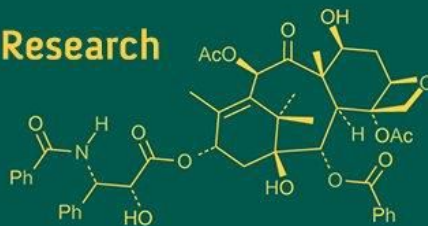


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Hematological and biochemical evaluation of effect of Mangiferin in Amitraz induced toxicity in rats

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Abstract

The present study was conducted to assess the protective role of mangiferin against amitraz-induced toxicity in female wistar rats. Forty rats were randomly allocated into five groups (n = 8): Group I (normal control), Group II [amitraz, 80 mg/kg] Group III (mangiferin, 30 mg/kg), Group IV (amitraz + mangiferin, 30 mg/kg), and Group V (amitraz + mangiferin, 60 mg/kg). Exposure to amitraz alone caused significant reductions in body weight gain, hematological indices, TEC and Hb along with a significant increase in liver and kidney function biomarkers (ALT, AST, ALP, BUN and creatinine) lipid peroxidation and total leukocyte count (TLC). Co-administration of mangiferin, particularly at a dose of 60 mg/kg, significantly mitigated amitraz-induced toxic effects by restoring hematological and biochemical parameters and reducing tissue damage. In conclusion, mangiferin demonstrated notable neuroprotective, hepatoprotective and nephroprotective effects against amitraz-induced toxicity suggesting its potential therapeutic value in managing pesticide-induced oxidative stress and toxicity in animals.

Keywords: Amitraz, mangiferin, hepatotoxicity, nephrotoxicity

Introduction

Parasitism represents a major challenge in veterinary medicine, particularly in hot and humid tropical regions. Among parasitic conditions, ectoparasitism causes substantial reductions in productivity in cattle and other livestock, leading to significant economic losses for farmers. In companion animals, ectoparasite infestations are equally problematic, as they compromise animal welfare and pose public health concerns due to their zoonotic potential (Dantas-Torres and Otranto, 2014) [9].

Formamidines are an important class of ectoparasiticides, with amitraz being the principal compound approved for veterinary use. Amitraz is widely employed for the control of ticks, mites, and lice in cattle, swine, and sheep, and is also used in dogs. The development of formamidine acaricides began with the introduction of chlordimeform in 1963 (Hollingworth, 1976) [14], followed by several related compounds, of which amitraz remains the most prominent. First synthesized in England in 1969 (Hollingworth, 1976) [14], amitraz continues to be extensively used, formamidine insecticides currently approved for veterinary applications.

Amitraz undergoes hepatic biotransformation, producing several metabolites, among which BTS 27271 is the most pharmacologically active. This metabolite exerts its effects primarily through binding to $\alpha 2A$ and $\alpha 2D$ adrenergic receptors (Abu-Basha *et al.*, 1999) [1]. In insects, formamidines such as chlordimeform and amitraz act by targeting octopaminergic receptors in the central nervous system. In mammals, however, toxicity is mainly attributed to amitraz acting as an $\alpha 2$ -adrenergic receptor agonist and a monoamine oxidase (MAO) inhibitor. Additional mechanisms have also been reported, including inhibition of histamine receptors, H1 suppression of prostaglandin synthase and adenylyl cyclase, activation of voltage-gated calcium channels, generation of reactive oxygen species (ROS), induction of cell death, and disruption of endocrine function (Evans and Gee, 1980; Chen *et al.*, 2007) [12, 33].

Numerous studies have established a link between insecticide exposure and enhanced production of reactive oxygen species, which can cause oxidative damage to vital organs such as the liver, kidneys and brain. Oxidative stress occurs when ROS generation exceeds

the capacity of the body's antioxidant defense systems. Antioxidants play a crucial role in neutralizing free radicals by donating electrons, thereby preventing cellular damage. Maintaining a balance between pro-oxidant and antioxidant mechanisms is essential for health and disease prevention (Srivastava and Dwivedi, 1998; Yu *et al.*, 2008) [30, 32]. Both enzymatic antioxidants such as catalase, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase, and glutathione reductase and non-enzymatic antioxidants, including reduced glutathione and total thiols, are vital in protecting cells from oxidative injury. Exposure to pesticides has been shown to significantly alter the activity of these antioxidant systems (Banerjee *et al.*, 2001; Bebe and Panemangalore, 2005) [5, 7].

