



Hepatoprotection

Original Research Article (Experimental)

Evaluation of the ameliorative effects of *Phyllanthus niruri* on the deleterious insecticide imidacloprid in the vital organs of chicken embryos

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ABSTRACT

Background: Insecticides are widely used in agriculture to curb the loss caused by insects. These insecticides are incorporated into the food chain and accumulate in the human body, as well disturb the various metabolic pathways. Imidacloprid is an insect neurotoxin commonly used in agriculture to control the insect pests. *P. niruri* is a traditional medicinal shrub widely used as an anti-inflammatory, antipyretic, and anti-lethality agent.

Objective: The present study is designed to evaluate the ameliorative effects of *Phyllanthus niruri* (Bhumi amla) on the deleterious Insecticide imidacloprid in the vital organs of Chicken embryos.

Materials and methods: The embryonated chicken eggs were divided into the four groups (one control and three treated groups); the chorioallantoic membranes of control received 200 μ l phosphate buffer saline, whereas group I and group II received 100 μ g imidacloprid and 200 μ l aqueous extract of *P. niruri* (PNE) respectively. Group III received both 100 μ g imidacloprid and 200 μ l PNE. The serum was collected on the 18th day its development; which was subjected to the biochemical analysis based on colorimetric assay in semi-automated biochemical analyzer using commercial kits.

Results: We observed significant *in ovo* effects of imidacloprid on chicken embryos; the values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), were increased in imidacloprid treated group I; histopathology also revealed damage to the liver (necrotic areas and dilated blood sinusoids). Alkaline phosphatase (ALP), amylase, cholesterol, triglycerides protein and albumin levels were also altered significantly ($p < 0.05$).

Conclusion: The serum biochemicals were returned back to the nearly normal levels. PNE has ameliorated and overcome the effects of imidacloprid reasonably with the subsequent treatment among group III. Hence, *P. niruri* may be used to minimize the effects of an accidental exposure of imidacloprid.

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1. Introduction

Plants have been sources of food and medicine since antiquity. These play important roles in the hunger satiety, treatment of

various diseases, as well as the maintenance of human health. At present, development of the pharmaceutical industry and production of over 50 % of modern chemical drugs depend on the various phytochemicals.

Phyllanthus niruri (commonly known as 'bhumiymalaki' in Ayurveda, 'chancapiedra' in Spanish, and 'enyikwonwa' in the Ibo dialect of southeastern Nigeria) belongs to the family Euphorbiaceae; it has been studied intensively and is being used in traditional folk medicine. The plant is distributed across almost

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all tropical and subtropical regions including America, India, and Nigeria. It is a common weed that grows mostly in shady or moist and sunny places. It demonstrates therapeutic benefits and used for problems of the stomach, genitourinary system, liver, kidney, and spleen [1]. In Ayurveda, the Indian medicinal system, *P. niruri* is believed to act as a hypolipidemic, anti-lethality, antiviral, antibacterial, anti-malarial, antidepressant, antiseptic, diuretic, anti-diabetic, anti-inflammatory, wound-healing, and antipyretic agent; and is being used traditionally to treat diabetes, diarrhea, dysentery, fevers, jaundice, ulcers, wounds, ulcers, and urogenital diseases [2–8].

Insecticides are the chemical substances used with the explicit intention to prevent or destroy the insect pests; various insecticides in use include pyrethroids, organophosphates, acephates, cyfluthrin, and neonicotinoids. Imidacloprid is a member of new chemical class of neonicotinoids, developed with the intention of high toxicity against insects but low to the mammals. It is used most commonly to control soil and sap-sucking insects [9]. Direct or indirect exposure to insecticides is not only detrimental to human health but also to plants [10]. Pesticides, including fungicides, rodenticides, and insecticides, have been classified into various groups on the basis of their mode of action, structure, and origin [11]. These are used to increase plant productivity; however, its increased concentrations in food and the environment poses safety concerns [12,13].

