ±0.01
CD Value	1%	0.928	0.829	-	0.984	-	-	-	-	-	-	-	-
	5%	0.676	0.609	-	0.715	-	-	-	-	-	-	-	-
Statistics		HS	HS	NS	HS	NS	NS	NS	NS	NS	NS	NS	NS

Means bearing similar superscripts in column do not differ significantly ($P< 0.05$) ($P< 0.01$).

Where,

Group I - Healthy control Group II - Profenofos control

Group III – *T. purpurea* control Group IV - Profenofos + *T. purpurea* treatment

Gross and Histo-pathological alterations

At both 14th and 28th day of experiment, the gross and histopathological examination of organs of sacrificed birds of group I and group III did not show any considerable pathological changes. Grossly, liver of sacrificed birds of group II showed diffuse congestion and areas of hemorrhages (Petechial) at the borders of liver (Plate 1). Whereas kidneys, intestinal loop and lungs revealed mild, diffuse congestion and focal hemorrhages.

Histopathological examination of organs from birds of group II comprising liver sections revealed moderate to severe congestion of hepatic capillaries, congested and dilated centrilobular vein in the liver parenchyma and dilated central hepatic vein. There were focal cystic changes in the hepatic parenchyma. Dilated portal vein, fibrous connective tissue proliferation, hyperplasia of bile duct, newly formed bile ducts and lymphocytic aggregations around triad were the significant changes observed in portal triad areas of liver (Plate 2-4). There were diffuse degenerative changes (Both granular and vacuolar) in hepatocytes and small and rounded focal areas of coagulative necrosis in the liver parenchyma. Kidney section revealed diffuse degenerative changes (Acute cellular swelling & hydropic degeneration) and multifocal areas of necrotic changes in tubular epithelial cells of kidney. At places, tubular epithelial hyperplasia was evident. Diffuse congestion of intertubular capillaries, and focal areas of hemorrhages and increased glomerular cellularity were also noticed in some kidney sections(Plate 5-6). In addition to the changes noticed in earlier interval at 28th day of experiment, there were widely distributed areas of coagulative necrosis in renal parenchyma. The histopathological examination of heart sections revealed mild to moderate focal areas of zenkers degeneration. Brain sections revealed mild to moderate congestion of microcapillaries and vacuolation in the cytoplasm of few neurons.

At 14th and 28th of experiment, the sections of intestine of birds from group II revealed desquamation of tops of the villi, scanty exudate and infiltration of inflammatory cells (mostly mononuclear cells) along with few erythrocytes in the lumen are the changes noticed in mucosa of intestine (Plate 7). The lung sections showed diffuse severe congestion of pulmonary and parabronchial capillaries and parabronchiolar hemorrhages. Bursal mucosa was intact and in the lymphoid follicle of bursa vacuoles of varied size were evident indicating depopulation of lymphocytes. Spleen sections of the birds did not reveal any significant changes, except for mild to moderate congestion of sinusoidal capillaries.

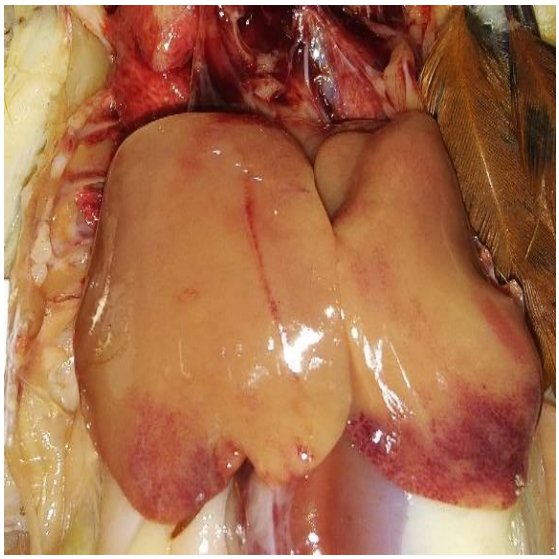

Plate 1: Congestion and Petechial hemorrhages at the borders of liver of a bird from group II at 14th day