Xanthenes are secondary metabolites found in certain higher plants, lichens, and fungi and have attracted considerable interest due to their therapeutic potential and taxonomic significance (Negi *et al.*, 2013) [22]. Structurally and chromatographically similar to flavonoids, xanthenes exhibit diverse pharmacological activities, including anti-inflammatory, antifungal, antibacterial, antioxidant, and antiviral effects (Saini *et al.*, 2022) [25]. Mangiferin, a xanthone glucoside, is predominantly isolated from the tropical fruit *Mangifera indica* (mango), native to India and has a molecular weight of 422.34 g/mol. Although mango is its primary source, mangiferin is also present in several other plant species (Jaiswal *et al.*, 2015) [16].

Mangiferin exhibits a broad spectrum of therapeutic properties, including antioxidant, anti-inflammatory, antidiabetic and anticancer activities. Traditionally, it has been used in Indian medicine for the treatment of melancholia and nervous debility (Bhattacharya *et al.*, 1972) [8]. Furthermore, mangiferin is a key active component in over 40 polyherbal formulations used in traditional Chinese medicine (Alduhisa and Demayo, 2019) [4]. Studies have demonstrated that mangiferin enhances glutathione levels by upregulating gamma-glutamyl cysteine synthetase, the rate-limiting enzyme in glutathione synthesis. Its antioxidant action is also associated with the downregulation of NF- κ B, resulting in reduced tumor necrosis factor-induced ROS production (Sarkar *et al.*, 2004) [26]. Additionally, mangiferin has been shown to interact directly with catalase, thereby significantly reducing tissue damage (Sahoo *et al.*, 2015) [24]. Considering the toxic potential of amitraz and the antioxidant, anti-inflammatory and anti-apoptotic properties of mangiferin, the present study was designed to evaluate the protective effect of mangiferin against amitraz-induced subacute toxicity in rats, with the following objectives:

- To assess hematological and serum biochemical associated with amitraz-induced toxicity in rats.

Materials and Methods

The experiment was conducted in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA) after obtaining prior approval from the Institutional Animal Ethics Committee. The study was carried out at the Small Animal House, Hassan Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University (KVAFSU) Bidar with prior approval of the Institutional Animal Ethics Committee (IAEC), Veterinary College, Hassan with No HVC/IAEC/15/2025 Female wistar rats aged 6–8 weeks, non-pregnant and nulliparous were used for the experiment. The animals were maintained under controlled

environmental conditions with a temperature of 22 ± 3 °C, relative humidity of 50–60%, and a 12-hour light/12-hour dark cycle. Standard laboratory feed and water were provided ad libitum throughout the study period.

A total of 40 rats were randomly allocated into five groups, with eight animals in each group. Group I (normal control) received normal saline orally. Group II (positive control) was administered amitraz dose of 80 mg/kg body weight where amitraz of pure form was dissolved in normal saline where normal saline was used as a vehicle. Group III received mangiferin alone at a dose of 30 mg/kg body weight. Group IV was treated with amitraz (80 mg/kg body weight) in combination with mangiferin (30 mg/kg body weight). Group V received amitraz (80 mg/kg body weight) along with a higher dose of mangiferin (60 mg/kg body weight).

Body weight Measurement

The body weight of all the experimental animals were recorded weekly once and the dosing was adjusted accordingly.

Hematology

The blood samples collected in EDTA vials on 14th and 28th day of the experiment were subjected for haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), total platelet count, packed cell volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) estimation using Mindray BC-2800VET auto Haematology auto analyser in the Department of Teaching Veterinary Clinical Complex, Veterinary College, Hassan.

Serum Biochemistry

For serum biochemical parameters analysis, blood was collected on 14th and 28th day into serum vacutainers, centrifuged at 3000 rpm for 15 min and serum separated and stored at -20 °C till further analysis. Biochemical estimation for AST, ALT, ALP, Urea, BUN and creatinine levels were analysed using standard laboratory procedure by Robonik, India Semi-automatic Biochemistry Analyzer (Prietest Compact) using reagent kits from Lifechem, Ahmedabad.

Results

General observations

The amitraz was administered orally in rats for 28 days did not result in mortality. However, animals in Group II showed noticeable clinical signs, including roughened hair coats and reduced feed intake compared with Group I. During the last two weeks of treatment, Group II rats exhibited anorexia, lethargy, weakness, abnormal gait, aggressive behavior and dizziness. In contrast, rats in Groups III, IV, and V displayed normal behavior and activity throughout the experimental period.

