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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(8): 818-824
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www.thepharmajournal.com
Received: 25-06-2021
Accepted: 27-07-2021

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Evaluation of the extracts of *Anthocephalus cadamba, Brassica juncea* and *Pithecellobium dulce* against general toxicity induced by sub-acute exposure of Fipronil in rats

Devendra Singh, Shweta Anand, Rahul Swarnkar, Abhishek Choudhary and Sunil Boghia

Abstract
The present investigation was carried out to evaluate ameliorating potential of extracts of *Anthocephalus cadamba* (Kadamba), *Brassica juncea* (Mustard) and *Pithecellobium dulce* (Jungle jalebi) against general toxicity induced by sub-acute exposure of Fipronil in rats. Fipronil is a new generation insecticide of phenylpyrazole group used for pest management in agriculture practices. The ameliorative effect of *Anthocephalus cadamba* (300 mg/kg B.w.), *Brassica juncea* (300 mg/kg B.w.) *and Pithecellobium dulce* (300 mg/kg B.w.) were seen against Fipronil (10 mg/kg B.w.). The main clinical signs observed due to toxicity of Fipronil were anorexia, dizziness, abnormal gait, lethargy, weakness and aggressive behaviour. Sub-acute intoxication caused decrease in body weight both absolute and relative body weight which was significantly ameliorated by extracts of Kadamba, Mustard and Jungle jalebi. A significant increase in absolute and relative weight of liver, kidney and lung except spleen due to fipronil was significantly restored by Kadamba, Mustard and Jungle jalebi extracts. It can be concluded that Kadamba (@300 mg/kg B.w.), Mustard (@300 mg/kg B.w.) and Jungle jalebi (@300 mg/kg B.w.) extracts has potential to ameliorate toxicity caused by Fipronil (@10 mg/kg B.w.) induced sub-acute toxicity in rats and fruits extract of Jungle jalebi (@300 mg/kg B.w.) has greater ameliorating potential in comparison to Kadamba and Mustard.

Keywords: Kadamba, mustard, jungle jalebi, Fipronil and rat

1. Introduction
Pesticides assumed significance in modern agricultural practices by preventing pre-harvest and post-harvest losses. A lot of new chemical insecticides are appearing on the market now a day. Examples of such insecticides are: phenylpyrazoles, pyrethroids of 4th generation, avermectins, diamides and spinosyns etc. Phenylpyrazoles and neonicotinoids accounted for one-third of the global insecticide market in 2010 [37]. Fipronil is a phenylpyrazole insecticide that acts on γ-aminobutyric acid (GABA) receptors [23]. It is quite dangerous to rats (LD_{50} 97 mg/kg B.w.) and mice (LD_{50} 95 mg/kg B.w.). At sufficiently high dosages, fipronil leads to excessive neural excitation, paralysis, and death [32, 37]. With respect to pesticides there is great concern regarding the effects of neurotoxic systemic pesticides, in particular neonicotinoids and fipronil [31, 32].
Plant materials are composed of a vast array of bio-active principles which are responsible for the therapeutic activities of medicinal plants and provide unlimited opportunities for new drug leads because of their unmatched availability and chemical diversity [10]. *Anthocephalus cadamba* is used in the treatment of various diseases. It primarily consists of indole alkaloids, terpenoids, sapogenins, saponins, terpenes, steroids, fats and reducing sugars [6, 22, 34, 42,]. *Brassica juncea* is one such economically important plant well known in India for centuries for its nutritive and medicinal values [26]. Secondary metabolites of *Brassica juncea* together with glucosinolates, numerous polyphenolics, often considered to be its major therapy relevant bioactive components [9, 19]. *Pithecellobium dulce* Benth, a most versatile medicinal plant, has attracted a worldwide prominence in recent years. Its fruit pericarp contain anthocyanin, flavanoids and as a major source of polyphenol antioxidants. Anthocyanin and phenolic content indicated free radical scavenging activity of *Pithecellobium dulce* between fruit pods [35].

This study were conducted to study the general toxicity parameters induced by sub-acute exposure of Fipronil in rats and to evaluate the extracts of *Anthocephalus cadamba*, *Brassica juncea*and *Pithecellobium dulce* against general toxicity induced by sub-acute exposure of Fipronil in rats.

2. Material and Methods

2.1 Experimental animals

The study was conducted in 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. Before the start of the experiment, animals were kept in laboratory conditions for a period of 7 days for acclimatization. The prior approval of the institutional animal ethics committee was obtained for the use of animals in this study.

2.2 Collection of Plant material and Authentication

All required plant material such as leaves of *Anthocephalus cadamba*, seeds of *Brassica juncea* and pods/fruits of *Pithecellobium dulce* were procured from farms and were authenticated from Department of Horticulture, Maharana Pratap University of Agriculture Technology, Udaipur (Rajasthan).

2.3 Extraction of plants

Plant materials were cleaned to remove dirt and extraneous matter dried afterwards in the shade and cleaned materials were grinded by the use of a blender in order to get a pulverized powder form. Then powdered materials were macerated for seven days by using their proper solvent. After the seven days, ethanol extract was filtered with Whattman filter paper no. 1, and the filtrate was allowed to evaporate by a rotatory vacuum evaporator.

