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Efficacy of Extracts of *Anthocephalus cadamba*, *Brassica juncea* and *Pithecellobium dulce* against Fipronil-induced Histopathological Changes in Wistar Rats

Devendra Singh[1*], Pratishtha Sharma[2], Shweta Anand[3], Rahul Swarnkar[4] and Naresh Singh Kuntal[5]

[1]Ph.D. Scholar, Department of Veterinary Pharmacology and Toxicology, CVAS, Bikaner (Rajasthan), India.
[2]Assistant Professor, Department of Veterinary Pharmacology and Toxicology, CVAS, Bikaner (Rajasthan), India.
[3]Assistant Professor, Department of Veterinary Pharmacology and Toxicology, SVPUA & T, Meerut (Uttar Pradesh), India.
[4]Veterinary Officer, Animal Husbandry Department, Banswara (Rajasthan), India.
[5]Veterinary Medical Officer, Bulandshahr, Animal Husbandry Department (Uttar Pradesh), India.

(Corresponding author: Devendra Singh)*
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ABSTRACT: Fipronil is a new generation broad spectrum insecticide of phenylpyrazole group used for pest management in agriculture practices widely. The current study aimed to assess the ameliorating effects of *Anthocephalus cadamba* (Kadamba), *Brassica juncea* (Mustard), and *Pithecellobium dulce* (Jungle jalebi) extracts on histopathological changes induced by sub-acute Fipronil exposure in rats. Rats of either sex were randomly divided into five groups (6 rats/group). Group I served as a control in which corn oil (acting as a vehicle of Fipronil) was administered @10 ml/kg body weight daily for 28 days. Group II served as Fipronil treated group @10 mg/kg body weight, daily for 28 days. Fipronil along with extracts of *Anthocephalus cadamba* leaves, *Brassica juncea* seeds and *Pithecellobium dulce* fruits @300 mg/kg body weight, daily for 28 days were administered in groups III, IV, and V, respectively. Histopathological findings revealed that fipronil produced mild to moderate degenerative changes in the liver, kidney, brain, and spleen which were minimized by the co-treatment with Kadamba, Mustard, and Jungle jalebi in fipronil-intoxicated rats. Extracts of all three plants were found effective to ameliorate the histopathological alterations of major organs affected by sub-acute toxicity induced by fipronil in rats. Among these, extract of *Pithecellobium dulce* fruits exhibits the highest ameliorative potential compared to *Anthocephalus cadamba* leaves, *Brassica juncea* seeds extracts.

Keywords: Kadamba, Mustard, Jungle jalebi, fipronil.

INTRODUCTION

Pesticides have gained importance in contemporary agricultural practices by effectively preventing pre-harvest and post-harvest losses. Many new chemical insecticides are emerging in the market today like phenylpyrazoles, 4th generation pyrethroids, avermectins, diamides, spinosyns etc. Fipronil, classified as a phenylpyrazole insecticide, operates on γ-aminobutyric acid (GABA) receptors (Law and Lightstone 2008). It poses significant toxic effects in rats (LD_{50} 97 mg/kg body weight) and mice (LD_{50} 95 mg/kg body weight). At elevated doses, fipronil induces heightened neural excitation, paralysis, and eventual fatality in non targeted species. (Pisa *et al.,* 2015; Simon-Delso *et al.,* 2015). It is a matter of great concern regarding the effects of neurotoxic systemic pesticides, particularly for neonicotinoids and fipronil (Simon-Delso *et al.,* 2015; Pisa *et al.,* 2021). Medicinal plants play a crucial role in maintaining the health of a significant portion of the global population. *Anthocephalus cadamba* (kadamba) possesses analgesic, anti-inflammatory, antimicrobial, antioxidant, antimalarial, antihepatotoxic, antidiarrheal, and wound-healing properties (Umachigi *et al.,* 2007; Alam *et al.,* 2008a; Alam *et al.,* 2008b). *Brassica juncea*, commonly known as Indian mustard, stands as a prominent oilseed crop cultivated worldwide in tropical, subtropical regions, also in the Indian subcontinent (Tiwari *et al.,* 2022). It is an economical and nourishing food source enriched with bioactive components like glucosinolates and their breakdown products, polyphenols (flavonoids and anthocyanins), substantial dietary fiber, chlorophylls, β-carotene, ascorbic acid, minerals, and volatile compounds (Yokozawa *et al.,* 2003). The fruit of *Pithecellobium dulce* is endowed with both nutritional and medicinal values and is widely consumed as a food in various regions of India. It contains therapeutically potential bioactive phytocomponents, including naringenin, quercetin, rutin, gallic acid, stigmasterol, clonazepam, quinolinone, nootkatone, junipene, calarene, eremophiline, valencene, and baicalin (Pradeepa *et al.,* 2013). This study was conducted to examine the