The Body weights were recorded weekly and are presented in Table 1.1, 1.2, 1.3. The rate of body weight gain was significantly influenced by amitraz administration, both alone and in combination with mangiferin. From the second week onward, the mean body weight of rats in Group II was significantly lower compared with Groups I, III, IV, and V. Conversely, Group III animals showed a progressive increase in body weight throughout the study period.

Absolute organ weights

A significant increase in absolute liver weight was observed in Group II compared with Group I. In Groups IV and V, liver weights were significantly reduced relative to Group II, while liver weights in Group III were comparable to those of Group I.

Similarly, the absolute kidney weight was significantly higher in Group II than in Group I. In Groups IV and V, kidney weights were significantly lower compared with Group II, whereas a marginal increase was noted in Group III relative to Group I.

Relative organ weights

The relative weights of the liver and kidneys were significantly increased in Group II compared with Group I. In contrast, Groups IV and V showed a significant reduction in relative liver and kidney weights when compared with Group II. No significant differences in relative liver or kidney weights were observed between Group III and Group I.

Hematological parameter

The mean values of hematological parameters with standard error of mean on 14th and 28th day are presented in Table 2.1, 2.2. On days 14 and 28, Group II showed a significant decrease in total erythrocyte count (TEC) compared to Groups I, IV, and V. In contrast, TEC levels were significantly higher in Groups IV and V when compared with Group II. TEC levels in Group III were similar to those in Group I. During the same periods, total leukocyte count (TLC) was significantly increased in Group II compared to Group I. However, Groups IV and V showed a significant decrease in TLC levels compared to Group II. No significant difference in TLC levels was observed between Group III and Group I. Hemoglobin (Hb) levels on days 14 and 28 were significantly lower in Group II compared to Group I. In contrast, Hb levels were significantly increased in Groups IV and V relative to Group II. Group III showed Hb levels was comparable to Group I.

Serum Biochemistry

The mean values of serum biochemistry parameter values with standard error of mean on 14th and 28th day were presented in Table 3.1, 3.2, 3.3 Serum ALT, AST, and ALP levels were significantly elevated in Group II compared to Group I. In contrast, Groups IV and V showed a significant reduction in these enzyme levels relative to Group II. Enzyme levels in Group III remained comparable to those in Group I. Renal function markers, including urea, blood urea nitrogen (BUN) and creatinine, were significantly increased in Group II compared to Group I. However, Groups IV and V exhibited a significant decrease in these parameters when compared with Group II. No significant differences were observed between Group III and Group I.

Discussion

Body weight gain is an important indicator of growth. In the present study, rats were treated with amitraz alone (Group II) showed a significant reduction in both absolute and percentage body weight gain after 28 days compared to the control group (Group I). Although Group III showed an increase in body weight gain, the difference was not statistically significant compared to Group I. These findings are consistent with earlier reports showing reduced body

weight gain following amitraz exposure (Ueng *et al.*, 2004; Institoris *et al.*, 2007) [31, 15]. The reduction in body weight may be due to the metabolic and hormonal effects of amitraz through activation of α 2-adrenergic receptors, leading to increased norepinephrine release and elevated metabolism (Del Pino *et al.*, 2015) [11]. Similar effects on body weight have also been reported in fipronil-induced toxicity in rats (Mohan, 2023) [21]. Relative organ weight is a key indicator of organ toxicity. In this study, a significant increase in relative liver weight was observed in Group II compared to Group I, which may be attributed to inflammatory changes and liver enlargement caused by amitraz exposure, as previously reported (Ueng *et al.*, 2004) [31].

Similarly, Group II showed a significant increase in relative kidney weight compared to Group I. This increase may result from inflammatory cell accumulation and altered renal function, consistent with earlier findings (Ueng *et al.*, 2004; Institoris *et al.*, 2007; Filazi *et al.*, 2004) [31, 15, 13].

Hematological analysis is commonly used to assess disease conditions and toxic effects caused by chemicals and pesticides (Al-Attar *et al.*, 2010) [3]. Erythrocytes play a vital role in oxygen transport and hemoglobin levels are closely associated with total erythrocyte count (Pasini *et al.*, 2010) [23]. In the present study, Group II showed a significant decrease in TEC and Hb levels, along with an increase in TLC, compared to Group I. These findings are consistent with earlier reports on amitraz toxicity (Barretto *et al.*, 2017; Institoris *et al.*, 2007) [6, 15] and similar hematological alterations reported in fipronil-induced toxicity (Mohan, 2023) [21]. The reduction in hemoglobin may indicate regenerative anemia due to interference with erythropoiesis, while decreased TEC and increased TLC could be attributed to oxidative stress-induced damage to red blood cells (Barretto *et al.*, 2017; Institoris *et al.*, 2007) [6, 15]. Similar protective effects of mangiferin on these parameters have also been reported by Mohan (2023) [21], supporting the present findings.