2.4 Experimental design

48 rats were randomly divided into eight groups (6 rats/group). The test dose of Fipronil was selected on the basis of its oral LD_{50}. Oral LD_{50} of technical grade Fipronil in rat has been reported to be 97 mg/kg B.w. [45]. Accordingly, a test dose of Fipronil in the 28 day sub-acute toxicity study was 10 mg/kg. The extracts of leaves of Kadamba, the extract of seeds of Mustard and the extract of fruits of Jungle jalebi were given at the same dose rate of 300 mg/kg [12, 35, 38] by oral gavage for consecutive 28 days. Corn oil was used as a vehicle for Fipronil while the plant extracts were dissolved in distilled water. Each dose was adjusted as per body weight of each rat by adjusting the gavage volume (10ml/kg). Fipronil and plants extract doses were prepared on seven (7) days.

2.5 Toxicity Parameters

2.5.1 Clinical signs/symptoms

Rats of all groups were closely observed twice a day throughout the period of experiment for clinical signs and mortality.

2.5.2 Body Weight and organ weight

Body weight of each rat was recorded on day 0 and at an interval of one week till the completion of the experiment. Organ weight was represented as absolute organ weight and relative organ weight. Relative organ weight was calculated as mentioned below.

Relative organ weight = Organ weight (g)/Body weight (g)

2.5.3 Statistical analysis

Statistical differences between respective means for various parameters were evaluated by using Microsoft Excel and SPSS statistic software. Comparisons among the treatment groups were made by using one way Annova with Duncan multiple comparisons as a post hoc test. All p values <0.05 were considered to be statistically significant.

3. Results

3.1 Clinical signs and symptoms

Treatment of rats with Fipronil over a period of 28 days did not show any mortality. Body coat was roughened and feed intake was reduced in Fipronil treated group in comparison to control group. Rats in the group treated with Fipronil, exhibited anorexia, dizziness, abnormal gait, lethargy, weakness and aggressive behaviour during last two weeks of treatments. All the other groups exhibited normal activities and behaviour during the experimental period.

3.2 Body weight

The body weights recorded at weekly time intervals. The rate of body weight gain were significantly ($p<0.05$) affected by Fipronil alone and in combination with Kadamba, Mustard and Jungle jalebi respectively. The average body weight of Fipronil treated rats was significantly less ($p<0.05$) on 2nd week onwards as compared to all other groups *viz.* group I, III, IV, V, VI, VII and VIII. Body weight gain of rats in plant control groups (VI, VII, VIII) were increased during experiment period.

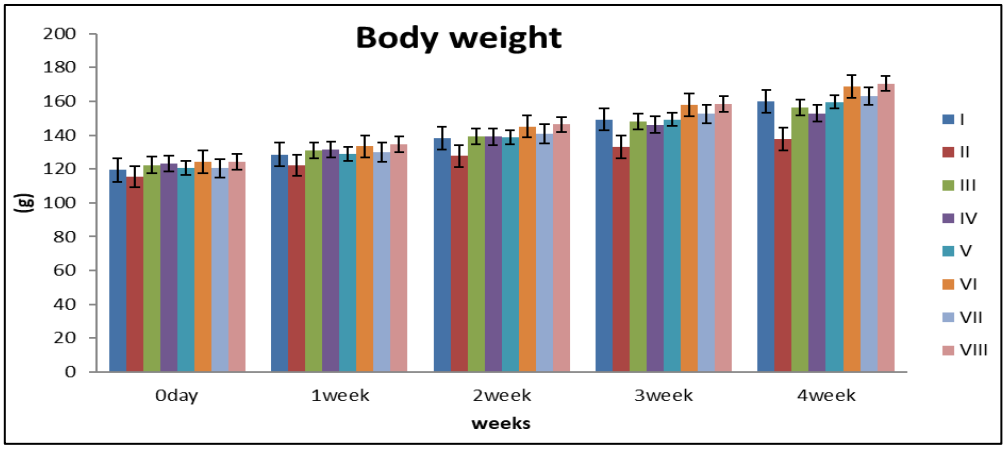

Fig 1: Effects of extracts of Kadamba, Mustard and Jungle jalebi on weekly recorded body weights (g) against toxicity induced by sub-acute exposure of Fipronil in albino rats (Mean ±S.E.)

Table 1: Effects of extracts of Kadamba, Mustard and Jungle jalebi on weekly recorded body weights (g) against toxicity induced by sub-acute exposure of Fipronil in albino rats

Groups	Treatment	0th day	1st week	2nd week	3rd Week	4th week
I.	Control	$119.33^{a} \pm 6.84$	$128.50^{a} \pm 6.85$	$138.33^{ab} \pm 6.71$	$149.17^{ab} \pm 6.43$	$159.83^{a} \pm 6.73$
II.	Fipronil	$115.33^{a} \pm 6.21$	$122.00^{a} \pm 6.24$	$127.67^{b} \pm 6.41$	$133.17^{b} \pm 6.65$	$137.67^{b} \pm 6.90$
III.	Fipronil+ Kadamba	$122.33^{a} \pm 4.74$	$130.83^{a} \pm 4.74$	$139.33^{ab} \pm 4.62$	$148.00^{ab} \pm 4.7$	$156.33^{a} \pm 4.88$
IV.	Fipronil + Mustard	$123.33^{a} \pm 4.58$	$131.33^{a} \pm 4.66$	$139.00^{ab} \pm 4.82$	$146.17^{ab} \pm 4.92$	$152.83^{ab} \pm 4.95$
V.	Fipronil +Jungle jalebi	$120.67^{a} \pm 4.13$	$128.83^{a} \pm 4.30$	$138.83^{ab} \pm 4.11$	$149.33^{ab} \pm 4.07$	$159.67^{a} \pm 4.01$
VI.	Kadamba	$124.17^{a} \pm 6.71$	$133.5^{a} \pm 6.45$	$145.17^{ab} \pm 6.59$	$157.67^{a} \pm 6.72$	$168.83^{a} \pm 6.85$
VII.	Mustard	$120.33^{a} \pm 5.54$	$130.00^{a} \pm 5.54$	$140.67^{ab} \pm 5.57$	$152.50^{a} \pm 5.35$	$163.00^{a} \pm 5.20$
VIII	Jungle jalebi	$124.17^{a} \pm 4.68$	$134.67^{a} \pm 4.63$	$146.33^{a} \pm 4.54$	$158.33^{a} \pm 4.48$	$170.5^{a} \pm 4.40$

