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Evaluation of antioxidant potential of *Pithecellobium dulce* fruit against fipronil induced toxicity in rats

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Abstract
Oxidative stress is considered as a biochemical event playing crucial role in the upset of the oxidant/antioxidant balance in the sense of exceeding the antioxidant defense capacity. The present investigation was carried out to evaluate antioxidative effect of the fruit extract of *Pithecellobium dulce* (Jungle jalebi) (300mg/kg B.w.) against oxidative stress induced by sub-acute exposure of Fipronil (10mg/kg B.w.) in rats. Sub-acute oxidative stress was conducted in adult male wistar rats. Rats were randomly divided into four groups (6 rats/group).Group I served as control in which corn oil (acting as a vehicle of Fipronil) was administered @10ml/kg B.w.Group II served as Fipronil treated group @10 mg/kg B.w. In Group III Fipronil along with *Pithecellobium dulce* fruits extract @300 mg/kg B.w. was administered and in Group IV *Pithecellobium dulce* fruits extract @300mg/kg B.w. was administered.Vehicle, Fipronil and *Pithecellobium dulce* were administered daily to the rats orally by gavage for 28 days. The dose of fipronil was selected on the basis of LD_{50} in rats. Lipid peroxidation, reduced GSH, SOD, catalase and glutathione reductase levels were estimated. Fipronil produced toxicity in the form of enhanced lipid peroxidation and reduced GSH, SOD and catalase levels. *Pithecellobium dulce* was significantly effective in restoration of these parameters towards normal**.** The study suggested that *Pithecellobium dulce* has ameliorating potential on oxidative stress following subacute exposure to fipronil in rats.

Keywords: Oxidative stress, free radical, fipronil, Jungle jalebi, ameliorating potential, rat model

Introduction
The word pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and more. In developing countries pesticide poisoning is a significant problem due to indiscriminate use of pesticide and handling practices. Farmers may be exposed to pesticides from storing them in or near the residence. A huge farming population may suffer from several health issues, primarily neurological abnormalities, respiratory ailments, impaired intelligence, reproductive, endocrinological, and dermal problems [1].

Fipronil was first discovered by Rhone-Poulenc Agro in 1987 and is used worldwide in agriculture. It is a member of the phenylpyrazole class of pesticides that acts through a different mechanism compared to other conventional insecticides. It is poorly water soluble, delivered in very small amounts and does not leach into the groundwater. It is used in combination with methoprene (9.8% Fipronil/11.8% methoprene for cats; 9.8% Fipronil/8.8% methoprene for dogs) for additional control of immature flea stages. It is also formulated as insect bait for roaches, ants and termites; sprays for pets. At present Fipronil is widely used in agriculture for soil treatment and seed coating [2]. It is more effective than organophosphate, carbamate and pyrethroids insecticides against several species of Lepidoptera, Orthoptera and Coleopteran [36]. In mammalian systems, the mechanism of Fipronil is quite different from other classes of insecticides, and it is better understood in insects than in mammals.

The extracellular or intracellular adverse events, represented by alterations of cellular physiological processes, may lead to increase synthesis of toxic molecules known as the Reactive Oxygen Species (ROS). Due to their high reactivity, free radicals generate harmful effects on the body, and are involved in numerous pathology among which the most feared are cancer, atherosclerosis, chronic inflammation and diabetes [3].

Plants are widely used in many indigenous systems of medicine for therapeutic purposes and increasingly becoming popular in modern society as alternatives to synthetic medicines. Plant materials are composed of a vast array of bioactive 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 [4]. Plant products are known to exert their protective effects by scavenging free radicals in biological membranes and modulating antioxidant defense system.

Pithecellobium dulce Benth, a most versatile medicinal plant, has attracted a worldwide prominence in recent years. All plant parts of the *Pithecellobium dulce* elaborates a vast array of biologically active compounds and have been demonstrated to exhibit antidiabetic, antivenom, free radical scavenging, protease-inhibitor, antiinflammatory, antibacterial, antimycobacterial, anticonvulsant, antiulcer, antidiarrheal, antifungal, antitubercular, antitumor and antioxidants properties. *Pithecellobium dulce* 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 [5]. There are plenty of reports about the protective effect of herbal extract in insecticide induced toxicity and oxidative stress. However, there is a paucity of data on the protective effect of these antioxidants present in the fruits of *Pithecellobium dulce* in Fipronil induced toxicity or oxidative stress.The present work was designed to investigate the ameliorating potential of *Pithecellobium dulce* against oxidative stress induced by Fipronil in rats.