A significant increase in AST, ALT, ALP, creatinine, urea and BUN levels was observed in Group II compared to Group I. These findings are consistent with previous reports showing amitraz-induced liver and kidney toxicity in rodents (Kanbur *et al.*, 2016) [17]. Elevated ALT and AST levels indicate liver cell damage, which may be associated with bile duct hyperplasia, hepatic congestion, Kupffer cell hyperplasia, and altered membrane permeability. Similar hepatocellular changes through histopathological observations. Increased ALP levels, which are present on hepatocyte membranes and bile duct epithelium, serve as a sensitive indicator of toxic liver damage (Ueng *et al.*, 2004) [31].

The rise in urea levels observed in this study supports the findings of Kanbur *et al.* (2016) [17] and correlates with kidney tissue damage. Elevated BUN and creatinine levels further indicate impaired renal function, likely due to reduced glomerular filtration and tubular dysfunction (Mahmood and Mohammad, 2019; Kanbur *et al.*, 2016) [20, 17].

Meanwhile, Mangiferin is a natural xanthone compound known for its antioxidant, anti-inflammatory and protective properties. It reduces toxicity by scavenging free radicals and strengthening antioxidant defense mechanisms (Shashirekha *et al.*, 2015; Ajay *et al.*, 2023) [28, 2]. Based on these properties, this study evaluated the protective effect of mangiferin against sub-acute amitraz-induced toxicity in

Wistar rats. Groups IV and V showed a significant increase in absolute and percentage body weight gain compared to Group II, indicating that mangiferin reduced amitraz-induced oxidative stress and neurotoxicity. Group III showed values comparable to Group I. Similar protective effects of mangiferin on body weight were reported by Song *et al.* (2020) [29]. Relative liver and kidney weights were significantly reduced in Groups IV and V compared to Group II, suggesting hepato- and nephroprotective effects of mangiferin. This may be due to inhibition of the NF- κ B inflammatory pathway and reduced reactive oxygen species formation (Li *et al.*, 2019) [19]. These findings are consistent with earlier reports on the protective role of mangiferin (Song *et al.*, 2020) [29].

Serum biochemical markers, including ALT, AST, ALP, urea, creatinine and BUN, were significantly lowered in Groups IV and V compared to Group II, supporting the protective effects of mangiferin on liver and kidney function (Li *et al.*, 2013) [18].

Hematological parameters such as TEC, Hb, and TLC were also significantly improved in Groups IV and V relative to Group II. These results agree with previous studies showing the hemoprotective and immunoprotective effects of mangiferin, likely through enhanced erythropoiesis and reduced oxidative damage to blood cells (Das *et al.*, 2011; Sellamuthu *et al.*, 2014; Mohan, 2023) [10, 27, 21].

Table 1.1: Body weights (g; Mean $\pm$ SEM) of experimental groups (n=8) of rats

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
I	182.06 $\pm$ 4.49 ^a	192.31 $\pm$ 4.35 ^a	201.81 $\pm$ 4.16 ^{ab}	216 $\pm$ 4.34 ^b	228.63 $\pm$ 5.33 ^b
II	181 $\pm$ 3.47 ^a	189.44 $\pm$ 3.58 ^a	195 $\pm$ 3.56 ^a	204 $\pm$ 3.99 ^a	206.06 $\pm$ 3.96 ^a
III	182.25 $\pm$ 3.12 ^a	192.31 $\pm$ 2.97 ^a	203.56 $\pm$ 3.05 ^b	217.13 $\pm$ 3.57 ^b	232.65 $\pm$ 3.84 ^b
IV	182.5 $\pm$ 2.86 ^a	191.75 $\pm$ 2.77 ^a	199.19 $\pm$ 3.08 ^{ab}	210.88 $\pm$ 3.18 ^{ab}	223.44 $\pm$ 3.16 ^b
V	181.44 $\pm$ 2.84 ^a	190.94 $\pm$ 2.91 ^a	199.50 $\pm$ 2.87 ^{ab}	212.31 $\pm$ 2.92 ^b	225.13 $\pm$ 1.88 ^b

Note: Mean $\pm$ SEM values of body weight within the same column bearing different superscripts differ significantly at $p \leq 0.05$

Table 1.2: Absolute organ weight (g; Mean $\pm$ SEM) of liver and kidney of experimental groups (n=8) of rats