Pesticides can enter the human body through inhalation, dermal exposure, and ingestion [14], leading to the numerous detrimental effects [15] such as respiratory problems; skin, gastrointestinal, and neurological effects; diabetes; fetal diseases; genetic disorders; and numerous biological changes before clinical manifestations appear [16]. Millions of pesticide poisonings and deaths have been reported annually in developing countries [17]. Numerous biological changes, many destructive or degenerative, occur in various vital organs, including the liver, kidneys, lungs, brain, testes, and skin, and enzymatic biomarkers of organ function can also be altered following exposure to pesticides [18]. Deleterious harmful effects may be due to several insecticides such as organophosphate and carbamate; which are accountable for 94 % of hospital admissions of the affected population and among them 98% died. Three compounds (paraquat, dimethoate and fenthion) account for 47 % of the total deaths due to the exposure of pesticides [19].

The toxicity of imidacloprid is due to its binding with the nicotinic acetylcholine receptors of the central nervous system; due to which no further nerve impulses are propagated. Among mammals, its binding affinity to the nicotinic acetylcholine receptors is far less than insects. It is rapidly absorbed followed by ingestion; however, very little systemic absorption is seen through skin. Rapid metabolism occurs in liver and only 10%–16 % of the unaltered dose is excreted [20]. There are two major pathways through which the imidacloprid is metabolized. In one pathway, its oxidative cleavage results into 6-chloronicotinic acid and imidazolidine; the later is excreted through urine, whereas 6-chloronicotinic acid is converted into mercaptonicotinic acid and hippuric acid [21]. Another pathway includes the hydroxylation of the imidazolidine ring. Variable level of toxicity in human has been observed due to individual variation present in cytochrome P450 isoenzymes responsible for oxidative metabolism of the pesticide [22].

In the present investigation, the effects of *P. niruri* and imidacloprid treatment on some serum markers of pathological investigations such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and amylase enzymes, as well as cholesterol, creatinine, triglycerides, uric acid, protein, and albumin were studied on chicken embryo model.

2. Materials and methods

2.1. Plant material

The plant, *P. niruri* (Fig. 1), was collected from the campus of the Barkatullah University, Bhopal (M.P.), India. Plants were identified according to taxonomic and morphological features [23].

2.2. Preparation of aqueous extract and phytochemical analysis

The entire *P. niruri* plant was thoroughly washed in running tap water and shade dried; it was then powdered using a mortar and pestle. One gram of plant powder was soaked with 20 mL of distilled water in a test tube and heated at 70 °C for 3 h. After incubation, the aqueous extract was centrifuged at 2000 g and the supernatant was used for experimentation. This supernatant was referred to as *P. niruri* extract (PNE). It was screened for the presence of different phytochemicals such as steroids, terpenoids, saponins, flavonoids, anthocyanin, and leucoanthocyanin following methods described by Billmery et al. [24].

2.3. Chemicals

Imidacloprid A.I. 17.8% SL, 1[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine, was obtained from Risiga Agro (India) Pvt. Ltd. (Trade name: DIVIDER). The commercial kits used to determine the enzymatic activity of AST, ALT, ALP, and amylase, as well as the amount of creatinine, cholesterol, urea, protein, albumin and triglycerides were procured (Erba Mannheim, GmbH).

2.4. In ovo study

The embryonated chicken eggs ($n = 40$; 61 ± 3 g of approximately age of 10 days) were procured from a state poultry farm, raisen road, Bhopal (MP), India. Eggs were wiped with disinfectant and candled to screen for the living embryos. Live embryos were divided into four experimental groups with 10 eggs each (one control group and three treatment groups I–III).