Material Methods

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. Bedding material (wheat straw) was changed on alternate day. All the experimental animals were kept under constant observation during the entire period of study and handled as per the institutional animal ethic guidelines. The prior approval of the institutional animal ethics committee was obtained for the use of animals in this study.

Toxicological and pharmacological agents

Fipronil of technical grade (>98% purity) was offered generously by Sat Shri Sai Crop Protection Science, Delhi. Corn oil was procured from Deve Herbs, Delhi. The pods/fruits of *Pithecellobium dulce* (fig.1) was procured from farms and was authenticated from Department of Horticulture, Maharana Pratap University of Agriculture Technology, Udaipur (Rajasthan)

Fig 1: fruit of Jungle jalebi

Preparation of Herbal Extracts

The hydroalcoholic fruit extract of *Pithecellobium dulce* was prepared by macerating about 500 g of air dried ground homogenous powder of the fruits in 70 per cent ethanol for a week. The extract was filtered with Whattman filter paper no. 1, and the filtrate was allowed to evaporate by rotatory vacuum evaporator [6].

Experimental design

Rats were randomly divided into four groups (6 rats/group).Group I served as control in which corn oil (acting as a vehicle of Fipronil) was administered @10ml/kg B.w.Group II served as Fipronil treated group @10 mg/kg B.w. [7]. In Group III Fipronil along with *Pithecellobium dulce* fruits extract @300 mg/kg B.w. was administered [5] and in Group IV *Pithecllobium dulce* fruits extract @300mg/kg B.w. was administered.

Table 1: Experimental design for the ameliorating potential of *Pithecellobium dulce* against oxidative stress and genotoxicity induced by sub-acute exposure of Fipronil in rats.

Groups	Treatment	No. of rats	Dose (mg/kg B.w.)	Feeding schedule
I	Control (corn oil)	6	10ml/kg	0-28 days
II	Fipronil toxicity	6	10	0-28 days
III	Fipronil toxicity + Extract of Jungle jalebi fruits Papaya	6	10+300	0-28 days
IV	Extract of Jungle jalebi fruits	6	300	0-28 days

Doses and Exposure schedules

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. [7]. Oral LD_{50} was experimentally determined by preliminary dose range finding study and was found to be 100 mg/kg B.w. in rats. Accordingly, a test dose of Fipronil in the 28 day subacute toxicity study was 10 mg/kg. The extract of fruits of Jungle jalebi was given at the same dose rate of 300 mg/kg [5] 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 (10 ml/kg). Fipronil and plants extract doses were prepared on seven (7) days.

The dosing was performed between 09:00 and 11:00 h as far as possible. Rats in the group III was treated with Jungle jalebi 3 to 4 h after administering Fipronil (i.e.10 mg/kg).

Assessment of Oxidative Stress

Estimation of different oxidative stress-related biochemical parameters in liver, kidney, spleen and brain was carried out. A double beam UV-VIS spectrophotometer was used for recording the absorbance of the test samples.

Preparation of Liver, Kidney, Spleen and Brain Homogenates

About 500 mg of tissue was weighed and taken in 5 ml of ice-cold PBS (pH 7.4). Another 100 mg of sample was weighed separately and taken in 1 ml of 0.02 M ethylene diamine tetra acetic acid (EDTA) solution for reduced glutathione (GSH) estimation. The homogenates (10%) prepared with IKA

Homogenizer under ice-cold condition were centrifuged for 10 min at 3000 rpm. The supernatant was stored at -20°C until assayed for different oxidative stress-related biochemical parameters. A double beam UV-VIS spectrophotometer was used for recording the absorbance of the test sample.

Assays for Measuring the Activities of Antioxidant Enzymes

Lipid peroxidation (LPO) was evaluated in terms of malondialdehyde (MDA) production by using thiobarbituric acid-reactive substances (TBARS) test [8]. Reduced glutathione (GSH) was determined by estimating free-SH groups, using 5-5' dithiobis 2- nitrobenzoic acid (DTNB) [9]. For estimation of GSH, 10% homogenate was made in 0.02 M EDTA. Superoxide dismutase (SOD) was estimated as per the method described by [10]. The catalase activity in tissue supernatant was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2, the substrate of the enzyme [11]. Glutathione reductase (GR) activity was assayed by the method of [12].