Values are Mean ± S.E; n=6; Values bearing common superscripts within a column do not differ significantly ($p<0.05$)

3.3 Effect on absolute weight gain (g) and per cent weight gain

Table 2: Effects of extracts of Kadamba, Mustard and Jungle jalebi on the absolute weight gain (g) and per cent body weight gain (%) against toxicity induced by sub-acute exposure of Fipronil in albino rats

Groups	Treatment	Initial B.w. (g)	Final B. w. (g)	Absolute B. w. gain (g)	Per cent B.w. gain (%)
I.	Control	$119.33^{a} \pm 76.84$	$159.83^{a} \pm 6.73$	$40.50^{cd} \pm 0.50$	$34.52^{a} \pm 2.09$
II.	Fipronil	$115.33^{a} \pm 6.21$	$137.67^{b} \pm 6.90$	$22.33^{g} \pm 1.17$	$19.49^{d} \pm 0.99$
III.	Fipronil+ Kadamba	$122.33^{a} \pm 4.74$	$156.33^{a} \pm 4.88$	$34.00^{e} \pm 0.97$	$28.00^{bc} \pm 1.41$
IV.	Fipronil + Mustard	$123.33^{a} \pm 4.58$	$152.83^{ab} \pm 4.95$	$29.50^{f} \pm 0.96$	$24.06^{cd} \pm 1.07$
V.	Fipronil + Jungle jalebi	$120.67^{a} \pm 4.13$	$159.67^{a} \pm 4.01$	$39.00^{d} \pm 0.63$	$32.55^{ab} \pm 1.48$
VI.	Kadamba	$124.17^{a} \pm 6.71$	$168.50^{a} \pm 7.09$	$44.33^{ab} \pm 0.95$	$36.15^{a} \pm 1.79$
VII.	Mustard	$120.33^{a} \pm 5.54$	$163.00^{a} \pm 5.20$	$42.67^{bc} \pm 1.02$	$35.88^{a} \pm 2.10$
VIII.	Jungle jalebi	$124.17^{a} \pm 4.68$	$170.50^{a} \pm 4.40$	$46.33^{a} \pm 0.80$	$37.39^{a} \pm 1.85$

Values are Mean ± S.E; n=6; Values bearing common superscripts within a column do not differ significantly ($p<0.05$)

Absolute and per cent body weight gain were significantly ($p<0.05$) lower in group II as compared to control group. While in the group III, IV and V, it was significantly ($p<0.05$) higher as compared to group II but significantly lower than control. Absolute body weight gain and per cent weight gain were increased in group III, IV and V. It was observed that there was much better restoration of body weight in group V as compared to group III and IV. On the other hand, significant ($p<0.05$) difference in absolute body weight gain were observed among the group VI, VII and VIII as compared to control group while there was no significant difference in per cent body weight gain.

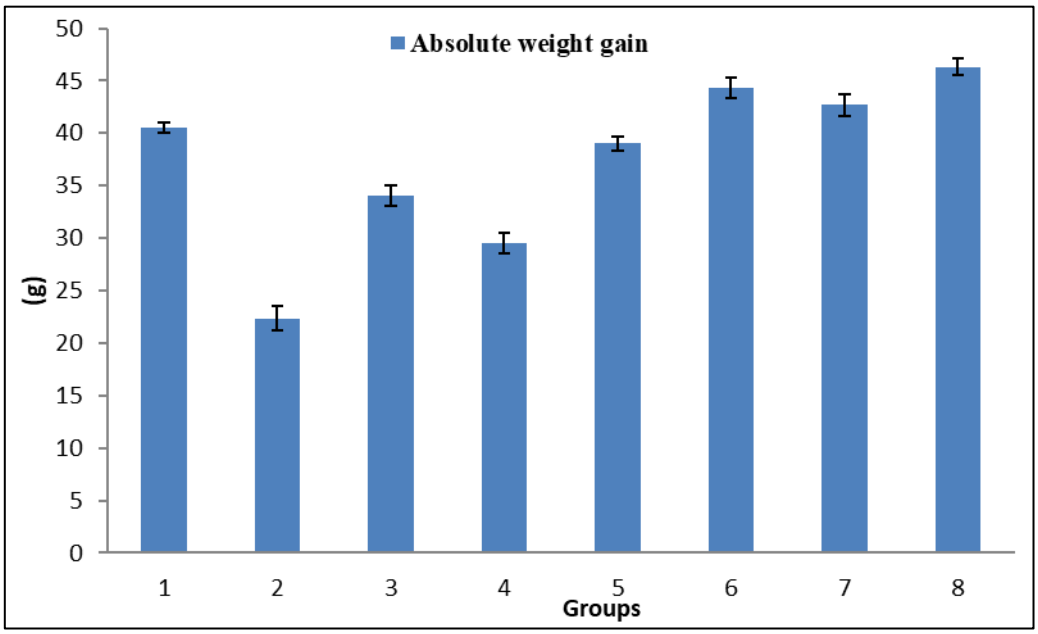

Fig 2: Effects of extracts of Kadamba, Mustard and Jungle jalebi on absolute weight gain (g) against toxicity induced by sub-acute exposure of Fipronil in albino rats (Mean ±S.E.)