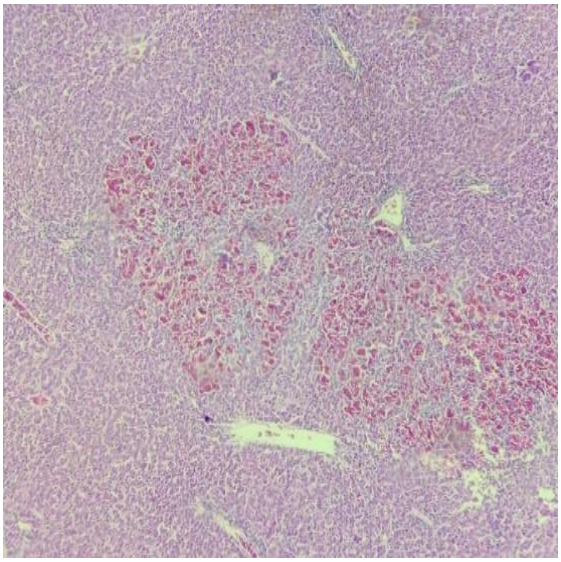

Plate 2: Areas of congestion of hepatic capillaries in liver parenchyma of bird of group II at 14th day (H & E × 100)

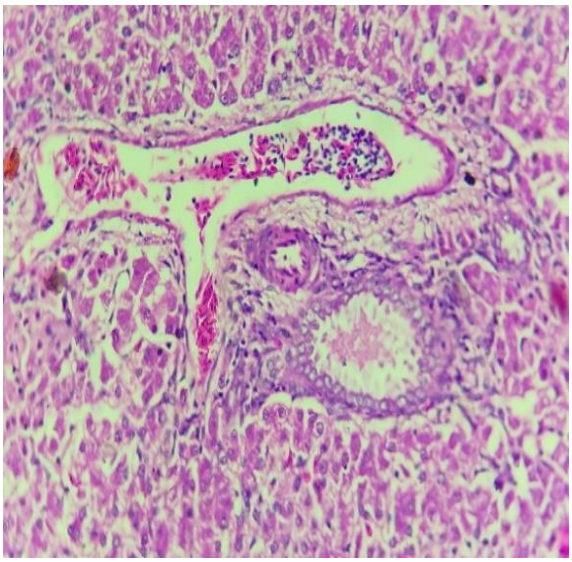

Plate 3: Dilatation of portal vein and hyperplasia of bile duct in a section of liver of bird in group II at 14th day interval (H & E × 400)

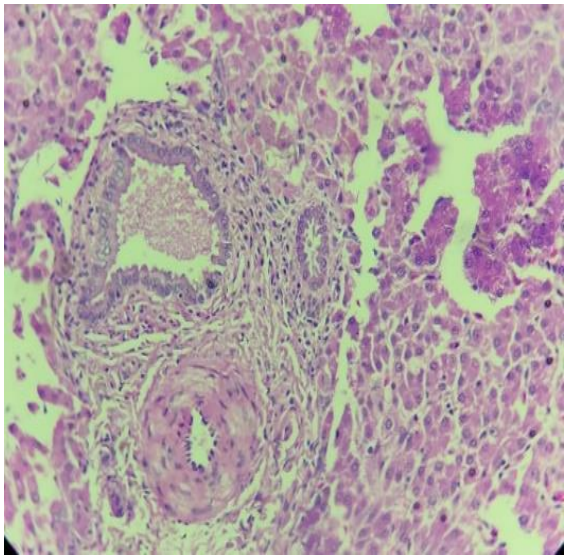

Plate 4: Section showing hyperplasia of bile duct in liver parenchyma of bird of group II at 28th day of experiment (H & E × 400)

