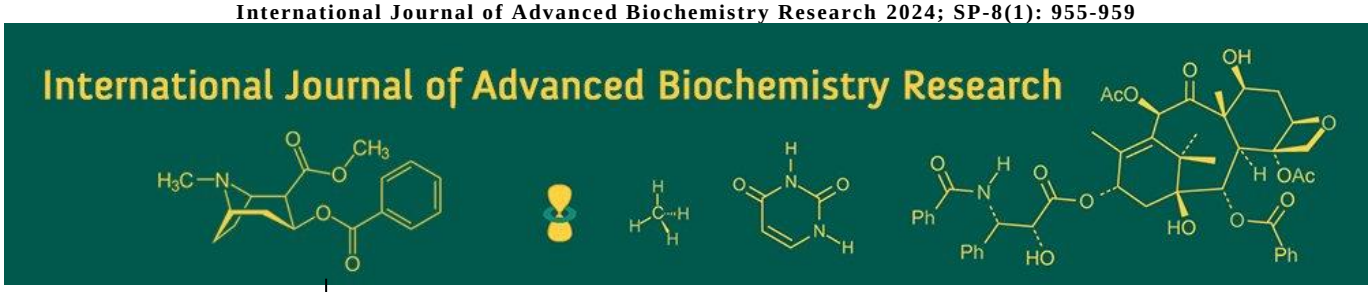

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Ameliorative effect of *Anthocephalus cadamba, Brassica juncea* and *Pithecellobium dulce* against fipronil-induced hemato-biochemical alterations in rats

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Abstract
Exposure to pesticides can lead to a range of disorders, spanning from simple topical irritations to intricate systemic illnesses. Current study was designed to explore the toxic impact of fipronil, a phenylpyrazole insecticide, on hematological and serum biochemical parameters and the potential alleviating effects of extracts from *Anthocephalus cadamba* (Kadamba), *Brassica juncea* (Mustard), and *Pithecellobium dulce* (Jungle jalebi) in rats. Rats were randomly divided into eight groups, each having 6 rats. Group I served as the control group, receiving only corn oil (serving as the vehicle for fipronil) @ 10 ml/kg b.w daily for 28 days. Group II served as fipronil treated group @ 10 mg/kg b.w. daily for 28 days. In Groups III, IV, and V, Fipronil was administered orally (10 mg/kg b.w. daily for 28 days) along with extracts of leaves of *A. cadamba*, seeds of *B. juncea*, and fruits of *P. dulce*, respectively (each @ 300 mg/kg b.w. daily for 28 days). While Groups VI, VII and VIII received only extracts of leaves of *A. cadamba*, seeds of *B. juncea*, and fruits of *P. dulce*, respectively (@ 300 mg/kg b.w. daily for 28 days). Fipronil treatment led to a significant decrease in TEC, Hb, PCV, MCV, MCH, DLC, TLC and Lymphocytes while significant increase in Neutrophil, Monocyte, and Eosinophil counts. Biochemical parameters *viz*. ALT, AST, LDH and, creatinine kinase were significantly increased in fipronil treated rats. Administration of Kadamba, Mustard, and Jungle Jalebi extracts effectively restored these altered haemato-biochemical values. It was concluded that Kadamba, Mustard, and Jungle Jalebi extracts are having a potential protective or mitigating role against the deleterious impact of fipronil exposure in rats. Jungle Jalebi exhibited superior ameliorative effects on serum haemato-biochemical parameters compared to groups treated with Mustard and Kadamba.

Keywords: Fipronil, rats, Kadamba, mustard and jungle Jalebi

Introduction
Pesticides constitute the exclusive category of chemical toxins deliberately introduced into the environment for the purpose of improving food production by combating insect pests and managing disease vectors [1]. These compounds exhibit non-specificity towards targets, and prolonged exposure to them can trigger numerous abnormalities in diverse organisms including humans [2, 3]. Fipronil, a broad-spectrum insecticide, belongs to the phenylpyrazole group of chemicals. The documented toxicity resulting from fipronil is extensive and well-established in mice, rats, rabbits, as well as in carnivores such as cats and dogs, along with its impact on wildlife. The presumed cause of fipronil toxicity lies in the inhibition of a neurotransmitter, specifically a $GABA_A$-gated chloride channel. This leads to a deficient neurological threshold in the organism, ultimately culminating in fatality [35]. Previous findings have indicated that fipronil may be implicated in hindering the metabolic enzymatic systems, commonly recognized for containing sulfhydryl groups, as well as disrupting the oxidative phosphorylation in the mitochondrial complex [5]. Additionally, recent findings have exposed that fipronil induces severe mitochondrial dysfunctions, DNA damage, and micronuclei formation in freshwater fish and mammals [6, 7]. Hemato-biochemical indices serve as an important tool for evaluating the health status of animals and discerning the potential effects of various contaminants. These parameters can serve as rapid tools for estimating and diagnosing the insecticide toxicity.