Results and Discussion

Effect on L.P.O. level

LPO is known to disturb the intergrity of cellular membranes and implicates in the pathogenesis of various kidney and liver injuries [13-14]. Therefore, it has been used as biomarkers of pesticides-induced oxidative stress and suggested as one of the molecular mechanisms involved in pesticides-induced toxicity. Effect of extracts of Jungle jalebi on LPO in liver, kidney, spleen and brain against toxicity induced by sub-acute exposure of Fipronil in rats are presented in terms of MDA in Table 2.

In the present study there was increase in the values of MDA, indicator of lipid peroxidation in kidney, liver, brain and spleen in Fipronil treated groups. The results were in complete agreement to [15] who reported that, there was significant increase in MDA levels in the liver and kidney of Fipronil treated mice. Similar findings were also reported by [16] whereas Fipronil exposure significantly increased MDA level in erythrocytes and co-exposure of Fipronil and NaF also produced additive effect on LPO level [17]. Also suggested that Fipronil exposure caused excessive lipid peroxidation. The increased MDA level was also observed by [18] due to exposure of Fipronil. MDA level was significantly increased by Fipronil [19-21] found that prolonged exposure to Fipronil increased LPO in liver of pregnant rats and their offspring. The results were also in accordance with [22] who reported that there was a significant increase in LPO level in Fipronil exposed rats.

Jungle jalebi co-treatment restored the increased LPO values. Similar findings were reported by [23] where treatment with aqueous extract of *P. dulce* decreased the levels of lipid peroxidation suggesting the anti-oxidant nature of aqueous extract of *P. dulce* in CCL_4 –induced hepatic oxidative stress. [24] suggested that the levels of MDA in the gastric tissue of rats treated with *Pithocellobium jiringa* were significantly decreased compared to the ulcer control rats.

Table 2: Effect of extracts of Jungle jalebi on Lipid peroxidation (in terms of MDA) levels against toxicity induced by sub-acute exposure of Fipronil in rats

Groups	Treatment	MDA (nmole/g)			
		Liver	Kidney	Spleen	Brain
I.	Control	$42.29^{f} \pm 0.52$	$31.22^{f} \pm 0.32$	$60.85^{g} \pm 0.43$	$71.93^{e} \pm 0.46$
II.	Fipronil	$66.78^{a} \pm 1.15$	$65.21^{a} \pm 0.89$	$81.38^{a} \pm 1.04$	$91.89^{a} \pm 1.29$
III.	Fipronil + Jungle jalebi	$52.75^{d} \pm 0.76$	$45.85^{c} \pm 0.76$	$69.77^{cd} \pm 0.83$	$79.1^{c} \pm 0.69$
IV.	Jungle jalebi	$44.96^{f} \pm 0.79$	$34.44^{e} \pm 0.65$	$63.81^{f} \pm 0.83$	$73.19^{e} \pm 0.65$

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

Effect on Reduced glutathione (GSH) level

Glutathione (GSH) is the most abundant nonprotein thiol in organisms and it plays a key role in intracellular protection against toxic compounds, such as reactive oxygen intermediates and other free radicals. GSH plays a major role in antagonizing the oxidative action of the herbicides or insecticides. Effect of extract of Jungle jalebi on reduced glutathione (GSH) (mmol/ml) level in liver, kidney, spleen and brain against toxicity induced by sub-acute exposure of Fipronil in rats are presented in Table 3. In the present study, there was decrease in the level of GSH in Fipronil treated groups which are in accordance with [25] where Fipronil treatment significantly decreased GSH level in kidney and brain of mice [16]. Reported that Fipronil significantly decreased blood GSH level. Similar findings were also found by [22] where, there was significant decrease in level of GSH in Fipronil treated rats. Glutathione is the cell's natural antioxidant, which destroys free radicals formed in cells. Significant dose dependent depletion of GSH levels and perturbations in antioxidant enzyme levels further confirmed the potential of Fipronil to induce oxidative stress.

In the present study, Jungle jalebi co-treated with Fipronil brought the values towards normalcy. According to [23] post treatment with aqueous extract of *P. dulce* has the potential to prevent the toxin induced alteration in intracellular thiol status and GSH levels.