histopathological changes induced by sub-acute exposure to fipronil in rats and to assess the effectiveness of extracts from *Anthocephalus cadamba*, *Brassica juncea*, and *Pithecellobium dulce* in mitigating the histopathological alterations caused by sub-acute exposure to fipronil in rats.

MATERIAL AND METHODS

Experimental animals. The study was conducted on adult male and female Wistar rats weighing 100-250 g procured from Birds Park Meerut Cantt. (U.P.), India. The animals were maintained under standard management conditions and provided feed and water *ad libitum*. Animals were kept in laboratory conditions for 7 days for acclimatization before the start of the experiment. The bedding material (wheat straw) was changed every alternate day. Throughout the entire study, the experimental animals were continuously observed and handled according to institutional animal ethics guidelines. The use of animals in this study received prior approval from the institutional animal ethics committee.

Preparation of extracts. *Anthocephalus cadamba* leaves and *Pithecellobium dulce* pods were collected from in and around the campus of the College of Veterinary and Animal Science, Navania, Udaipur. Dried seeds of *Brassica juncea* were procured from the local market. All plant materials (Leaves, pods, and seeds) were authenticated by the Department of Horticulture, Maharana Pratap University of Agriculture and Technology in Udaipur, Rajasthan. Plant materials were cleaned, shade-dried, and ground to make a coarse powder. The extracts were prepared through maceration using distilled water for *Anthocephalus cadamba* leaves, and 70 percent ethanol for *Pithecellobium dulce* pods and *Brassica juncea* seeds. After the seven-day maceration, the aqueous extract of the *Anthocephalus cadamba* and hydroethanolic extracts of *Pithecellobium dulce* pods and *Brassica juncea* seeds were filtered through Whatman filter paper no. 1, and the filtrate was subjected to evaporation using a rotary vacuum evaporator (Macro Scientific Works Pvt. Ltd.).

Experimental design:

Table 1: Experimental design for the study of ameliorating potential of *Anthocephalus cadamba, Brassica juncea,* and *Pithecellobium dulce* against histopathological alterations induced by sub-acute exposure of fipronil in rats.

Groups	Treatment	No. of rats	Dose (mg/kg B.w.)	Feeding schedule	Route of administration
I	Control (Corn oil)	6	10ml/kg	0-28 days	Orally
II	Fipronil	6	10	0-28 days	Orally
III	Fipronil + aqueous extract of Kadamba leaves Papaya	6	10+300	0-28 days	Orally
IV	Fipronil + hydroalcoholic extract of Mustard seeds	6	10+300	0-28 days	Orally
V	Fipronil + hydroalcoholic extract of Jungle Jalebi fruits	6	10+300	0-28 days	Orally

Histopathology. At the end of the experiment, tissues of the liver, kidney, brain, and spleen of rats from each treatment groups were collected in 10 percent formaldehyde solution. Each sample was treated with graded alcohol concentrations for dehydrate, cleared with xylene and embedded in paraffin to make tissue blocks. The sections with 5-6 µm thickness obtained from tissue blocks of each organ were stained with haematoxylin and counterstained with eosin.

RESULTS AND DISCUSSION

Histopathological assessments are commonly employed to identify organ-specific effects associated with chemical exposure (Crissman *et al.,* 2004). Fipronil seems to produce oxidative stress in the liver, kidney, brain, and spleen of rats. These findings also seem to be correlated with the histopathological changes observed in various organs *viz*. liver, kidney, brain, and spleen of rats as compared to control animals. Examination of all organs revealed significant alterations in the normal histological architecture.