Groups	Liver	Kidney
Group I	5.01 $\pm$ 0.23 ^a	0.77 $\pm$ 0.66 ^a
Group II	6.89 $\pm$ 0.18 ^c	1.29 $\pm$ 0.03 ^b
Group III	5.62 $\pm$ 0.26 ^{ab}	0.99 $\pm$ 0.06 ^{ab}
Group IV	5.52 $\pm$ 0.13 ^{ab}	1.05 $\pm$ 0.05 ^{ab}
Group V	5.39 $\pm$ 0.08 ^{ab}	0.99 $\pm$ 0.06 ^{ab}

Table 1.3: Relative organ weight (g/100g; Mean $\pm$ SEM) of liver and kidney of experimental groups (n=8) of rats

Groups	Liver	Kidney
Group I	2.16 $\pm$ 0.04 ^a	0.34 $\pm$ 0.02 ^a
Group II	3.29 $\pm$ 0.08 ^c	0.69 $\pm$ 0.01 ^c
Group III	2.39 $\pm$ 0.07 ^b	0.39 $\pm$ 0.02 ^b
Group IV	2.46 $\pm$ 0.05 ^b	0.46 $\pm$ 0.03 ^b
Group V	2.42 $\pm$ 0.02 ^b	0.46 $\pm$ 0.02 ^b

Note: Mean $\pm$ SEM values of relative organ weight within the same column bearing different superscripts differ significantly at $p \leq 0.05$

Table 2.1: TEC (x106/ μ l; Mean $\pm$ SEM) and TLC (x103/ μ l; Mean $\pm$ SEM) of experimental groups (n=8) of rats

Groups	Hematological parameters			
	TEC (x106/ μ l)		TLC (x103/ μ l)	
	14th Day	28th Day	14th Day	28th Day
Group I	7.35 $\pm$ 0.89 ^a	7.98 $\pm$ 0.15 ^a	11.41 $\pm$ 0.12 ^a	11.11 $\pm$ 0.80 ^a
Group II	6.56 $\pm$ 0.10 ^b	4.99 $\pm$ 0.18 ^c	14.01 $\pm$ 0.21 ^b	16.05 $\pm$ 1.92 ^b
Group III	7.63 $\pm$ 0.10 ^a	7.74 $\pm$ 0.14 ^a	11.33 $\pm$ 0.80 ^a	11.32 $\pm$ 0.55 ^a
Group IV	7.18 $\pm$ 0.85 ^a	7.15 $\pm$ 0.10 ^b	11.63 $\pm$ 1.00 ^a	10.92 $\pm$ 0.79 ^a
Group V	7.38 $\pm$ 0.09 ^a	7.16 $\pm$ 0.13 ^b	11.46 $\pm$ 0.77 ^a	10.63 $\pm$ 0.06 ^a

Note: Mean $\pm$ SEM values of TEC and TLC within the same column bearing different superscripts differ significantly at $p \leq 0.05$

Table 2.2: Hb (g/dl; Mean $\pm$ SEM) of experimental groups (n=8) of rats

Groups	Hb (g/dl)	
	14th Day	28th Day
Group I	12.51 $\pm$ 0.65 ^a	12.41 $\pm$ 0.46 ^b
Group II	9.04 $\pm$ 0.73 ^d	8.39 $\pm$ 1.17 ^d
Group III	11.64 $\pm$ 0.88 ^c	11.49 $\pm$ 1.18 ^c
Group IV	11.91 $\pm$ 0.173 ^b	11.97 $\pm$ 1.18 ^b
Group V	12.55 $\pm$ 0.169 ^a	13.12 $\pm$ 1.97 ^a

Note: Mean $\pm$ SEM values of Hb within the same column bearing different superscripts differ significantly at $p \leq 0.05$

Table 3.1: ALT and AST (IU/L; Mean $\pm$ SEM) of experimental groups (n=8) of rats

Groups	Serum biochemical parameters			
	ALT (U/L)		AST (U/L)	
	14th Day	28th Day	14th Day	28th Day
Group I	39.84 $\pm$ 1.89 ^{cd}	42.78 $\pm$ 2.01 ^b	82.41 $\pm$ 1.33 ^b	88.29 $\pm$ 1.18 ^b
Group II	71.19 $\pm$ 3.55 ^a	77.54 $\pm$ 2.64 ^a	119.15 $\pm$ 0.94 ^a	128.21 $\pm$ 1.26 ^a
Group III	31.20 $\pm$ 0.00 ^d	44.29 $\pm$ 0.79 ^b	60.51 $\pm$ 2.27 ^c	70.30 $\pm$ 2.79 ^c
Group IV	47.55 $\pm$ 1.30 ^{bc}	39.50 $\pm$ 1.48 ^b	77.46 $\pm$ 1.91 ^b	72.75 $\pm$ 1.58 ^c
Group V	49.86 $\pm$ 6.21 ^b	39.09 $\pm$ 1.82 ^b	66.56 $\pm$ 1.05 ^c	58.55 $\pm$ 0.91 ^d