Control group received 200 μ L phosphate buffer saline on the chorioallantoic membranes (CAM) using a 28-gauge hypodermic syringe to rule out any non-specific changes in the tested parameters. Group I was treated with 100 μ g imidacloprid and group II was treated with 200 μ L PNE (corresponding to 10 mg dried plant material for an egg of weight 60 g; hence, an equivalent dose of 167 mg/kg of human body weight). Administration of imidacloprid in the group I revealed the effect of insecticide and the same also served as positive control to study the detrimental effect of insecticide. In group II, administration of PNE alone is an indicative of the



Fig. 1. The plant *Phyllanthus niruri*.

Table 1
Biochemical parameters of serum of chick embryos (control group and treated groups).

	Control group	Group I	Group II	Group III	F ratio	p value
AST (U L ⁻¹)	127.24 ± 40.24	187.4 ± 28.79	115.76 ± 48.76	137.6 ± 41.75	2.43	NS
ALT (U L ⁻¹)	153.8 ± 50.89	204.04 ± 57.84	159.58 ± 53.08	156 ± 17.04	1.01	NS
ALP (U L ⁻¹)	1197.4 ± 166.18	1801.2 ± 273.20	1180.2 ± 383.16	1336.8 ± 291.08	4.43 ^a	<0.05
Amylase (U L ⁻¹)	95.6 ± 19.89	264.18 ± 104.51	148.04 ± 52.17	204.92 ± 35.66	5.52 ^a	<0.05
Creatinine (μmol L ⁻¹)	108.0224 ± 23.41	97.4156 ± 44.51	103.602 ± 11.17	104.324 ± 5.92	0.12	NS
BUN (mmol L ⁻¹)	3.19 ± 0.77	4.55 ± 0.72	3.712 ± 0.29	4.089 ± 1.80	1.77	NS
Cholesterol (mmol L ⁻¹)	2.3514 ± 0.94	5.2592 ± 2.19	3.1746 ± 0.65	4.3893 ± 0.95	3.63 ^a	<0.05
Triglycerides (mmol L ⁻¹)	1.875 ± 0.39	4.4352 ± 0.40	2.0074 ± 0.23	3.4118 ± 0.62	31.33 ^a	<0.00001
Protein (g L ⁻¹)	42.2 ± 7.44	65.24 ± 17.12	69.2 ± 30.50	61.6 ± 16.60	1.48	NS
Albumin (g L ⁻¹)	23.8 ± 4.45	30.46 ± 0.89	22.6 ± 5.08	27.16 ± 3.94	3.24 ^a	<0.05

Values are means ± S.D.

^a Significant values.

effects of the plant extract; this group was included to observe the detrimental effects of PNE, if any. In the study, we anticipated the ameliorative effect of PNE against Imidacloprid administration; hence in the group III eggs, both insecticide and plant extract were administered (100 μg imidacloprid + 200 μL PNE) on the CAM, so that improvements in the detrimental effects of insecticide may be observed. In order to prevent any kind of bacterial or fungal contaminations, 10 μl solution containing antibiotics (50 μg/ml ampicillin + 10 μg/ml streptomycin) and antimycotic solution (10 μg/ml amphotericin) were also added on to the CAM of the control as well as treated groups. CAM are water permeable membranes that allow the entry of test compounds into the allantoic fluid, which immediately surrounds the chicken embryo and plays an important role in lipid and vitamin metabolism and metal ion transport [25].

The eggs were resealed using cellophane tape and incubated up to 18th day of its development in a humid incubator chamber at 37 ± 1 °C. The eggs were opened post-incubation; embryos were excised, and blood was collected using 2 mL hypodermic syringe and allowed to clot. The isolated serum was subjected to aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), amylase, creatinine, blood urea nitrogen (BUN), cholesterol, triglyceride, protein, and albumin analysis using an automated biochemical analyzer (Erba EM360). All the parameters were tested using commercial kits from Erba Mannheim, GmbH.

2.5. Data analysis

Analysis of obtained data was carried out using one-way analysis of variance (ANOVA) for independent measures using the online tool (<https://www.socscistatistics.com/tests/anova/Default2.aspx>). Statistical significance was obtained using the p values.