The observed results provide a quantitative measure of stress in the blood and organs of individuals exposed to stressors [8]. Various studies have illustrated the toxic effects of pesticides on these indicators [9-11].

Medicinal plants serve as a repository of bioactive compounds possessing therapeutic properties, utilized in the treatment of diverse health conditions. *Anthocephalus cadamba*, also known as Kadamba, is a substantial evergreen tree abundant in India, featuring expansive leaves and captivating ball-shaped fruits. The leaves, fruits, and bark of Kadamba harbor noteworthy pharmacological properties attributed to the diverse array of chemical constituents present in them. Phytochemical assessment of its leaf powder indicates the presence of saponins, alkaloids, tannins, and phenolics [12]. *Brassica juncea*, widely recognized as mustard, belongs to the Brassicaceae family and holds significant prominence as a key edible oilseed crop globally, primarily cultivated in India. The major bioactive components with therapeutic significance in *Brassica juncea* are often considered to be its polyphenolic secondary metabolites, along with glucosinolates [13, 14]. *Pithecellobium dulce*, locally identified as Jungal Jalebi, is an evergreen tree extensively distributed throughout a significant portion of India and Southeast Asia. The fruits of this plant found extensive application in Ayurvedic medicines and household remedies due to presence of saponins, flavonoids and phenols. The flavonoid and phenolic compounds contain hydroxyl functional groups, which exhibit radical scavenging abilities, serving to prevent oxidative stress [15].

Hence, the current study aimed to assess the potential ameliorative effects of *Anthocephalus cadamba* (kadamba), *Brassica juncea* (Mustard), and *Pithecellobium dulce* (Jungle jalebi) against the sub-acute toxicity induced by fipronil in rats. This investigation may offer a foundation for identifying novel pharmacologically active compounds to address hemato-biochemical alterations resulting from insecticide exposure.

Materials and Methods

Experimental Animals

The research involved adult male and female Wistar rats, with weights ranging from 100 to 250 g, obtained from Birds Park in Meerut Cantt, Uttar Pradesh, India. The animals were kept in standard management conditions and provided feed and water *ad libitum*. The animals were acclimatized in laboratory conditions for duration of 7 days prior to the commencement of the experiment. Bedding material containing 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 research 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 College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur. Dried seeds of *Brassica juncea* were procured from the local market. All plant materials (Leaves, pods and seeds) were authenticated by Department of Horticulture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan. All 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 per cent 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.

Experimental design

Groups	Treatment	No. of rats	Dose (mg/kg body weight)	Feeding schedule	Route of administration
I	Control (Corn oil)	6	10 ml/kg	0-28 days	Orally
II	Fipronil toxicity	6	10	0-28 days	Orally
III	Fipronil toxicity + Extract of Kadamba leaves Papaya	6	10+300	0-28 days	Orally
IV	Fipronil toxicity + Extract of Mustard seeds	6	10+300	0-28 days	Orally
V	Fipronil toxicity + Extract of Jungle jalebi fruits	6	10+300	0-28 days	Orally
VI	Extract of Kadamba leaves	6	300	0-28 days	Orally
VII	Extract of Mustard seeds	6	300	0-28 days	Orally
VIII	Extract of Jungle jalebi fruits	6	300	0-28 days	Orally

Haematological estimations

Experimental rats were anesthetized by diethyl ether 24 h after the last treatment/dose and blood samples were collected (2 ml by a direct cardiac puncture) in vials containing anticoagulant, EDTA. Various hematological parameters *viz.* Hemoglobin concentration (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined with the help of fully automatic haematological analyzer (MINDRAY BC-2800 VET) while Differential leukocyte count (DLC) was carried out manually.