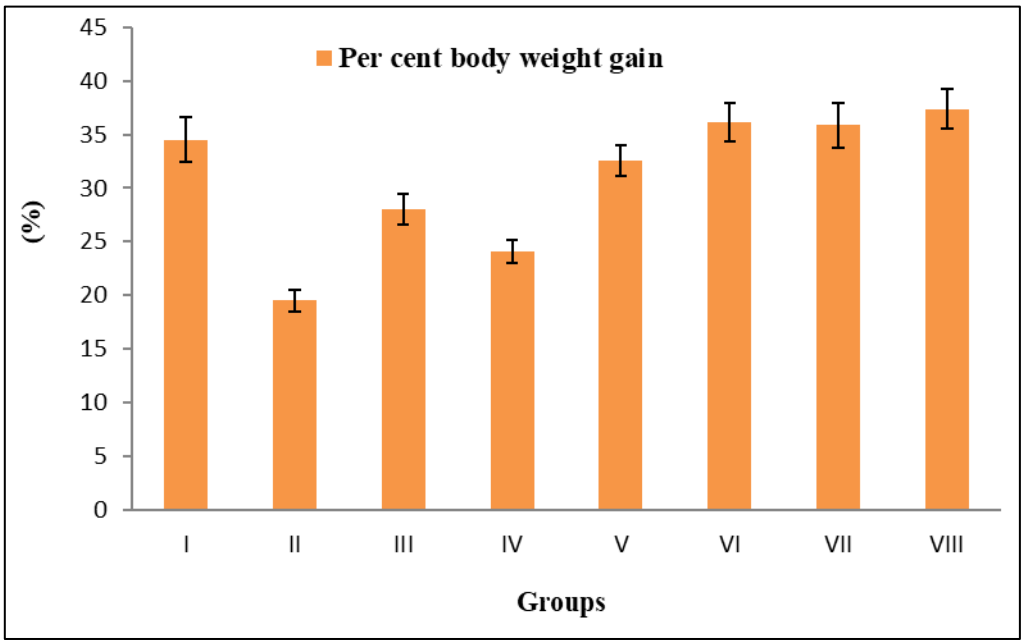

Fig 3: Effects of extracts of Kadamba, Mustard and Jungle jalebi on per cent weight gain (%) against toxicity induced by sub-acute exposure of Fipronil in albino rats (Mean ±S.E.)

3.3 Effect on absolute organ weight

Table 3: Effects of extracts of Kadamba, Mustard and Jungle jalebi on absolute organ weight (g) against toxicity induced by sub-acute exposure of Fipronil in albino rats

Groups	Treatment	Liver	Kidney	Spleen	Brain	Heart	Lung
I	Control	$3.47^{e} \pm 0.05$	$0.48^{f} \pm 0.02$	$0.44^{ab} \pm 0.02$	$0.82^{a} \pm 0.03$	$0.52^{a} \pm 0.02$	$0.71^{d} \pm 0.04$
II	Fipronil	$4.38^{a} \pm 0.11$	$0.85^{a} \pm 0.03$	$0.17^{d} \pm 0.02$	$0.87^{a} \pm 0.04$	$0.52^{a} \pm 0.03$	$0.92^{a} \pm 0.04$
III	Fipronil + Kadamba	$3.97^{bc} \pm 0.06$	$0.69^{bc} \pm 0.02$	$0.30^{c} \pm 0.02$	$0.89^{a} \pm 0.04$	$0.56^{a} \pm 0.03$	$0.85^{ab} \pm 0.02$
IV	Fipronil + Mustard	$4.06^{b} \pm 0.05$	$0.63^{cd} \pm 0.04$	$0.27^{c} \pm 0.02$	$0.89^{a} \pm 0.03$	$0.53^{a} \pm 0.02$	$0.86^{ab} \pm 0.02$
V	Fipronil + Jungle jalebi	$3.83^{cd} \pm 0.02$	$0.72^{b} \pm 0.02$	$0.38^{b} \pm 0.02$	$0.88^{a} \pm 0.02$	$0.54^{a} \pm 0.01$	$0.82^{bc} \pm 0.01$
VI	Kadamba	$3.75^{d} \pm 0.04$	$0.59^{de} \pm 0.02$	$0.44^{ab} \pm 0.03$	$0.88^{a} \pm 0.03$	$0.57^{a} \pm 0.02$	$0.76^{cd} \pm 0.02$
VII	Mustard	$3.79^{d} \pm 0.03$	$0.55^{ef} \pm 0.02$	$0.40^{b} \pm 0.03$	$0.87^{a} \pm 0.02$	$0.56^{a} \pm 0.01$	$0.79^{bcd} \pm 0.01$
VIII	Jungle jalebi	$3.73^{d} \pm 0.05$	$0.56^{def} \pm 0.02$	$0.47^{a} \pm 0.02$	$0.89^{a} \pm 0.02$	$0.56^{a} \pm 0.03$	$0.77^{cd} \pm 0.02$

Values are Mean ± S.E; n=6; Values bearing common superscripts within a column do not differ significantly ($p<0.05$)