Findings of our study were found similar with Kammon *et al.*, (2010) [9], Kammon *et al*, (2011) [10], Kafle *et al.*, (2018), Sodhi *et al*., (2008) and Begum *et al.*, (2015) [2]. Rhayf *et al.*, (2012) in their study on dimethoate induced histopathological changes in local layer chickens. Liver might be affected due to the irritant and toxic nature of the OP compound, as it was the major organ for biotransformation and detoxification of most of the xenobiotics in body [18]. Also, disturbances in oxido-reduction processes in mitochondria might be responsible for cellular degenerative changes in liver parenchyma as reported by Ghodke *et al.*, (2019) [5] in subacute Acephate toxicated broiler chickens. Lesions in the kidneys were revealing of nephrotoxic effects of the profenofos and its metabolites as kidneys are the major route for elimination for most of the OP compounds [8]. Tubular lesions observed due to the direct toxic effect of OP pesticide on the cell function in kidney tissue as well as the reactive free radical or oxidative stress caused due to OP pesticides were also responsible for altered histopathological changes in kidney [8]. The lipid nature of the brain and lipophilic property of the OP pesticides might be responsible for crossing the blood brain barrier by the OP pesticide. Highest oxygen metabolic rate and inadequate defense system against oxidative stress such as considerably lower catalase activity in the brain makes the brain tissue highly susceptible for OP toxicity as stated by Wani *et al.*, (2017) [24]. Also, Kafle *et al.*, (2018) [8] noted that pulmonary edema was a common microscopic lesion in most organophosphate pesticides toxicated animals. Shahzad *et al.*, (2013) [19] noted in their study that the histopathological damage to lymphoid organs (bursa) might be due to the modulation of nervous system consequences to altered production of lymphocytes, phosphorylation and oxidative damage.

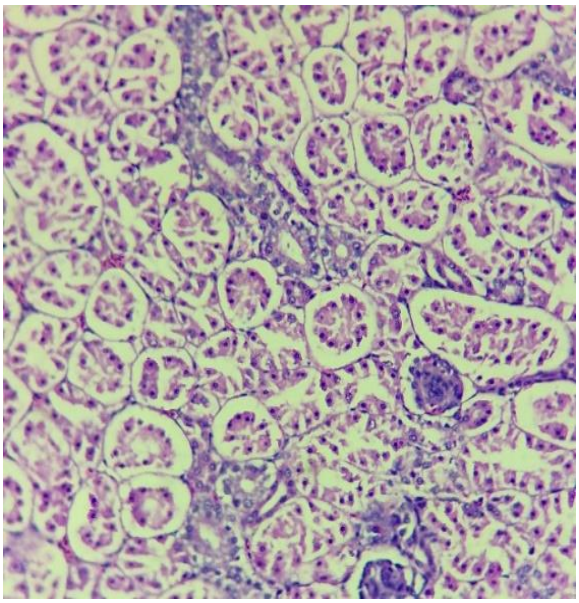

Plate 5: Note varied degree of necrotic changes in tubular epithelial cells in kidney of birds of group II at 14th day interval (H & E × 400)

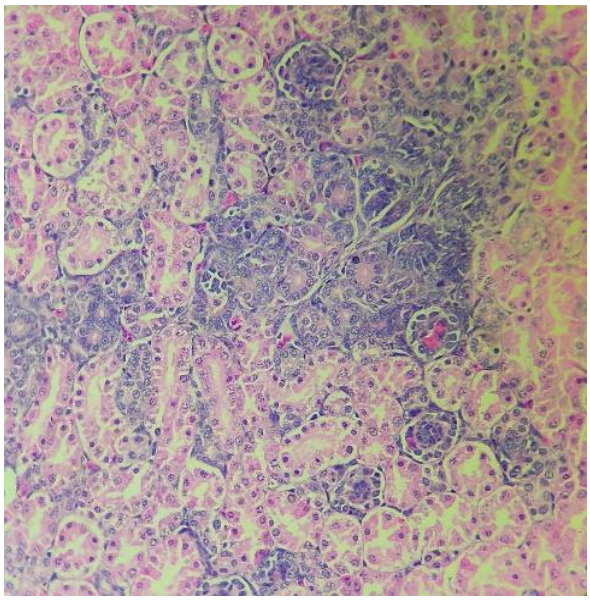

Plate 6: Hyperplasia in few tubular epithelial cells and degenerative changes in many tubules in kidney of bird in group II at 14th day (H & E × 400)