Serum biochemical estimations

For serum, 0.5-1 ml of blood was collected from the posterior vena cava and heart of rats at the time of sacrifice in the sterile micro centrifuge tubes and allowed to clot. These samples were then centrifuged at 3000 rpm for 10 min to separate serum. Serum samples were stored at -20 °C till the estimation of biochemical parameters. Various biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine and lactate dehydrogenase (LDH) was assessed using a fully automatic biochemistry analyzer 'Turbo Chem 100, Awareness Technologies, USA', employing the dedicated iChem 100 reagent kits.

Statistical analysis

Statistical variances among the means for different parameters were evaluated utilizing Microsoft Excel and SPSS statistical software Treatment group comparisons were performed using one-way ANOVA, followed by Duncan multiple comparisons as a post hoc test. Statistical significance was established at p-values > 0.05 for all analyses.

Results

Hematological Parameters

Effect on TEC, Hb, PCV, MCV, MCH and MCHC

The sub-acute toxicity study of fipronil demonstrated a significant (p<0.05) decrease in TEC values in Group II when compared to the control group. Conversely, in groups III, IV, and V exhibited a significant rise in TEC values in comparison to Group II. Hb and PCV levels exhibited a significant (p<0.05) decrease in Group II compared to the control group. Conversely, in groups III, IV, and V, there was a significant (p<0.05) increase in both Hb and PCV levels when compared to Group II. MCV levels were found to be significantly lower in all fipronil treated groups as compared to control, while in groups III, IV and V its level was significantly (p<0.05) higher in comparison to the group II. MCH levels were significantly lower in groups II, III, IV, and V compared to the control group, while in groups III, IV, and V, the MCH level was significantly higher than in Group II. The MCHC levels were significantly elevated in groups II, III, IV, and V compared to the control group. However, in groups III, IV, and V, the MCHC level was significantly lower when compared to Group II. It was observed that group III showed much better amelioration in hematological parameters as compared to group IV and V (Table 1).

Effect on TLC and DLC

The impact of Kadamba, Mustard, and Jungle Jalebi extracts in groups II, III, IV, and V indicated a significant (p<0.05) reduction in TLC levels compared to the control group. On the other side, groups III, IV, and V exhibited a significant (p<0.05) increase in TLC values compared to Group II. The lymphocyte level in groups II, III, IV, and V was significantly (p<0.05) lower compared to the control group. However, in groups III, IV, and V, it was significantly (p<0.05) increased compared to Group II. Neutrophils, Monocyte, Eosinophil, Basophil were also significantly (p<0.05) higher in all treatment groups when compared to control group and there was significant (p<0.05) decrease in these groups as compared to group II. It was also observed that group III significantly restored TLC count and DLC count as compared to group IV and V (Table 2).

Table 1: Impact of Kadamba, Mustard, and Jungle Jalebi extracts on hematological parameters (TEC, Hb, PCV, MCV, MCH, and MCHC) in response to sub-acute Fipronil exposure in rats

Groups	Treatment	TEC (x10^6/µL)	Hb (g/dl)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dl)
I.	Control	7.11^{a} ±0.09	13.65^{a}±0.15	42.85^{a}±0.25	60.32^{a}±0.50	20.31^{a}±0.23	31.52^{e}±0.13
II.	Fipronil	5.99^{d} ±0.03	11.08^{e}±0.04	33.30^{g}±0.15	55.61^{e}±0.29	17.97^{f}±0.14	34.11^{a}±0.21
III.	Fipronil+ Kadamba	6.81^{c} ±0.01	13.13^{c}±0.05	39.38^{e}±0.14	58.07^{c} 0.12	19.39^{cd}±0.05	32.65^{c}±0.17
IV.	Fipronil + Mustard	6.74^{c}±0.01	12.97^{c}±0.04	38.90^{f}±0.11	57.80^{cd}±0.15	19.17^{de}±0.03	33.12^{b}±0.07
V.	Fipronil + Jungle jalebi	6.78^{c}±0.03	12.93^{d}±0.03	38.79^{f}±0.09	57.14^{d}±0.09	18.98^{e}±0.04	33.37^{b}±0.05
VI.	Kadamba	6.99^{b}±0.03	13.60^{a}±0.04	42.09^{b}±0.11	60.08^{a}±0.20	19.78^{b}±0.05	31.72^{e}±0.12
VII.	Mustard	6.95^{b}±0.04	13.42^{b}±0.05	41.44^{c}±0.14	59.63^{a}±0.31	19.57^{bc}±0.05	32.22^{d}±0.13
VIII	Jungle jalebi	6.94^{b}±0.03	13.38^{b}±0.03	40.67^{d}±0.12	58.90^{b}±0.08	19.50^{bc}±0.04	32.36^{cd}±0.15