Statistically significant ($p<0.05$) increase in liver weight was observed in group II, III, IV and V as compared to the control group. In Group III, IV and V liver weight was significantly ($p<0.05$) reduced as compared to the group II. Liver weight in group VI, VII and VIII was comparable to control group. The absolute weight of kidney was significantly ($p<0.05$) higher in group II, III, IV, V as compared to the control group, while in group III, IV and V the kidney weight was significantly ($p<0.05$) lower as compared to the group II. It was slightly increased in groups VI, VII and VIII as compare to control group. A statistically significant ($p<0.05$) decrease in absolute spleen weight was observed in groups II, III, IV and V as compared to control group, while in group III, IV and V increase was significant ($p<0.05$) as compared to the group II. Spleen weight was slightly increased in group VIII and slightly decreased in group VI as compared to control group. There was no significant change in group VI as compared to control group. No significant change in absolute weight of brain and heart was observed in all the groups. The absolute weight of lungs was also found to be significantly ($p<0.05$) higher in different treatment groups as compared to control group, while it was non significantly lower in treatment group III and IV and it was significantly lower in treatment group V as compare to group II. The relative weight of lung in groups VI, VII and VIII were comparable to control group.

3.4 Effect on relative organ weight

3.4.1 Liver: The relative weight of liver was significantly ($p<0.05$) higher in groups II, III, IV and V as compared to control group. While in groups III, IV and V liver weights was significantly ($p<0.05$) decreased and towards amelioration in comparison to group II. In plant control groups there was no significant difference observed in liver weight.

3.4.2 Kidney: The relative weight of Kidney was significantly ($p<0.05$) higher in groups II, III, IV and V as compared to control group. While in groups III, IV and V Kidney weights was significantly ($p<0.05$) decreased and towards amelioration in comparison to group II while kidney weight was slightly increased in groups VI, VII and VIII. Fipronil treatment significantly increased the relative kidney weight in comparison to control.

3.4.3 Brain and Lung: The relative weight of brain and lung were significantly ($p<0.05$) higher in groups II, III, IV and V as compared to control group, while in groups III, IV and V brain and lung weights were significantly ($p<0.05$) lower as compared to group II. On the other hand, no significant change was determined among the group III, IV and V in brain and lung weight

3.4.4 Spleen: The relative weight of the spleen was significantly ($p<0.05$) decreased in group II as compared to control group, while in group III, IV and V there was significant ($p<0.05$) increase in spleen weight as compared to group II. In groups VI, VII and VIII spleen weight were comparable to control.

Table 4: Effects of extracts of Kadamba, Mustard and Jungle jalebi on relative organ weight (g/100 g B.w.) by sub acute exposure of Fipronil in albino rats

Groups	Treatment	Liver	Kidney	Spleen	Brain	Lung
I	Control	$2.18^{e} \pm 0.06$	$0.30^{f} \pm 0.00$	$0.27^{a} \pm 0.00$	$0.48^{f} \pm 0.02$	$0.44^{f} \pm 0.01$
II	Fipronil	$3.20^{a} \pm 0.08$	$0.62^{a} \pm 0.01$	$0.11^{e} \pm 0.01$	$0.63^{a} \pm 0.01$	$0.69^{a} \pm 0.02$
III	Fipronil + Kadamba	$2.54^{bc} \pm 0.04$	$0.43^{b} \pm 0.00$	$0.19^{d} \pm 0.01$	$0.56^{bc} \pm 0.01$	$0.54^{bc} \pm 0.01$
IV	Fipronil + Mustard	$2.66^{b} \pm 0.06$	$0.41^{c} \pm 0.01$	$0.17^{d} \pm 0.01$	$0.58^{b} \pm 0.00$	$0.56^{b} \pm 0.01$
V	Fipronil + Jungle jalebi	$2.40^{cd} \pm 0.05$	$0.45^{b} \pm 0.00$	$0.23^{c} \pm 0.01$	$0.55^{cd} \pm 0.00$	$0.52^{cd} \pm 0.01$
VI	Kadamba	$2.24^{de} \pm 0.07$	$0.34^{d} \pm 0.01$	$0.26^{ab} \pm 0.01$	$0.52^{e} \pm 0.01$	$0.49^{de} \pm 0.01$
VII	Mustard	$2.33^{de} \pm 0.05$	$0.33^{de} \pm 0.00$	$0.25^{a} \pm 0.01$	$0.53^{d\,e} \pm 0.00$	$0.51^{d} \pm 0.01$
VIII	Jungle jalebi	$2.19^{e} \pm 0.03$	$0.32^{e} \pm 0.01$	$0.27^{a} \pm 0.00$	$0.52^{e} \pm 0.00$	$0.47^{e} \pm 0.01$

Values are Mean ± S.E; n=6; Values bearing common superscripts within a column do not differ significantly ($p<0.05$)

4. Discussion

4.1 Clinical signs

In the present study rats with sub-acute exposure of Fipronil did not show any mortality, but signs of toxicity were exhibited viz anorexia, dizziness, abnormal gait, lethargy, weakness and aggressive behaviour. It may be due to impaired permeability of blood vessels and less absorption of nutrients from intestine under the effect of drug leading to dizziness, weakness and lethargy [4]. Fipronil led to decrease in food consumption and body weight [24]. Common clinical signs of Fipronil toxicity are of CNS hyper excitability including tremors, convulsions, seizures and death [14, 17, 20].