Mean $\pm$ SEM values of body ALT and AST within the same column bearing different superscripts differ significantly at $p \leq 0.05$

Table 3.2: ALP (IU/L; Mean $\pm$ SEM) and UREA (mmol/L) of experimental groups (n=8) of rats

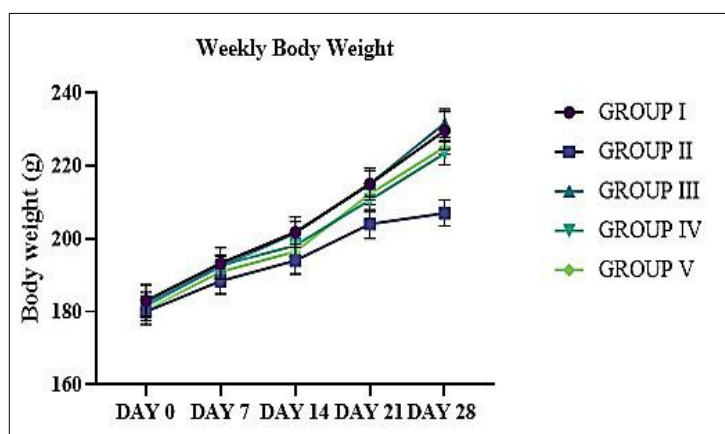
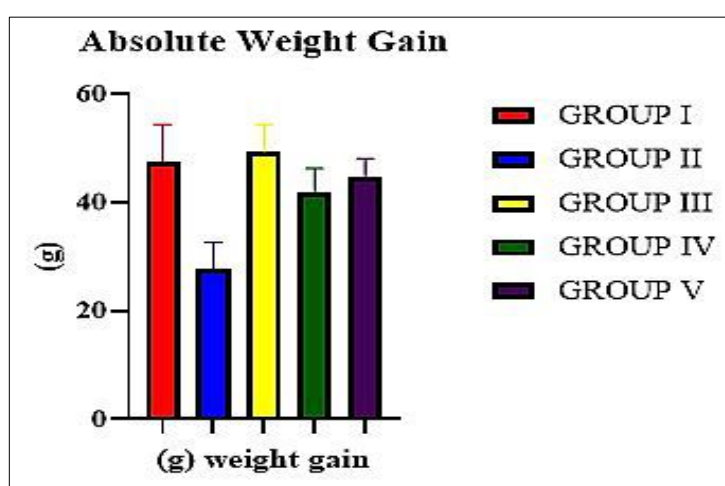
Groups	Serum biochemical parameters			
	ALP (U/L)		UREA (mg/dl)	
	14th Day	28th Day	14th Day	28th Day
Group I	101.52 $\pm$ 3.07 ^b	126.96 $\pm$ 3.09 ^b	26.25 $\pm$ 1.41 ^b	26.27 $\pm$ 0.86 ^c
Group II	164.36 $\pm$ 1.46 ^a	250.39 $\pm$ 1.02 ^a	64.34 $\pm$ 3.78 ^a	78.49 $\pm$ 2.99 ^a
Group III	81.80 $\pm$ 4.30 ^c	71.86 $\pm$ 3.88 ^c	60.74 $\pm$ 1.75 ^a	58.57 $\pm$ 1.55 ^b
Group IV	91.17 $\pm$ 1.66 ^c	78.91 $\pm$ 2.68 ^c	61.86 $\pm$ 1.57 ^a	61.20 $\pm$ 1.04 ^b
Group V	85.49 $\pm$ 2.53 ^c	79.89 $\pm$ 2.44 ^c	63.01 $\pm$ 1.80 ^a	62.470 $\pm$ 1.67 ^b

Note: Mean $\pm$ SEM values of ALP and UREA within the same column bearing different superscripts differ significantly at $p \leq 0.05$

Table 3.3: BUN and Creatinine (mg/dl; Mean $\pm$ SEM) of experimental groups (n=8) of rats