The values are presented as Mean ± S.E; n=6; Values with common superscripts within a column are not significantly different (p<0.05)

Table 2: Effect of extracts of Kadamba, Mustard and Jungle jalebi on TLC (x10^3/µL) and DLC (%) against toxicity fipronil-induced sub-acute toxicity in rats

Groups	Treatment	TLC (x10^3/µL)	Lymphocytes (%)	Neutrophils (%)	Monocyte (%)	Eosinophils (%)	Basophils (%)
I.	Control	8.80^{a}±0.04	74.16^{a}±0.26	21.66^{e}±0.24	2.51^{e}±0.03	1.41^{f}±0.05	0.23^{e}±0.02
II.	Fipronil	6.44^{f}±0.03	63.59^{f}±0.44	28.02^{a}±0.25	5.75^{a}±0.17	2.23^{a}±0.04	0.41^{a}±0.04
III.	Fipronil+ Kadamba	8.35^{c}±0.03	72.07^{c}±0.27	23.01^{e}±0.23	2.87^{cd}±0.02	1.86^{d}±0.02	0.28^{de}±0.02
IV.	Fipronil + Mustard	8.12^{d}±0.02	70.95^{d}±0.06	23.75^{c}±0.04	3.03^{bc}±0.03	1.96^{c}±0.02	0.34^{c}±0.01
V.	Fipronil + Jungle jalebi	7.99^{e}±0.02	69.93^{e}±0.05	24.41^{b}±0.03	3.19^{b}±0.02	2.11^{b}±0.02	0.36^{ab}±0.01
VI.	Kadamba	8.74^{a}±0.03	74.05^{ab}±0.12	21.59^{d}±0.12	2.67^{de}±0.02	1.44^{e}±0.04	0.25^{de}±0.01
VII.	Mustard	8.57^{b}±0.02	73.96^{ab}±0.05	21.50^{e}±0.04	2.70^{de}±0.02	1.58^{e}±0.02	0.26^{de}±0.01
VIII	Jungle jalebi	8.42^{c}±0.02	73.50^{b}±0.03	21.66^{e}±0.03	2.76^{d}±0.01	1.79^{de}±0.01	0.30^{cd}±0.01

The values are presented as Mean ± S.E; n=6; Values with common superscripts within a column are not significantly different (p<0.05)

Serum biochemical parameters

Effect on ALT, AST, LDH (IU/L), Creatinine Kinase (mg/dl) enzymes activity in serum

In our study, fipronil treatment in group II, III, IV and V caused significantly (p<0.05) higher serum ALT and AST activities as compared to the control group. On the other hand, in group III, IV and V significantly lower the ALT, AST, activities were observed in comparison to the group II. There was a non-significant increase in the levels of ALT and AST in groups VI, VII, and VIII, which were comparable to the control group. The activity of ALT and AST in group V showed greater ameliorative effect in comparison to group III and IV. The levels of LDH and creatinine kinase activities were significantly (p<0.05) elevated in groups II, III, IV, and V compared to the control group. However, in groups III, IV, and V, these levels were significantly (p<0.05) lower compared to Group II. Conversely, levels of LDH and creatinine kinase (CK) showed a slight increase in groups VI, VII, and VIII compared to the control group (Table 3).

Table 3: Effects of Kadamba, Mustard and Jungle Jalebi extracts on ALT, AST, LDH (IU/L) and Creatinine Kinase (mg/dl) in serum against fipronil-induced sub-acute toxicity in rats