4.2 Body weight

The weight gain in animals serves as index of growth rate [30]. A significant reduction in both absolute (g) and per cent body weight gain was observed in the present study in the rats treated with Fipronil alone in 28 days exposure. Significant decrease in body weight may be due to the oxidative stress and neurotoxicity of Fipronil. Similar findings have been reported by Mossa *et al.* [29]. Where, there was a slight decrease in the body weight after 45 days of treatment. Significant decrease in body weight gain was also reported by Gupta *et al.* [16]. WHO [46] suggested some signs of maternal toxicity (decreased body weight gain, decreased food consumption and reduced efficiency of food use) in rabbits treated with Fipronil by gavage at doses of 0, 0.1, 0.2, 0.5 or 1 mg/kg body weight per day. WHO [46] also reported a subacute toxicity study, in which Fipronil was administered in gelatin capsules to dogs for 13 weeks at doses of 0, 0.5, 2 or 10mg/kg body weight per day. Inappetence and decreased body weight gain and food consumption were noted in females at 2 and 10 mg/kg body weight per day.

Kadamba, Mustard and Jungle jalebi co-treated with Fipronil significantly increased the weight gain of rats in 28 days trial. In the present work treatment with Kadamba showed protective effects which were in agreement with Alam *et al.* [3] reports where extract of *Anthocephalus cadamba* prevented the weight loss in alloxan-induced diabetic rats. Significant improvement in weight gain was observed by methanolic extract of *Anthocephalus cadamba* as compared to diabetic rats [39]. Treatment with extract of *Anthocephalus cadamba* resulted in an improvement in the body weight as comapred to toxic groups which may be due to its protective effect in controlling muscle wasting.

Likewise, Mustard also helps to prevent body weight loss by its capability of affording protection against depression and body weight loss [41]. *Brassica juncea* treated mice showed significant weight gain [15]. Extract of Jungle jalebi also produced a significant increase in body weight gain and indicated its beneficial effects by controlling muscle wasting. Similar findings were reported by Pradeepa *et al.* [33] where, the body weight of rats was significantly increased in STZ-induced diabetic rats.

4.3 Relative Organ Weight

In toxicological studies, relative organ weights are important criteria for evaluation of organ toxicity [11, 43]. Generally in subacute toxicity studies relative weight of affected organ increases.

4.3.1 Liver: Liver is the major organ responsible for the metabolism of drugs and toxic chemicals, thus it is the primary target organ for nearly all toxic chemicals. A statistically significant increase was observed in relative weight of liver in Fipronil treated groups. The results were in accordance with Silva and Koshlukova [36], the observation of increased liver weight was reported at 0.13 mg/kg B.w. day Fipronil dose. Fipronil exposure to rats resulted in a significant elevation in relative liver weights [29], Which could be attributed to reduction in body weight gain of experimental animals [27, 28]. In another study, Swelam *et al.* [40] also reported that Fipronil treatment induced significant increase in relative liver weight.

In the present study, Kadamba, co-treatment significantly decreased the relative liver weight as compared to Fipronil group. The findings corroborates with Acharyya *et al.* [1] where a similar hepatoprotective result of *A. cadamba* extract was observed in rats after induction of diabetes with alloxan.

Likewise, Mustard co-treatment also lowered the liver weight significantly as compared to Fipronil treated animals. The findings are in agreement with Agnel *et al.* [21] who evaluated the hepatoprotective activity of aqueous extract of Mustard seeds against CCL_4 induced hepatic damage in albino rats. It has also been reported that ethanolic extract of *Brassica oleracea* showed hepatoprotective activity against simvastatin induced hepatotoxicity [21]. It may be suggested that the presence of terpenoids and flavonoids are the possible reasons for hepatoprotective activity of Mustard.

Similarly, Co-treatment with Jungle jalebi extract also had a restorative effect on liver. The findings were in accordance with Manna *et al.* [25] where, CCL_4 induced toxicity reduced relative liver weight and was ameliorated by aqueous extract of *Pithecellobiumdulce*. This observable effect may be due to the presence of flavinoids, saponins, phenolics and steroids in plant extract.

4.3.2 Kidney: Fipronil treatment significantly increased the relative kidney weight in comparison to control. The findings are in agreement with the study of Mossa *et al.,* [29] and Swelam *et al.* [40] where a significant elevation in relative weight of kidney was observed after exposure to Fipronil.
Kadamba, Mustard and Jungle jalebi co-treatment lowered the relative kidney weight in the present study as compared to Fipronil treatment group. The results are in accordance with Acharyya *et al.* [1] where the nephroprotective effect of Kadamba was observed in alloxan induced diabetic rats. Decrease in relative kidney weight after treatment with these plant extracts, shows their renoprotective nature. This is possibly due to the anti-oxidant potential of these plants.

4.3.3 Brain and Lung: In the present study, significant increase in the relative weight of brain and lung was decreased, indicating the toxic nature of Fipronil. Kadamba, Mustard and Jungle jalebi co-treatment lowered the relative weight of these organs as compared to Fipronil treatment group. It may be attributed to the protective effect of these plants on brain and lung by reducing oxidative stress.
Spleen: The weight of spleen was reduced in Fipronil treated rats as compared to control the decline in spleen weight in animals exposed to Fipronil may be due to damage of spleen cells, which is reflected by the decreased weight of the organ [18]. The results were in agreement with the study of Badgujar [7] where it was observed that the spleen of mice exposed to Fipronil reduced absolute and relative weight of spleen both in male and female mice in a dose dependent manner with high dose producing significant decrease in spleen weight. Acetamiprid alone treatment resulted in a decrease in organ weight of spleen [5].
In the present study, Kadamba, Mustard and Jungle jalebi significantly increased spleen weight. The results indicated a protective effect of these plants on spleen.