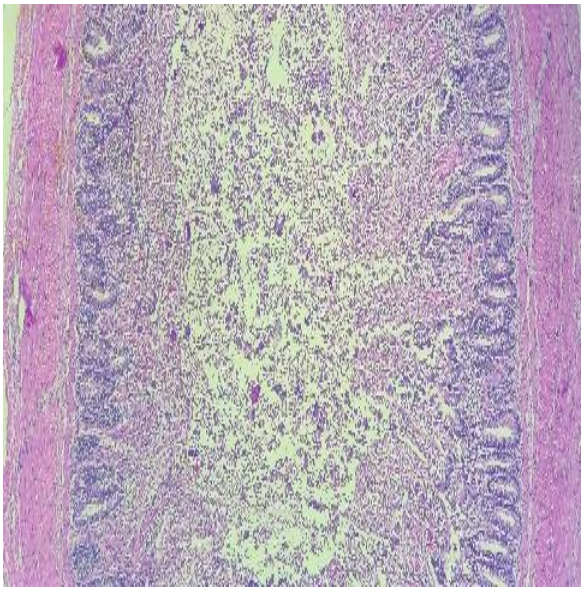

Plate 7: Necrotic enteritis with full of desquamated epithelial cells, scanty exudate and inflammatory cells in lumen of a bird from group II at 28th day of study (H & E × 100)

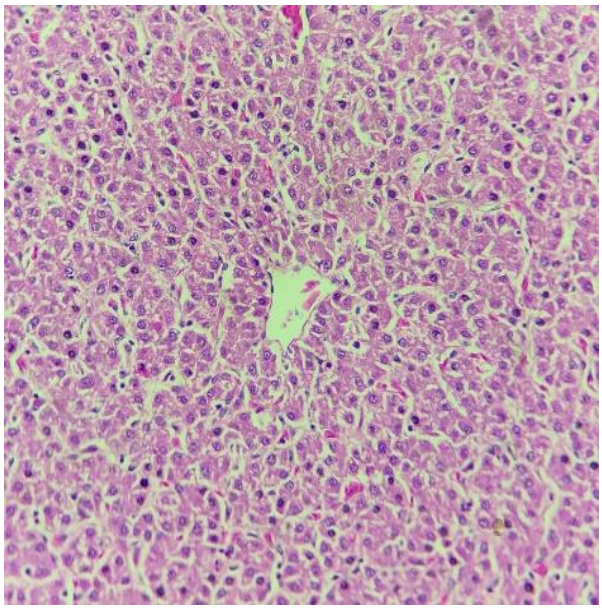

Plate 8: The compact histo-architecture of liver parenchyma and hepatic vein in bird of group IV at 14th day of interval (H & E × 100)

Histopathological examination of organs from birds of group IV showed that overall histoarchitecture was maintained at both the sacrifices as compare to sections from birds of group II (Plate 8-11). In the liver section of birds of group IV, it was observed that the degenerative & necrotic changes in hepatocytes around central vein were minimal as compare to profenofos treated group (group II). However, the congestion and dilatation of hepatic vein in portal area, hyperplasia of bile duct, moderate lymphocytic proliferation in the area were still observed. Histoarchitectural changes in renal, brain and intestinal sections of birds in group IV revealed not much improvement after addition of *T. purpurea*, in the diet of toxicated birds. Overall lungs, bursal and spleen sections of birds of group IV at both sacrifices revealed little improvement in the histoarchitecture. However, at places some hyperplastic changes were evident in renal tubular epithelia & necrotic changes were moderate in tubular epithelia as compared to purely toxin treated group II.