Groups	Treatment	Serum biochemical parameters			
		ALT	AST	LDH	C. K.
I.	Control	$36.47^{e}\pm0.59$	$96.10^{f}\pm0.67$	$225.83^{d}\pm3.06$	$290.00^{e}\pm3.82$
II.	Fipronil	$77.73^{a}\pm2.37$	$174.78^{a}\pm3.45$	$270.83^{a}\pm3.92$	$333.67^{a}\pm4.74$
III.	Fipronil+ Kadamba	$62.83^{b}\pm1.62$	$132.12^{c}\pm2.53$	$253.33^{bc}\pm2.99$	$323.83^{b}\pm3.2$
IV.	Fipronil + Mustard	$66.85^{b}\pm1.44$	$164.47^{b}\pm1.34$	$259.00^{c}\pm2.65$	$324.33^{b}\pm2.82$
V.	Fipronil + Jungle jalebi	$56.18^{c}\pm1.96$	$122.02^{d}\pm1.48$	$251.33^{bc}\pm1.89$	$320.83^{bc}\pm2.34$
VI.	Kadamba	$40.98^{d}\pm0.79$	$109.87^{e}\pm3.42$	$249.17^{c}\pm4.02$	$310.67^{d}\pm3.49$
VII.	Mustard	$41.22^{d}\pm1.06$	$108.52^{e}\pm3.21$	$250.17^{bc}\pm3.02$	$312.33^{cd}\pm3.05$
VIII	Jungle Jalebi	$39.57^{de}\pm1.00$	$105.72^{e}\pm2.16$	$244.83^{c}\pm2.34$	$318.00^{bcd}\pm1.53$

The values are presented as Mean ± S.E; n=6; Values with common superscripts within a column are not significantly different ($p<0.05$)

Discussion

Blood and serum biochemical parameters are recognized as the foremost biomarkers for assessing the toxic effects of pollutants. They function as indicators of the internal homeostasis and physiological state of organisms under exposure. [16, 17]. The levels of Hb and PCV generally serve as reliable indicators of the extent to which the circulating red blood cell mass are decreased [18]. The reduction in hemoglobin (Hb) concentration observed in the present experiment implies that Fipronil may have disrupted the process of erythropoiesis. The observed results were in agreement to [19]. The findings in the present study showed a significant reduction RBC, Haemoglobin, PCV and MCV values. The significant decline in Hb levels and TEC in the Fluoride treated groups in the present study was in agreement to [20, 21]. The reduction in MCH may be due to destruction of RBC and decrease in Hb synthesis and hemoglobin content. The findings in the present study showed a significant decline in TLC values, whereas a significant alteration in DLC, which include severe reduction in per cent lymphocytes and increase in per cent neutrophils, monocytes, eosinophils and basophils. It may be due to immunosuppressive effect of Fipronil. It also led to decrease in hematocrit count [22]. There was a noteworthy increase in TEC, Hb, PCV, MCV and MCH, in co-treatment groups with Kadamba, Mustard and Jungle jalebi. The results were in accordance with Khandelwal *et al.,* (2015) [23], Toudji *et al.,* (2017) [24], Pradeepa *et al.,* (2013) [25] and Dolai *et al.,* (2016) [26].

In the present study, there was a significant increase in AST, ALT, LDH and creatinine kinase after sub-acute exposure of Fipronil. These liver enzymes are widely recognized as superior biomarkers, routinely utilized to assess the degree of hepatic damage [27]. The elevation in the activity of these enzymes indicates liver dysfunction, suggesting an augmented release from hepatocytes owing to mitochondrial membrane damage [28]. Elevated levels of these enzymes following exposure to fipronil have been previously documented in rats [28], mice [29] and Japanese quail [30]. In this study, the co-treatment with Kadamba significantly reduced the levels of ALT, AST, LDH, and Creatinine Kinase compared to the administration of Fipronil alone. The results were in accordance with previous studies [23, 31-32], where extract of *Anthocephalus cadamba* significantly reduced the elevated level of these parameters. Similarly, co-treatment with Jungle Jalebi in Fipronil-administered rats led to a reduction in the serum levels of liver biomarker enzymes The findings corroborate to Toudji *et al.,* (2017) [24], Pradeepa *et al.,* (2013) [25] and Bhoopendra and Nitesh, (2014) [33] where similar decrease in these enzyme activity were observed in the Wistar rats. Co-treatment with mustard seeds similarly led to a significant reduction in serum biochemical parameters, aligning with the findings reported in reference [34].

Conclusion

The current experimental study demonstrated that the insecticide fipronil induces adverse effects on hematological and serum biochemical parameters, highlighting its hepatotoxic and nephrotoxic nature. However, co-treatment with *Anthocephalus cadamba*, *Brassica juncea*, and *Pithecellobium dulce* significantly alleviated these effects. The co-treatment involving *Pithecellobium dulce* (Jungle Jalebi) demonstrated superior ameliorative effects on serum biochemical parameters when compared to groups treated with Kadamba and Mustard.

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