5. Conclusion
The main clinical signs *viz* anorexia, dizziness, abnormal gait, lethargy, weakness and aggressive behaviour were observed throughout the period (28 days) in Fipronil treated alone group. Fipronil treatment significantly decreased absolute and relative body weight gain of albino rats which were significantly increased by Kadamba, Mustard and Jungle jalebi. It was observed that the group treated with extract of Jungle jalebi restored body weight better than group treated with Mustard and Kadamba. The relative liver and kidney weight were found significantly higher in Fipronil treated group which was towards restoration by Kadamba, Mustard and Jungle jalebi co-treatment groups. No significant change in relative heart weight was observed by Fipronil exposure. Significant increase was observed in relative weight of brain and lungs by Fipronil exposure which was restored by co-treatment with Kadamba, Mustard and Jungle jalebi extracts. Fipronil significantly decreased the relative spleen weight which was reversed by Kadamba, Mustard and Jungle jalebi co-treatment. It was observed that the group treated with *Pithecellobium dulce* (Jungle jalebi) had better ameliorative effect than other treatment groups.

6. References

1. Acharyya S, Dash GK, Abdullah MS. Antihyperglycemic and antilipidemic activity of *Anthocephalus cadamba* (Roxb.) Miq. Roots. European Journal of Experimental Biology 2013;3(3):116-120.
2. Agnel, John A, Soba. Hepatoprotective activity of *Brassica juncea* (L) czer against carbon tetrachloride induced hepatotoxicity in albino rats. Pharmacologyonline 2011;3:609-621.
3. Alam MA, Subhan N, Chowdhury SA, Awal MA, Mostofa M, Rashid MA *et al*. *Anthocephalus cadamba* extract shows hypoglycemic effect and eases oxidative stress in alloxan-induced diabetic rats. Revista Brasileira de Farmacognosia Brazilian Journal of Pharmacognosy 2011;21(1):155-164.
4. Ali HM, Qureshi AS, Hussain R, Urbinati G, Mustafa MZ, Ali F *et al*. Effects of natural environment on reproductive histo-morphometric dynamics of female dromedary camel. Animal Reproduction Science 2017;181:30-40.
5. Awasthy V. Evaluation of Acetamiprid-induced reproductive toxicity in male rats and its amelioration by quercetin. PhD Thesis. Indian Veterinary Research Institute, Izatnagar 2013.
6. Ayurvedic Pharmacopoeia of India Controller of publication, New Delhi 1999;1:64-65.
7. Badgujar PC. Elucidation of immunotoxic and genotoxic potential of fipronil following subacute exposure in mice and its amelioration by antioxidants. PhD Thesis. Indian Veterinary Research Institute, Izatnagar 2014.
8. PC Van Metre, DA Alvarez, BJ Mahler, Nowell L, Sandstrom M, Moran P. Complex mixtures of Pesticides in Midwest U.S. streams indicated by POCIS time-integrating samplers. Environ. Pollut 2017;220:431-440.
9. Cartea ME, Francisco M, Soengas P, Velasco P. Phenolic compounds in Brassica vegetables. Molecules 2010;16(1):251-280.
10. Chikezie PC, Ibegbulem CO, Mbagwu FN. Medicinal Potentials and Toxicity Concerns of Bioactive Principles. Medicinal and Aromatic plants 2015;4(3):202.
11. Crissman JW, Goodman DG, Hildebrandt PK, Maronpot RR, Prater DA, Riley *et al*. Best practice guideline. Toxicologic Pathology 2004;32:126-131.
12. Dubey A, Nayak S, Goupale DC. A Review on phytochemical, Pharmacological and Toxicological studies on Neolamarckia Cadamba. Scholars Research Library 2011;3(1):45-54.
13. Environmental Protection Agency. New Pesticide Fact Sheet 1996, 96-181516. epa 737-F-96-005.
14. Grant DB, Chalmers A, Wolff M, Hoffman H, Bushey D. Fipronil: action at the GABA receptor. Critical Reviews in Toxicology 1998;2:147-156.
15. Grover JK, Yadav SP, Vats V. Effect of feeding Murrayakoeingii and *Brassica juncea* diet kidney functions and glucose levels in streptozotocin diabetic mice. Journal of Ethnopharmacology 2003;85:1-5.
16. Gupta SK, Pal AK, Sahu NP, Jha AK, Akhtar MS, Mandal SC *et al*. Supplementation of microbial levan in the diet of Cyprinuscarpio fry (Linnaeus, 1758) exposed to sublethal toxicity of fipronil: effect on growth and metabolic responses. Fish Physiology and Biochemistry 2013;39:1513-1524.
17. Hainzl D, Cole LM, Casida JE. Mechanism for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. Chemical Research in Toxicology 1998;11:1529-1535.
18. Israa S, Al-Dabbagh, Layla JM, Al-Bahadyli. Study the behavioral changes and gravimetric changes for weight organs in liver, kidney and spleen exposure to insecticide