Liver. Histopathological lesions observed in the liver from groups I to V are depicted in Plate 1. The control group exhibited normal cellular architecture with identified hepatocyte structure arranged in cord pattern around the central vein. Congestion, dilation of blood vessels and sinusoids along with infiltration around portal triad in the liver were observed in fipronil treated group. Significant improvement was noticed in groups III, IV, and V with only mild congestion and infiltration of mononuclear cells. The results were in corroboration with another study (Mossa *et al.,* 2015) which reported that rats exposed to 10mg/L of fipronil showed degeneration of hepatocytes and portal infiltration with inflammatory cells. Similar histopathological changes were observed after 15 days of oral exposure of fipronil in Japanese quail (Ali *et al.,* 2016) and after 4 weeks of oral administration of fipronil in mice (Badgujar *et al.,* 2016). Similar findings were also observed in sub chronic acetamiprid toxicity in Wistar rats Chakroun *et al.* (2016). In the present study, Kadamba, Mustard, and Jungle jalebi co-treatment ameliorated the histopathological changes in the liver of fipronil-treated animals. These results were in agreement with the study where the *Anthocephalus cadamba* treated group showed relatively normal architecture of hepatocytes in alloxan-induced diabetic rats (Swarnkar *et al.,* 2016). Similar findings were also reported by others where treatment with aqueous extract of *Pithecellobium dulce* before and after the toxin exposure showed a considerable improvement in liver morphology (Manna *et al.,* 2011).

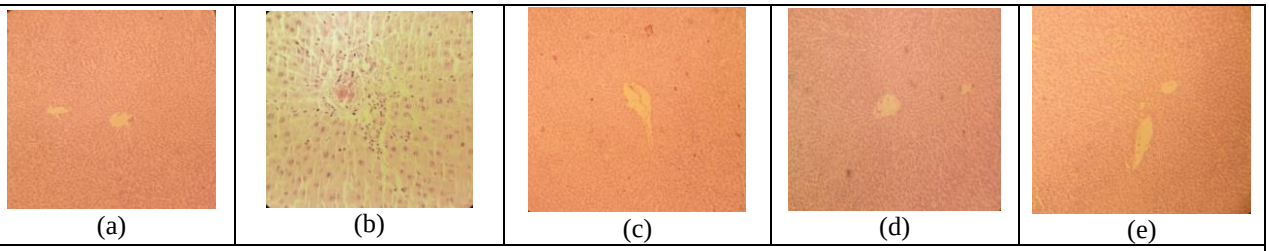

Plate 1: Photomicrograph of liver (a) showing normal hepatocyte arranged in cord pattern around the central vein in the liver of control (H&E x100); (b) showing congestion, dilation of blood vessel and sinusoids along with infiltration around portal triad in liver of Fipronil treated group (H&E x400); (c) Fipronil co-treatment with A. *cadamba* showing mild infiltration of mononuclear cells in parenchymatous tissue of liver (H&E x100); (d) Fipronil co-treatment with B. *juncea* showing mild congestion infiltration of neutrophil in the hepatic parenchyma around the portal triad (H&E x400); (e) Fipronil co-treatment with P. dulce showing congestion of blood vessel, sinusoids along with mild infiltration of neutrophil and lymphocyte (H&E x100).

Kidney. Histopathological lesions in the kidney of control and other treatment groups are presented in Plate 2. The control group exhibited intact glomeruli and renal tubules in the cortical area of kidneys whereas the fipronil-treated group showed tubular dilation, necrosis of tubular cell lining along with degeneration, increased Bowman's space, and formation of hyaline cast in the lumen of tubules in the cortical area of the kidney. Fipronil co-treatment with extracts of *A. cadamba*, *B. juncea*, and *P. dulce* groups exhibited improvement in histopathological changes of kidney by diminishing the necrosis and degeneration. Previous studies also reported dilation of collecting tubules, congestion, and severe degenerative changes along with necrosis of tubular lining cells in mice (Badgujar *et al.*, 2015) and alterations in the histopathological architecture of the kidney which included necrosis, inflammatory cell infiltration and vacuolation in rats exposed to fipronil (Mossa *et al.*, 2015). In the present study, Kadamba, Mustard, and Jungle jalebi co-treatment showed nephroprotective effect. Treatment with these plant extracts improved the kidney tissue histology with extremely mild necrosis of tubular lining cells. It may be attributed to the protective effect of these plants due to the presence of antioxidants in their extracts.