3. Results

The results of the serum biochemical analysis of the 18 days old chick embryos of control and treated groups with imidacloprid and PNE have been depicted in Table 1. These results revealed that AST and ALT levels were much higher in group I (imidacloprid-treated embryos) than those of the control or the PNE-treated embryos of the group II. In group II, AST and ALT levels were at par with the control group. However, the values of both AST and ALT were reduced in the group III embryos (treated with the mixture of imidacloprid and PNE). The effects of imidacloprid on the liver function test have also been depicted in Fig. 2; it is clearly visible that the values of both the AST (Fig. 2A) and ALT (Fig. 2B) among imidacloprid treated group I are much higher than the controls and PNE treated group II and the effects of the insecticide were ameliorated by the PNE in group III embryos (see Fig. 3).

Similar trends were also revealed for the level of ALP (Table 1); ALP level was significantly reduced (p < 0.05) in the group III relative to those of the group I, indicating the amelioration of the effects of the insecticide by PNE. However, the values were nearly similar in both the control and group II (PNE-treated) embryos. These results suggested that PNE possess detrimental effects on the levels of AST, ALT and ALP enzymes; and conversely, it has ability to protect against the degradative effects of insecticides on the liver.

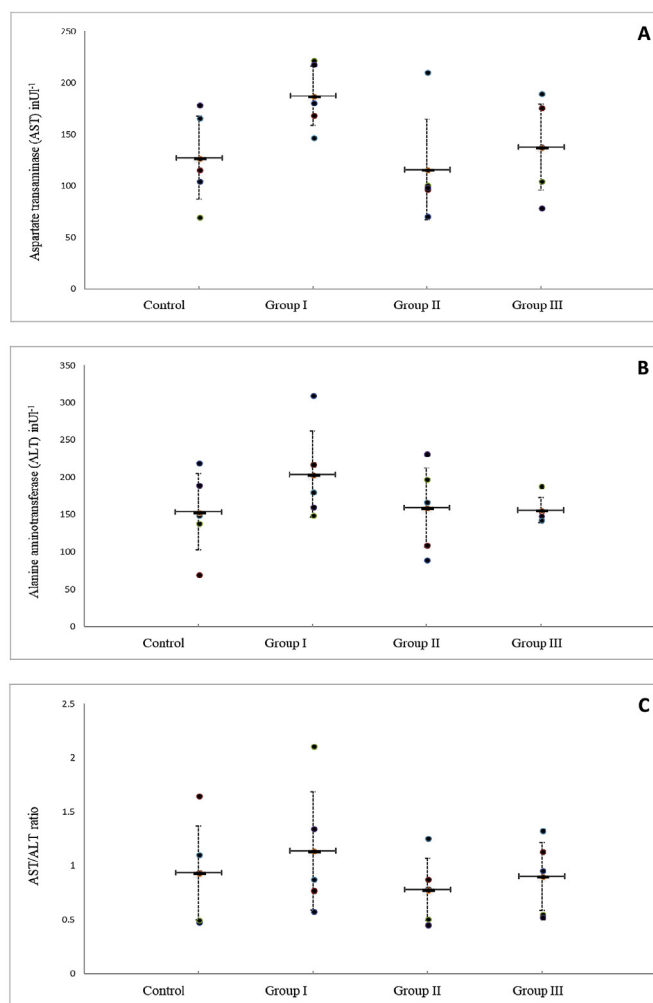


Fig. 2. Aminotransferases of liver function test [(A) aspartate aminotransferase (AST), (B) alanine aminotransferase (ALT)] and (C) AST/ALT ratio of 18 days chick embryos. Imidacloprid treated group I revealed higher values of AST and ALT in comparison to the controls and PNE treated group II; in group III, PNE has overcome the effects of imidacloprid to some extent showing the ameliorative role of *P. niruri*.