imidacloprid in the white mice. World Journal of Pharmaceutical Research 2015;4:114-22.
19. Jahangir M, Kim HK, Choi YH, Verpoorte R. Health-Affecting Compounds in Brassicaceae. Comprehensive Reviews in Food Science and Food Safety 2009;8:31-43.
20. Kamijima M, Casida JE. Regional modification of [3H] ethynylbicyclo-orthobenzoate binding in mouse brain GABA receptor by endosufan, fipronil and avermectin B. Toxicology and Applied Pharmacology 2000;160:188-194.
21. Kanathur N, Mathai MG, Byrd RP, Jr Fields CL, Roy TM. Simvastatin-diltiazem drug interaction resulting in rhabdomyolysis and hepatitis. Tennessee Medicine 2001;94(9):339-341.
22. Kirtikar KR, Basu BD. Indian medicinal plants. IInd ed. Lalitmohanbasu publishers, Allahabad 1999, 1250-1252.
23. Law RJ, Lightstone FC. Gaba receptor insecticide non-competitive antagonists may bind at allosteric modulator sites. Int J Neurosci 2008;118:705-734.
24. Lopez-Antia A, Ortiz-Santaliestra ME, Camarero PR, Mougeot F, Mateo R. Assessing the risk of fipronil-treated seed ingestion and associated adverse effects in the red-legged partridge. Environmental Science and Technology 2005;49(22):13649-13657.
25. Manna P, Bhattacharyya S, Das J, Ghosh J, Parames C. Phytomedicinal Role of Pithecellobiumdulce against CCl_4 mediated Hepatic Oxidative Impairments and Necrotic Cell Death. Evidence-Based Complementary and Alternative Medicine 2010, 832805:1-17.
26. Manohar P, Pushpan R, Rohini S. Mustard and its uses in Ayurveda 2009.
27. Mansour SA, Mossa AH. Oxidative damage, biochemical and histopathological alteration in rat exposed to chlorpyrifos and the role of zinc as antioxidant. Pesticide Biochemistry and Physiology 2010;96:14-23.
28. Mossa AH, Abbassy MA. Adverse haematological and biochemical effects of certain formulated insecticides in male rats. Research Journal of Environmental Toxicology 2012;6:160-168.
29. Mossa ATH, Swelam ES, Mohafrash SM. Sub-chronic exposure to fipronil induced oxidative stress, biochemical and histopathological changes in the liver and kidney of male albino rats. Toxicology reports 2015;2:775-784.
30. Palani V, Senthikumaran RK, Govindawamy S. Biochemical evaluation of antitumor effect of Muthumarunthua herbal formulation on experimental fibrosarcoma in rats. Journal of Ethnopharmacology 1999;65:257-265.
31. Pisa L *et al*. An update of the worldwide integrated assessment (WIA) on systemic insecticides. Part 2: impacts on organisms and ecosystems. Environ. Sci. Pollut 2017; Res. https://doi.org/ 10.1007/s11356-017-0341-3. (doi:10.1007/s11356- 017-0341-3)
32. Pisa LW *et al*. Effects of neonicotinoids and fipronil on non-target invertebrates. Environ. Sci. Pollut. Res 2015;22:68-102.
33. Pradeepa S, Subramanian S, Kaviyarasan V. Biochemical evaluation of antidiabetic properties of Pithecellobium dulce fruits studied in streptozotocin induced experimental diabetic rats. International Journal of Herbal Medicine 2013;1(4):21-28.
34. Prajapati, Purohit, Sharma, Kumar. A handbook of medicinal plants: A complete source book. Agrobios (India) publisher, Jodhpur 2007, 52-53.
35. Sharma S, Mehta BK. A review on pharmacological activities of Pithecellobium Dulce extract, and there effective doses. Journal of Medical Pharmaceutical and Allied Sciences 2013;05:37-45.
36. Silva MH, Koshlukova S. Comparative Toxicity of Endosuldan and Fipronil Insecticides: Utilizing In Vivo and In Vitro Data. Journal of Medical Toxicology and Clinical Forensic Medicine 2016; 1:1-9.
37. Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Wiemers M. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environmental Science and Pollution Research 2015;22(1):5-34.
38. Sindhoor KL, Gurram SK, Nagarjuna S, Reddy YP. Comparative Study of Anti-Inflammatory activity of Petroleum Ether and Ethanolic extracts of Brassica Juncea. International Journal of Pharm Tech Research 2012;4(3):1172-1176.
39. Singh HP, Irchhaiya R, Verma A, Pandey H, Singh PP. Phytochemical analysis, exploration of antidiabetic and antioxidant potential of *Anthocephalus cadamba* (Roxb.) International Journal of Research and Developement in Pharmacy and Life Sceince 2017;6(6):2800-2805
40. Swelam ES, Abdallah IS, Mossa ATH. Ameliorating effect of zinc against oxidative stress and lipid peroxidation induced by Fipronil in male rats. Journal of Pharmacology and Toxicology 2016;12:24-32.
41. Thakur AK, Chatterjee SS, Kumar V. Antidepressant-like effects of *Brassica juncea* L. Leaves in diabetic rodents. Indian Journal of Experimental Biology 2014;52:613-622.
42. The Wealth of India. A dictionary of Indian raw materials and industrial products. NISCAIR press publishers, New Delhi 2006, 305-308.
43. Timbrell JA. Biomarkers of organ toxicity. Archives of Industrial Hygiene and Toxicology 2000;33:295-302.
44. Tingle CC, Rother JA, Dewhurst CF, Lauer S, King WJ. Fipronil: environmental fate, ecotoxicology and human health concerns. Reviews of Environmental Contamination and Toxicology 2003;176:1-66.
45. Tomlin CDS. The Pesticide Manual: A World Compendium. 14th ed. Hampshire, England, British Crop Protection Council 2006, 462-546.
46. World Health Organization. Classification of pesticides by hazard. International Program on Chemical Safety 1998-199. WHO/IPCS/98.21.