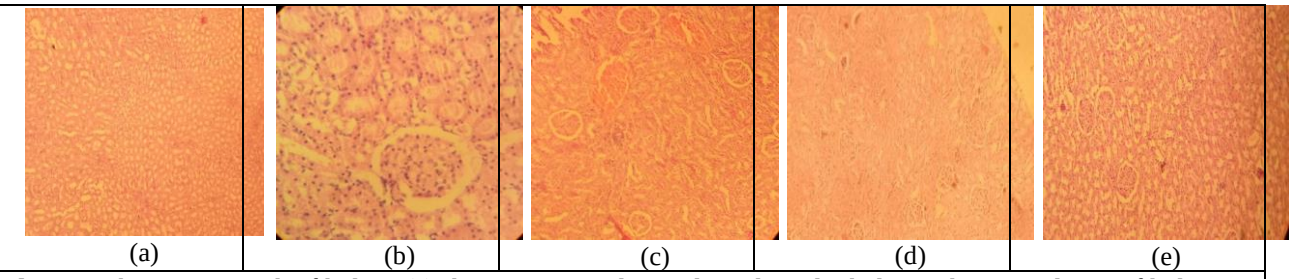

Plate 2 : Photomicrograph of kidney (a) showing intact glomeruli and renal tubules in the cortical area of kidney in control group (H&E x100); (b) showing tubular dilation, necrosis of tubular cell lining along with degeneration, increased bowman's space and formation of hyaline cast in the lumen of tubules in the cortical area of kidney in Fipronil treated group (H&E x400); (c) Fipronil co-treatment with *A. cadamba* showing mild interstitial haemorrhage, dilated tubules in the cortical area of kidney (H&E x100); (d) Fipronil co-treatment with *B. juncea* showing interstitial haemorrhage, infiltration, dilated tubules in medullary area of kidney (H&E x 400); (e) Fipronil co-treatment with *P. dulce* showing congested blood vessel, mild interstitial haemorrhage (H&E x 400).

Brain. Histopathological lesions in the brain of control and other treatment groups are presented in Plate 3. The control group displayed intact neuron and glial cells in the cerebral cortex of the brain while fipronil-treated group exhibited marked neuronal degeneration, vacuolation, and congestion of blood vessels in the cerebral cortex. Fipronil co-treatment with *A. cadamba*, *B. juncea* and *P. dulce* extracts provide improvement in histopathological changes with mild neuronal degeneration, vacuolation, and congestion of blood vessels. Previous studies also reported similar findings where high dose of fipronil caused severe vacuolation in the molecular layer, congestion of blood vessels in the cerebral cortex along with degeneration (Badgujar *et al.*, 2015). Cerebral hemisphere revealed changes comprising of mild neuronal degeneration with surrounding glial cells in *Gallus domesticus* due to thiacloprid toxicity (Goyal *et al.*, 2010). In the present study, co-treatment with Kadamba, Mustard, and Jungle jalebi in fipronil-treated rats restored the normal histopathological structure of the brain. From these results, it could be suggested that these plants had neuroprotective action.

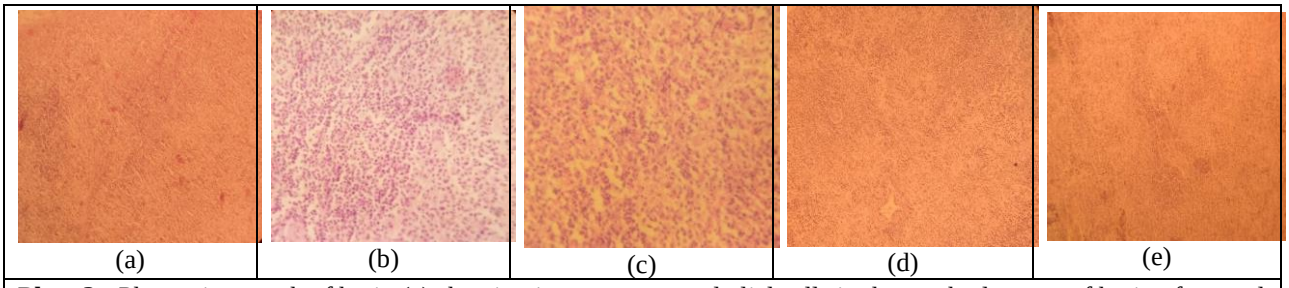

Plate 3 : Photomicrograph of brain (a) showing intact neuron and glial cells in the cerebral cortex of brain of control (H&E x100); (b) showing neuronal degeneration, vacuolation and congestion of blood vessel in cerebral cortex of brain in Fipronil treated group (H&E x400); (c) Fipronil co-treatment with *A. cadamba* showing mild neuronal degeneration and congestion with increase oligodendrocyte in cortex of brain (H&E x400); (d) Fipronil co-treatment with *B. juncea* showing mild increase in microglial cells along with neuronal degeneration (H&E x400); (e) Fipronil co-treatment with *P. dulce* showing vacuolar spaces around the few neuron (H&E x400).