Histopathological alterations of the liver corroborated the above findings. Damage to the liver was observed in group I (Fig. 3B); necrotic areas and dilated blood sinusoids are clearly visible. Whereas the group II (Fig. 3C) embryos possess nearly normal hepatic and sinusoidal architectures like those of the controls (Fig. 3A). The damage was much reduced in group III embryos (Fig. 3D) indicating the ameliorative role of *P. niruri*.

Among group I embryos, creatinine level was much lower than those of the controls, whereas group II and group III embryos revealed nearly equivalent values of creatinine to those of the controls. This indicates that PNE had no effect on creatinine levels; however, it ameliorated the effects of the imidacloprid on creatinine levels. Histologically, we did not find any differences in the kidneys of the control and the treated embryos.

Amylase levels were significantly less in the control group than the three treated groups. Group I exhibited the highest levels of amylase, followed by group III and group II (Table 1). Here, it is noteworthy that PNE also increased the level of amylase; however, this increase was not significantly higher than those of controls. However, group III revealed that PNE has reduced the amylase values when administered with the insecticide.

Further, the serum biochemical analysis of imidacloprid treated group I embryos revealed significantly higher values for the cholesterol, triglycerides, and albumin in comparison to the control and PNE-treated group II embryos. Serum protein, creatinine, and BUN also revealed the similar trends, but the differences were not significant.

The phytochemical analysis revealed the presence of saponins, tannins, terpenoids, steroids and flavonoids in the plant extract of *P. niruri*.

4. Discussion

Chicken embryos are considered as a model biological platform for human to understand the cancer biology, ischemia-reperfusion, engraftment of human leukemic stem cells, gastroschisis, consequences of environmental exposure, toxicopathological studies and as a model of non-clinical safety studies of pharmaceuticals [26–28]. The chicken embryo system presents an inexpensive, nutritionally self-sufficient and high throughput model that mimics human organ system and architecture [29].

P. niruri is a medicinal plant with numerous medicinal properties and it is being used for treating various ailments since long. The effect of imidacloprid and the curative effects of *P. niruri* on various vital organs have been described in the present report.

Imidacloprid exposure reduces the serum levels of glutathione (GSH), superoxide dismutase (SOD) enzyme activity, and enhances ALT, lactate dehydrogenase (LDH), uric acid, plasma tumor necrosis factor α (TNF α) and plasma acetylcholinesterase (AChEs) enzyme activities in the rock pigeon [30]. Similarly, in white leghorn (WLH) chick's administration of imidacloprid has increased the levels of AST, ALT and ALP enzymes [31]. Also, Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT), bilirubin and blood urea nitrogen (BUN) are significantly increased in serum of