Groups	Serum biochemical parameters			
	BUN (mg/dl)		Creatinine (mg/dl)	
	14th Day	28th Day	14th Day	28th Day
Group I	26.66 $\pm$ 0.80 ^a	25.96 $\pm$ 0.86 ^a	1.19 $\pm$ 0.05 ^a	1.13 $\pm$ 0.03 ^a
Group II	32.49 $\pm$ 1.09 ^c	39.26 $\pm$ 0.72 ^c	1.46 $\pm$ 0.02 ^c	2.05 $\pm$ 0.03 ^d
Group III	27.66 $\pm$ 0.83 ^c	27.43 $\pm$ 0.58 ^{ab}	1.24 $\pm$ 0.03 ^a	1.24 $\pm$ 0.05 ^b
Group IV	28.98 $\pm$ 0.65 ^b	28.14 $\pm$ 0.58 ^b	1.33 $\pm$ 0.02 ^b	1.50 $\pm$ 0.06 ^c
Group V	30.64 $\pm$ 0.92 ^b	29.51 $\pm$ 0.87 ^b	1.26 $\pm$ 0.02 ^a	1.31 $\pm$ 0.07 ^{bc}

Note: Mean $\pm$ SEM values of BUN and Creatinine within the same column bearing different superscripts differ significantly at $p \leq 0.05$

**Fig 1:** Body weights (g; Mean $\pm$ SEM) of experimental groups (n=8) of rats**Fig 2:** Absolute weight gain (g; Mean $\pm$ SEM) of experimental groups (n=8) of rats

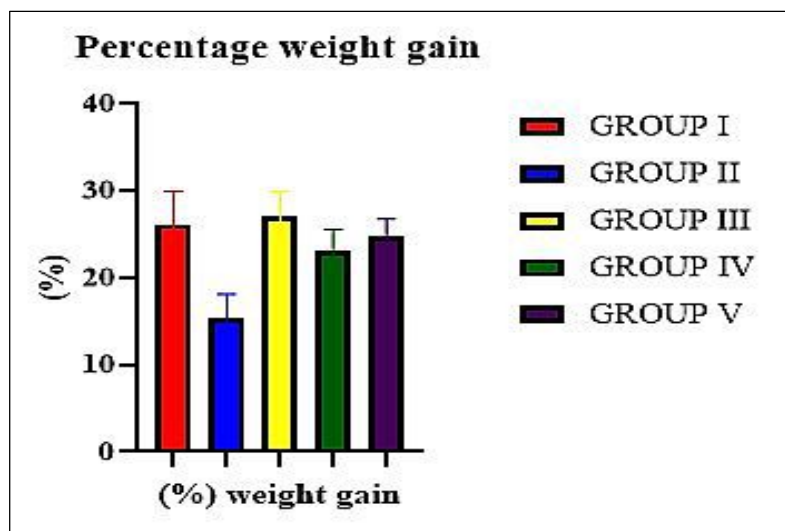


Fig 3: Per cent weight gain of experimental groups (n=8) of rats

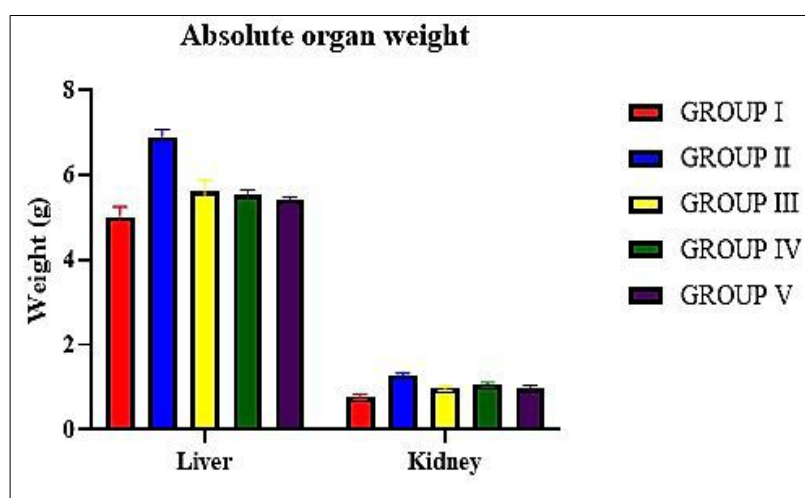


Fig 4: Absolute organ weight (g; Mean $\pm$ SEM) of Liver and Kidney of experimental groups (n=8) of rats