Spleen. Histopathological lesions in the spleen of control and other treatment groups are presented in Plate 4. The spleen of control rats showed normal histological architecture with abundant lymphocytes in lymphoid follicles in the white pulp whereas Fipronil treated group exhibited the presence of apoptotic bodies with rarefaction of lymphoid tissue. Fipronil co-treatment with *A. cadamba, B. juncea,* and *P. dulce* extracts showed mild depletion of lymphocyte and rarefication of lymphoid tissue. Other studies also reported depletion of lymphocytes in lymphoid follicles and, the presence of apoptotic bodies with severe degenerative and necrotic changes in lymphocytes in the spleen of mice exposed to fipronil at 10mg/kg dose rate (Badgujar, 2014). Depletion of lymphocytes and congestion in the white pulp of the spleen in imidacloprid-treated mice was also observed by Badgujar *et al.* (2013). Exposure to endosulfan caused depletion of lymphocytes and necrosis of the spleen of rabbits (Ozmen and More 2015). In the current study, a significantly improved histological architecture of the spleen was observed with the co-administration of Kadamba, Mustard, and Jungle Jalebi extracts along with fipronil.

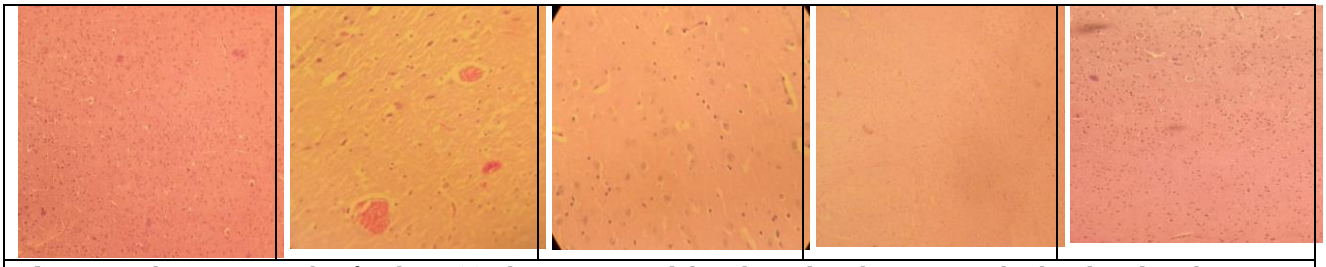

Plate 4 : Photomicrograph of spleen (a) showing normal histological architecture with abundant lymphocyte in lymphoid follicles in the white pulp of spleen in control group (H&E x100); (b) showing of apoptotic bodies with rarefaction of lymphoid tissue in Fipronil treated group (H&E x400); (c), (d), (e) Fipronil co-treatment with *A. cadamba, B. juncea* and *Pithecellobium dulce* respectively showing mild rarefaction and depletion of lymphoid tissue (H&E x400); (g) treatment with *B. juncea* showing abundant lymphocyte in lymphoid follicles in the white pulp of spleen (H&E x400).

CONCLUSIONS

The histopathological findings indicated that fipronil induced mild to moderate degenerative changes in the liver, kidney, brain, and spleen, which were subsequently reversed by the co-treatment with the extracts of Kadamba, Mustard, and Jungle Jalebi. Observations revealed that the group treated with *Pithecellobium dulce* (Jungle Jalebi) exhibited a superior ameliorative effect compared to the other treatment groups.

FUTURE SCOPE

Our findings suggest a promising avenue for the development of natural remedies to counteract the degenerative changes induced by Fipronil. Further research and exploration of the suggested areas can contribute to the development of novel therapeutic strategies with potential benefits for human health.

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Conflict of Interest. None.

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