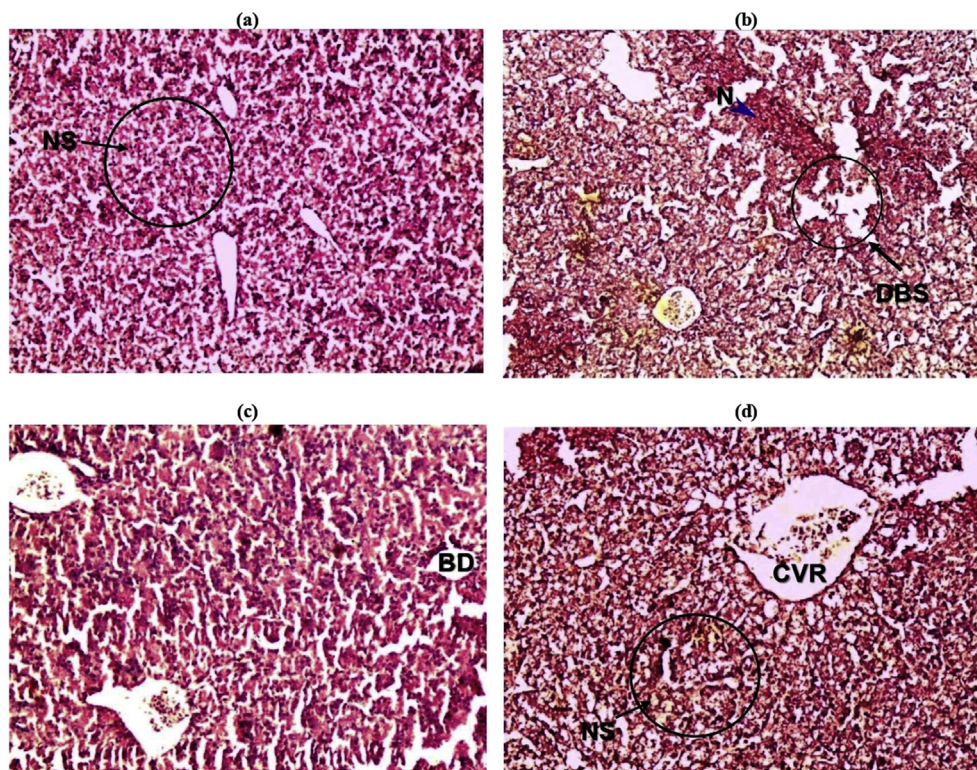


Fig. 3. Histopathology of hematoxylin–eosin-stained (H & E) sections of the chicken embryo liver (a) control group and (b–d) treated groups I–III respectively. Control group (a) showing normal hepatic and sinusoidal architectures (encircled NS), (b) Imidacloprid treated group I is showing necrotic areas (N) and dilated blood sinusoids (DBS); (c) group II receiving PNE only also showing near to normal sinusoidal architecture (d) Group III showing the restored sinusoidal architecture as well as reduced necrosis, when pesticide is given along with PNE, indicating the hepatoprotective role of *P. niruri*. (NS = normal sinusoid; N = necrosis; DBS = dilated blood sinusoid; BD = bile duct; CVR = central vein with RBCs).

female rats exposed for one time to imidacloprid [32]. Similar results were obtained by Bhardwaj et al. [33], they revealed an increase in serum ALT, AST, glucose and BUN. Our results are in concordance with the results of these researchers; hence, it is affirmed that chicken embryo model can be preferred like rodent model in such kind of studies.

Imidacloprid elevates serum transaminase, ALT, AST, LDH, ALP and/or glutamate dehydrogenase activities besides the changes in other analytical biomarkers. It induces pro-inflammatory cytokines such as TNF α in brain and liver and decreases the activities of LDH in exposed rodent model. Also, there is significant increase in MDA and SOD; and decrease in GSH level in serum. Other clinical chemistry parameters, such as uric acid, total protein, and albumin are also seen altered in case of imidacloprid administration [30].

However, in a double-blind clinical trial considering *P. niruri* for the treatment of chronic hepatitis B virus (HBV) infection had no apparent clinical benefits [34]. In Sprague–Dawley rats, 50% methanolic extracts of *P. niruri* showed strong anti-angiogenic effects in aortic ring assay and was able to ameliorate non-alcoholic fatty liver disease [35]. In broiler chickens infected with *Mycoplasma gallisepticum*, an oral administration of 65% meniran extract at a dose of 1 mL/kg body weight, reduced the total count of leucocytes that was increased due to infection and inflammation induced by the pathogen [36].

Pesticides are used worldwide in the modern agricultural sector to increase the productivity of plants. Their concentrations are also increasing in the food chain and environment. Imidacloprid (neonicotinoids) is a major class of potent insecticides that are used for crop protection and flea control because of its specific toxicity to arthropods and relative non-toxicity to vertebrates [37]. In the case of accidental human exposure, mild clinical effects such as tachycardia, nausea, hypertension, mydriasis, dyspnea, apnea, coma, CNS depression, hyperglycemia and vomiting have been observed. However, serious sequelae include respiratory failure, seizures, cyanosis, ventricular fibrillation, and even death [38,39].