Table 3: Effect of extracts of Jungle jalebi on Reduced glutathione (GSH) level in Liver, Kidney, spleen and brain against toxicity induced by sub-acute exposure of Fipronil in rats

Groups	Treatments	GSH (mM/g)			
		Liver	Kidney	Spleen	Brain
I.	Control	$3.10^{a} \pm 0.13$	$1.35^{a} \pm 0.05$	$4.37^{a} \pm 0.04$	$3.36^{a} \pm 0.05$
II.	Fipronil	$2.18^{d} \pm 0.06$	$1.07^{c} \pm 0.05$	$3.08^{e} \pm 0.06$	$2.14^{e} \pm 0.05$
III.	Fipronil + Jungle jalebi	$2.73^{b} \pm 0.03$	$1.27^{ab} \pm 0.02$	$3.94^{c} \pm 0.03$	$2.73^{c} \pm 0.03$
IV.	Jungle jalebi	$3.12^{a} \pm 0.03$	$1.32^{a} \pm 0.03$	$4.32^{ab} \pm 0.03$	$3.33^{a} \pm 0.03$

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

Effect on Superoxide dismutase (S.O.D.) level

SODs are metalloenzymes that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and prevents subsequent formation of hydroxyl radicals and important role in the cellular antioxidant defense mechanism [26]. Sub-acute effect of Fipronil on SOD level in liver, kidney, brain and spleen and its amelioration by Jungle jalebi in rats are presented in Table 4. In the present study, there was decrease in SOD levels in Fipronil treated group. A similar decrease in activity of SOD in rats was also reported with different pesticides namely chlorpyrifos, cypermethrin, carbofuran, dimethoate and malathion [17, 26, 27]. Consequently, the decreased and increased SOD activities might have reflected a cellular oxidative stress due to Fipronil exposure [35]. Concluded that decreased SOD activity in serum, liver and kidney of pigs suggested that the accumulation of superoxide anion radical might be responsible for increased lipid peroxidation following arsenic treatment.

The results were in accordance with [30] where Fipronil resulted in a dose dependent significant decrease in SOD levels in liver and kidney tissue. Similar findings were also reported by [25], where Fipronil treatment at medium and high doses significantly attenuated SOD activity in kidney of mice. Rat exposed to Fipronil showed significant reduction in the activity of SOD in liver tissue [22]. High doses of Fipronil decreased the gene expression of SOD1 and activity of SOD enzyme [15]. The decrease in the activity of SOD in Fipronil treated rats may be attributed to the utilization of this enzyme for converting the free radical formed O_2 to H_2O [27].

In the present study, Jungle jalebi co-treatment significantly increased SOD activity, the results were in accordance with [23] where post treatment with aqueous extract of *Pithecellobium dulce* normalized the levels of SOD as compared to control [24]. Also suggested that ethanolic extract of *Pithecellobium jiringa* increased the SOD levels in rats due to its ability to scavenge superoxide anions.

Table 4: Effect of extracts of Jungle jalebi on Superoxide dismutase (SOD) levels in Liver, Kidney, spleen and Brain against toxicity induced by sub-acute exposure of Fipronil in albino rats

Groups	Treatments	SOD (unit/mg)			
		Liver	Kidney	Spleen	Brain
I.	Control	77.96^{a}± 1.13	75.26^{a}± 0.78	56.36^{a}± 0.76	43.04^{a}±0.81
II.	Fipronil	54.26^{f}± 0.86	51.03^{e}± 0.72	38.39^{f}± 0.79	31.40^{e}±0.53
III.	Fipronil + Jungle jalebi	70.54^{d}± 0.45	68.47^{d}± 0.59	50.35^{cd}± 0.50	41.14^{cd}±0.42
IV.	Jungle jalebi	75.43^{b}± 0.55	73.42^{b}± 0.53	54.29^{b} ± 0.45	42.74^{ab}±0.50

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

Effect on Catalase level

Catalase is important enzymatic antioxidants that act against toxic oxygen free radicals such as hydroxyl ions in biological systems. It prevents oxidative hazards by catalyzing the formation of H2O and O2 from H2O2 [28]. GPx and Catalase are the major enzymes that remove H2O2 generated by SOD in cytosol and mitochondria by oxidizing GSH to GSSG. These enzymes prevent generation of hydroxyl radical and protect cellular constituents from oxidative damage. Effect of extracts of jungle jalebi on catalase level in liver, kidney, spleen and brain against toxicity induced by sub-acute exposure of Fipronil in rats are presented in Table 5.