Khatri *et al*., (2009) [11] also noted that the flavanoids present in *Tephrosia purpurea* could be responsible for the membrane stabilizing activity in hepatic cell, through potent antioxidative action. Antioxidant activity of *T. purpurea* helps to reduce increased oxidative stress and protects the tissues from the oxidative damage[6]. Poly phenolic compounds and flavonoids in leaves of *T. purpurea* having free radical scavenging activity protects cellular damage through antioxidant activity of by *T. purpurea* as reported by Gora *et al.,* (2014) and Mathews *et al.,* (2012) [6, 15]. Overall, ameliorative effects of feeding of plant leaf powder were evident on improvement of altered histoarchitecture caused by toxin in the liver section.

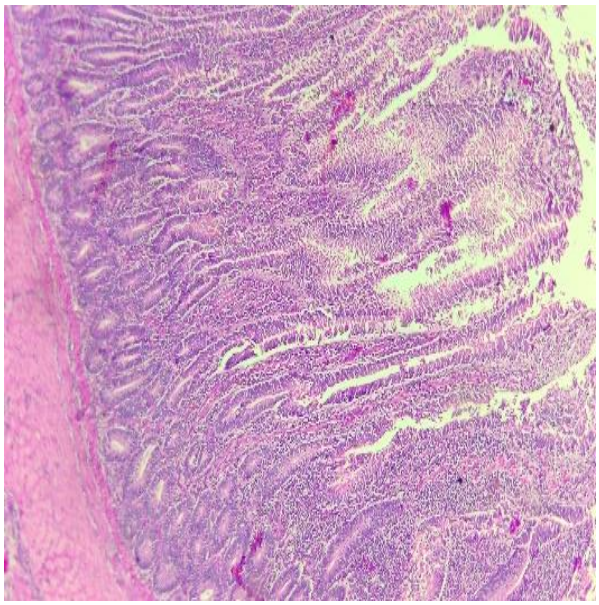

Plate 9: Sections of intestine revealed improved histo-architecture in a bird of group IV at 28th day of experiment (H & E × 40)

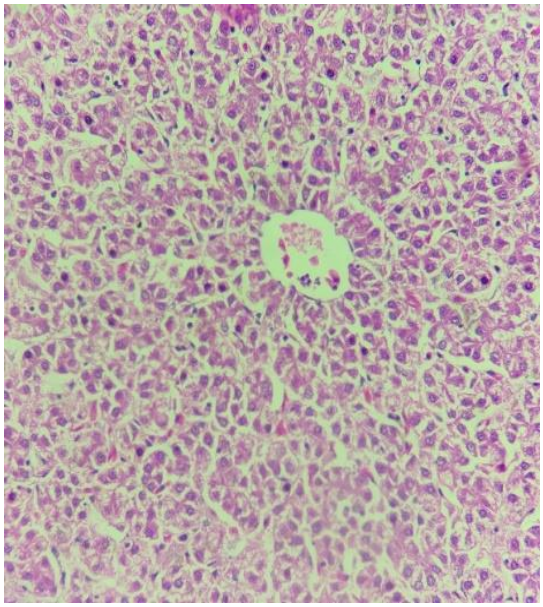

Plate 10: Note more compact histo-architecture of liver parenchyma of bird in group IV at 28th day interval of study (H & E × 400)