In our study also, the group of embryos treated with imidacloprid showed significantly altered levels of AST, ALT, ALP, creatinine, cholesterol, triglycerides, and pancreatic amylase. This confirms the damage caused by imidacloprid to the various vital organs such as the liver, pancreas, kidneys, and heart. The altered level of ALT, AST, ALP and deviances from normal values indicate pathological conditions. The enzyme ALP is present throughout the body, including in the liver, digestive system, kidneys, and bones, and in case of bone or liver disorder, ALP test is recommended by physician. In case of an inflamed pancreas, the quantity of amylase is disturbed. Different enzymes are used as biomarkers in organ function tests; the AST, ALT and gamma-glutamyl transferase (GGT) are biomarkers of liver damage [40], these are increased in serum after hepatic injury or toxicity. Serum pancreatic amylase is mainly used to diagnose acute pancreatitis or a flare-up of chronic pancreatitis [41]. In human, the BUN level higher than 100 mg/dL is pointing the severe kidney damage. Also, a higher BUN–creatinine ratio is observed in acute renal failure and pre-renal conditions [42].

Elevated liver enzymes in response to different drugs have been observed in several animal models [43,44]. A formulation containing the aerial parts of *P. niruri* has been shown to ameliorate the toxic effects of paracetamol and D-galactosamine [45] and to reduce CCl₄-induced hepato-renal toxicity [46]. In a thioacetamide-induced liver cirrhosis Sprague Dawley rat model, *P. niruri* treatment improved liver biochemical parameters, reduced oxidative stress enzyme and lipid peroxidation levels along, and restored histological and morphological patterns in livers [47], as well as in diabetic rats [48]. A possible explanation for the hepato-protective role is the restoration of impaired liver membrane function [49]. In the present study, PNE treatment (Group II) alone did not alter

normal AST and ALT values, whereas pesticide treatment (Group I) increased these levels. The increased ALP values in imidacloprid treated embryos indicate liver disease or bone disorders in the experimental animals. The PNE in group III ameliorated the altered enzyme levels; which contrasts the observations of Khan et al. [45] and Amin et al. [47], who found the minimal inflammation and normal lobular architecture in the livers of treated rats.

Upregulation of ALP enzyme due to its liver specific leakage has been reported to serve as a marker of adverse effect of aspirin administration and the deleterious effects which could be reversed by administration of *P. niruri* up to a dose of 10 mg/kg body weight. In rat model, liver damaging activities of alcohol and heated sunflower oil are evidenced by enhanced level of ALT and AST enzymes and treatment with *P. niruri* extract inhibited the upregulation of these enzymes and also reduced hyperlipidemia [50]. Increased level of serum pancreatic amylase, which is an indicative of pancreatic damage, was also significantly increased among imidacloprid-treated group I embryos. Strikingly in group II, PNE has also increased it, suggesting adverse effects of PNE on the pancreas. However, when the extract was administered with the pesticide, it reduced the amylase level by 22.4%. Our results are in contrast with the results of Okoli et al. [51], who demonstrated restoration of normal pancreas architecture in a histopathological study. In addition, these results contradict the findings of Tamil et al. [52], who showed *in vitro* amylase inhibition in alloxan-treated mice following treatment with *P. niruri*.

Increased amounts of BUN and creatinine are indicative of decreased kidney function. In different studies, various results have been obtained using PNE. In the present study, imidacloprid (Group I) and PNE (Group II) enhanced urea levels, indicating their harmful effects on kidneys. These results are in concordance with the previous studies of Manjrekar et al. [46], Ajibade and Famurewa [53], and Adedapo et al. [54]; their investigations revealed the adverse effects of *P. niruri* on renal and testicular organs despite of its hepatoprotective role. Different results were obtained by Asare et al. [55], who reported no effect of PNE on the urea levels. Creatinine levels were reduced by imidacloprid in group I embryos; however, the levels in group III embryos were similar to the controls. This revealed that PNE may restore the creatinine concentrations that decreased in response to imidacloprid. Freitas et al. [3] and Asare et al. [55], however, reported that creatinine clearance was unaffected by PNE, whereas, paradoxically, Ajibade and Famurewa [53] reported the enhancement of creatinine levels following the exposure of *P. niruri*.