In the present study there was reduction in the level of catalase in liver, kidney, brain and spleen in Fipronil treated groups. The results were in accordance with [15, 25] where Fipronil exposure significantly decreased activity of catalase enzyme in mice. Fipronil decreased CAT activity in liver [29]. Similar findings were also reported by [30, 22] where highly significant reduction in activities of CAT enzymes were found in rats exposed to Fipronil. The decrease in CAT activity in rats exposed to Fipronil could be explained as excess formation of oxygen free radicals which rapidly converted to H_2O by CAT. Previous studies also reported that exposure to insecticides such as chlorpyrifos [27], triazophos [14] and deltamethrin [31] resulted in decrease in SOD and CAT in liver and kidney of rats.

Co-treatment with Jungle jalebi in Fipronil treated groups restored the values of CAT. Similar protective results of Jungle jalebi were also found in CCL_4 induced hepatotoxicity in rats [23]

Table 5: Effect of extracts of Jungle jalebi on Catalase level in liver, kidney, spleen and brain against toxicity induced by sub-acute exposure of Fipronil in rats

Groups	Treatment	Catalase (mmole H_2O_2 utilized/min/mg)			
		Liver	Kidney	Spleen	Brain
I.	Control	369.17^{a}±4.35	488.50^{a}±4.16	328.00^{a}±3.43	541.00^{a}±3.83
II.	Fipronil	259.17^{g}±6.53	275.33^{g}±4.20	252.67^{e}±5.68	379.00^{f}±3.96
III.	Fipronil +Jungle jalebi	315.50^{de}±3.86	431.17^{de}±2.09	302.67^{c}±2.76	490.67^{d}±2.26
IV.	Jungle jalebi	344.33^{b}± 3.76	440.33^{cd}±3.50	312.33^{bc}±2.89	525.33^{b}±4.15

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

Effect on Glutathione reductase (GR) levels

Effect of extract Jungle jalebi on GR levels in liver, kidney, spleen and brain against toxicity induced by sub-acute exposure of Fipronil in rats are presented in Table 6.

In the present study there was decrease in levels of GR in liver, kidney, spleen and brain in Fipronil treated group. The results were in accordance with [32] where decreased level of GR by lambda-cyhalothrin induced toxicity has been seen in rats [33]. Also reported that level of GR was decreased in brain of rats after exposure to dichlorvos and lindane. Similar findings were also reported by [34] where decrease in level of GR in mthomyl induced oxidative stress in mice was observed. Treatment with Jungle jalebi significantly increased GR level in Fipronil induced toxicity in rats. The results were in accordance with [23] who suggested that aqueous extract of *P. dulce* against CCL_4 administration significantly restored the altered activities of GR enzyme. The observed antioxidant effects of these plants could be related to presence of different chemically defined compounds such as phenolic content and flavonoids in their extracts.

Table 6: Effect of extracts of Jungle jalebi on Glutathione reductase (GR) level (mM GSH/g tissue) in Liver, Kidney, spleen and Brain against toxicity induced by sub-acute exposure of Fipronil in rats

Groups	Treatment	GR (mM GSH/g tissue)			
		Liver	Kidney	Spleen	Brain
I.	Control	119.17^{a}±3.51	151.33^{a}±3.02	136.17^{a}±3.22	116.17^{a}±2.36
II.	Fipronil	81.33^{g} ± 2.67	92.83^{e} ± 2.87	99.00^{d} ± 2.54	86.33^{e} ± 3.09
III.	Fipronil + Jungle jalebi	100.17^{de}±1.90	115.00^{d}±1.46	110.50^{c}±2.09	102.83^{bcd}±2.36
IV.	Jungle jalebi	116.17^{ab}±1.87	140.67^{b}±2.11	133.33^{a}±1.43	116.83^{a} ± 1.22

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

Conclusion

Thus it was concluded that *Pithecellobium dulce* has ameliorating potential on oxidative stress following subacute exposure to fipronil in rats.

Conflicts of interest

The publication of this research paper the authors declare that there is no conflict of interest prevails.

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