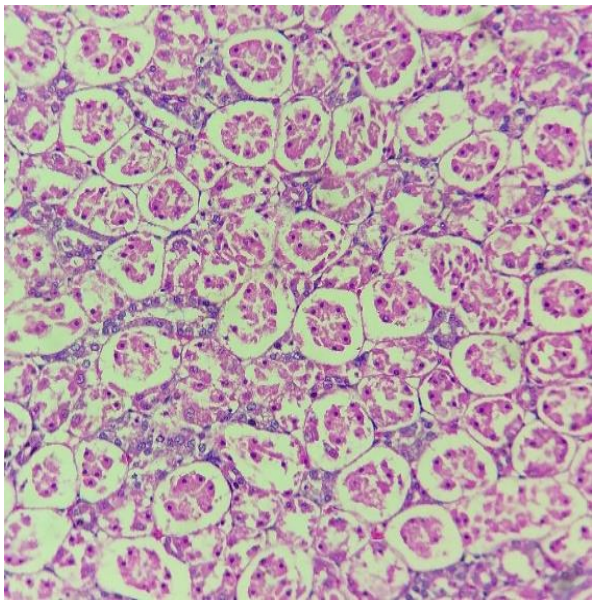

Plate 11: Note cellular swelling with early necrotic changes in tubular epithelial cells in kidney of bird from group IV at 14th day interval (H & E × 400)

Conclusions

- When Profenofos was fed @ 1.6 mg/kg bwt. daily through oral gavage for 28 days in Gramapriya birds, subacute toxicity was induced in experimental birds with prominent alterations in and clinical signs, organ weights, gross and histopathological alterations in vital organs.
- Feeding of *T. purpurea* to birds of group III (plant control) was found non-toxic and showed at par levels of clinical signs, organ weights, gross and histopathological observations in vital organs as compared to birds in healthy control group.
- The birds in treatment group (Profenofos @ 1.6 mg/kg bwt. + *T. purpurea* leaves powder @ 0.1% of feed daily for 28 experimental days) showed mild to moderate improvement in clinical signs, organ weights, gross and histopathological alterations in vital organs as compared to birds of a healthy control group.
- It was observed that the plant treatment was more of hepatoprotective as evidenced by pathological studies.