In general, pesticide exposure results in impairment of streamlined enzymatic pathways in the cytoplasm, mitochondria, and peroxisomes, which are involved in carbohydrate, fat, and protein metabolism [56]. For example, comparison of unexposed people and exposed to pesticides revealed higher levels of total protein with lower levels of serum total protein and albumin among Greek and Tunisian farm workers [57,58]. Similar results were obtained with Indian and Thai agricultural workers [59,60]. Our results are contrast with these results, although the difference in results in these studies might be attributed to the use of a pesticide other than imidacloprid. A study conducted on SD mice revealed that a dose higher than 5 mg/kg of weight induce toxicity on vital organs [55]. In human subjects, to treat other disorders like diabetes, hepatitis, high blood pressure, liver disease and urinary stones, a dose ranging between 0.8 and 3 g of dried powder of PNE has been suggested for three times a day up to three months. Besides that, toxicity in children is not studied. Hence in case of accidental exposure to Imidacloprid or in case of hyperlipidemia, a dose up to 3 g of PNE to a maximum of 3 dose per day may be suggested.

Some other plant extracts such as *Silybum marianum* and *Ginkgo biloba* have also been used to treat pesticide toxicity. In piscine model, *S. marianum* has been tested for hepatotoxicity induced by deltamethrin, that is a pyrethroid ester insecticide and the extract was found to normalized the altered levels of catalase, reduced glutathione and lipid peroxidation [61]. *G. biloba* plant extract ameliorated the toxicity induced by diazinon, an organophosphate pesticide, in rainbow trout [62]. Very less data is available on the plant extract mediated reversal of pesticide/insecticide toxicity and therefore it is presently not possible to check the ameliorative effects of other plant extracts in parallel with PNE against imidacloprid. The ingestion of the plant extract should be in optimum amount since at higher doses PNE is known to cause male hormone imbalance and testicular changes [63].

Zebrafish (*Danio rerio*) is also a good model for testing the effects of the toxicity. It has been used to determine the toxicity of ketoprofen (anti-inflammatory drug); phosalone and cypermethrin-based pesticides and other known mammalian hepatotoxic drugs such as carbaryl, isoniazid, and pyrazinamide; and the numerous studies revealed that ALT and AST enzymes were increased [64–66]. Post exposure increase in ALT, AST, ALP, urea, uric acid, albumin, and creatinine has been observed in triflumuron insecticide induced toxicity assay in mice [67]. The DDT exposure also caused elevated levels of liver enzyme ALT [68]. Co-exposure to deltamethrin pesticide and cadmium to mice significantly increased ALT and AST [69]. All the results of toxicity studies in zebrafish and mice are corroborated with the results of our study encompassing the use of chicken embryos as model system and results are also comparable as evidenced by altered profile of ALT and AST. Chicken embryo presents a very simple, easy to handle, cheap and highly reproducible system, that may be readily adapted for determining toxicity assays.

5. Conclusion

In conclusion, PNE helped reduce the levels of AST, ALT, ALP, amylase, triglycerides, and cholesterol in imidacloprid-treated embryos. Hence, PNE may be useful in imidacloprid poisoning cases. At the same time, the extract could not ameliorate the harmful effects of the pesticide on BUN. Protein levels were elevated in all treatment groups, whereas albumin concentrations were not affected by PNE alone, and PNE showed weak amelioration effects.

P. niruri may be used to treat accidental exposure to imidacloprid pesticide and to reduce hyperlipidemia. However careful use of the extract is suggested because of the elevated levels of uric acid found in serum after treatment with the extract. Results of this study suggest that *P. niruri* components might act as antidotes against the insecticide and reverse pesticide intoxication effects.

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Conflict of interest

None

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