References

1. Aulakh RS, Gill JPS, Bedi JS, Sharma JK, Joia BS, Ockerman HW. Organochlorine pesticide residues in poultry feed, chicken muscle and eggs at a poultry farm in Punjab, India. Journal of the Science of Food and Agriculture 2006;86:741-744.
2. Begum SA, Upadhyaya TN, Rahman T, Pathak DC, Sarma K, Barua CC *et al*. Hematobiochemical and pathological alterations due to chronic chlorpyrifos intoxication in indigenous chicken. Indian Journal of Pharmacology 2015;47(2):206-211.
3. Culling CFA. Handbook of histopathological and histochemical techniques, 3rd edn, Butterworth and Co. Ltd 1974,29-221.
4. Ghaffar AS, Ashraf R, Hussain T, Hussain M Shafique, Noreen S, Aslam S. Clinico-hematological disparities induced by triazophos (Organophosphate) in Japanese quail. Pakistan Veterinary Journal 2014;34(2):257-259.
5. Ghodke DD, Ingole RS, Hedau MS, Rathod PR, Hajare SW, Khose KK. Ultra-structural pathology of liver due to subacute acephate toxicity in broiler chickens. Indian Journal of Veterinary Pathology 2019;43(2):120-123.
6. Gora RH, Baxla SL, Kerketta P, Patnaik S, Roy BK. Hepatoprotective activity of *Tephrosia purpurea* against arsenic induced toxicity in rats. Indian Journal of Pharmacology 2014;46(2):197-200.
7. Jain NC. Schalm's Veterinary Haematology, 4th edn. Lea and Febiger, Philaldelphia 1986,15-81,356-404p.
8. Kafle A, Roy DC, Sarma J, Upadhyaya TN, Mahanta JD, Gogoi R. Histopathological Changes and Tissue Residue Deposition in Broiler Birds Following Profenofos Administration. International Journal Curr. Microbiol. App. Science 2018;7(01):206-213.
9. Kammon AM, Brar RS, Banga HS, Sodhi S. Patho-biochemical studies on hepatotoxicity and nephrotoxicity on exposure to chlorpyrifos and imidacloprid in layer chickens. Veterinary Arhiv 2010;80:663-672.
10. Kammon AM, Brar RS, Sodhi S, Banga HS, Singh J, Nagra NS. Chlorpyrifos chronic toxicity in broilers and effect of vitamin C. Open Veterinary Journal 2011;1:21-27.
11. Khatri A, Garg A, Agrawal SS. Evaluation of hepatoprotective activity of aerial parts of *Tephrosia purpurea L.* and stem bark of Tecomella undulate. Journal of Ethnopharmacology 2009;122:1–5.
12. Kumar SV, Md. Fareedullah, Sudhakar Y, Venkateswarlu B, Kumar EA. Current review on organophosphorus poisoning. Archives of Applied Science Research 2010;2(4):199-215
13. Kumar V, Singh S, Srivastava B, Patial PK, Kondalkar SA, Bharthi V. Volatile and semi-volatile compounds of *Tephrosia purpurea* and its medicinal activities, Experimental and computational studies. Biocatalysis and Agricultural Biotechnology [online] 2019, 20 Available through https://www.elsevier.com/locate/bab.
14. Luna LG. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd edn. McGraw-Hill, London 1968.
15. Mathews AM, Sujith K, Christina AJM, Muralidharan. Basic research on the herb *Tephrosia purpurea* (l) pers. - the translational challenges – a review. International Journal of Pharmaceutical and Chemical Sciences 2012;1(1):466-471.
16. Pica-ciamarra U, Otte J. Poultry, food security and poverty in India: looking beyond the farm-gate. World's Poultry Science Journal 2010;66:309-320.
17. Rath PK, Mandal KD, Panda P. Backyard poultry farming in India: a call for skill upliftment. Research journal of recent sciences 2015;4:1-5.
18. Rhayf A GH, Naji HA, Abdulrazzaq M. Protective effect of *Nigella sativa* seeds against dimethoate induces histopathological and hematological changes in local layer chickens. AL-Qadisiya Journal of Veterinary Medical Science of 5th conference 21-22 Nov. 2012. AL-Qadisiya.11 (3) (Available from Protective effect of nigella sativa seeds against dimethoate induces histopathological and hematological changes in local layer chickens researchgate.net) 2012.
19. Shahzad A, Khan A, Khan MZ, Mahmood F, Gul ST, Saleemi MK. Immuno-pathologic effects of oral administration of chlorpyrifos in broiler chicks. Journal of Immunotoxicology, [online] 2013,1-8. Available from: http://informahealthcare.com/imt [viewed 25th December 2020]. DOI: 10.3109/1547691X.2013.866706
20. Singh M, Poonia MK, Kumhar BL, Singh G. Livelihood

security of poor families through pratapdhan backyard poultry rearing in Kota district of Rajasthan. International Journal of Current Microbiology and Applied Sciences 2017;6(4):466-469.
21. Singh R, Srivastava AK, Gangwar NK, Giri DK, Singh R, Kumar R. Pathology of sub-chronic cadmium and chlorpyrifos toxicity in broilers. Indian Journal Veterinary Pathology 2016;40(4):331-336.
22. Sree EK, Nirmala TV, Reddy RVSK. Comparative performance of different varieties of chicken under backyard system of rearing in tribal and rural areas of west Godavari district, A.P. International Journal of Science, Environment and Technology 2017;6(2):1100 – 1104.
23. Verma N, Neeraj J, Singh. Evaluation of hepatoprotective activity of *Tephrosia purpurea* linn. Stem. International Educational Applied Scientific Research Journal 2017;2(7):2456-5040
24. Wani H, Rehman S, Shoukat S, Kour N, Dutta S. A study on chlorpyrifos induced oxidative stress in broiler chickens. International Journal of Livestock Research [online] 2017,7(3). Available through http://www.ijlr.org/