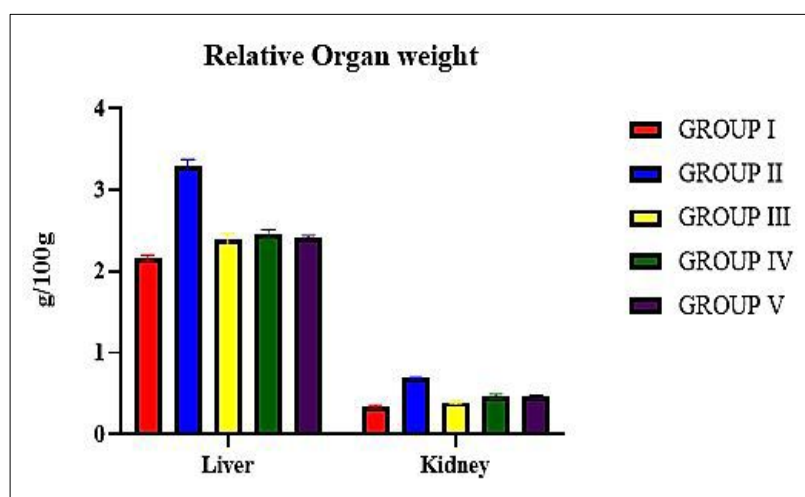


Fig 5: Relative organ weight (g/100g; Mean $\pm$ SEM) of liver and kidney in experimental groups (n=8) of rats

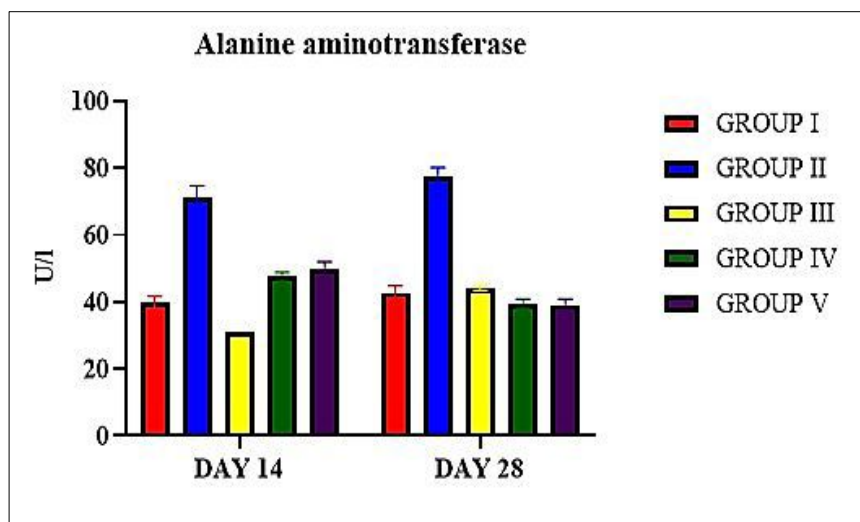


Fig 6: ALT (U/L; Mean $\pm$ SEM) of experimental groups (n=8) of rats

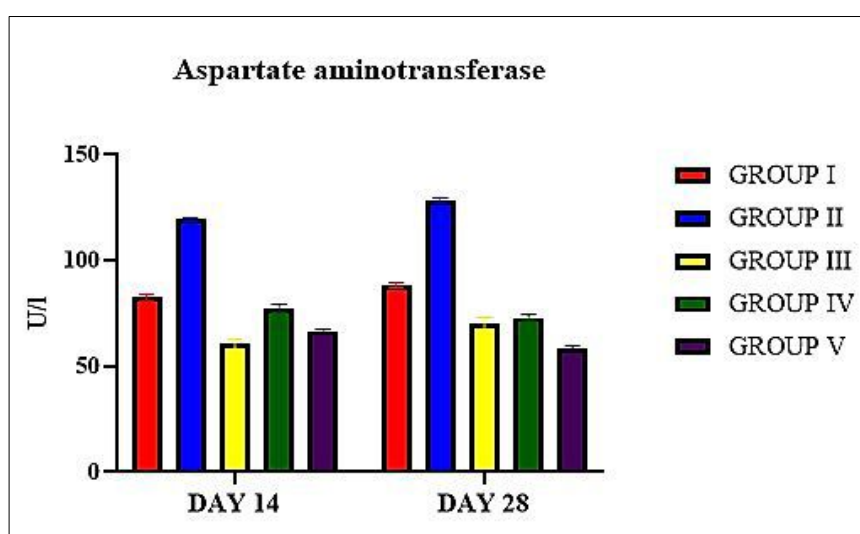


Fig 7: AST (U/L; Mean $\pm$ SEM) of experimental groups (n=8) of rats

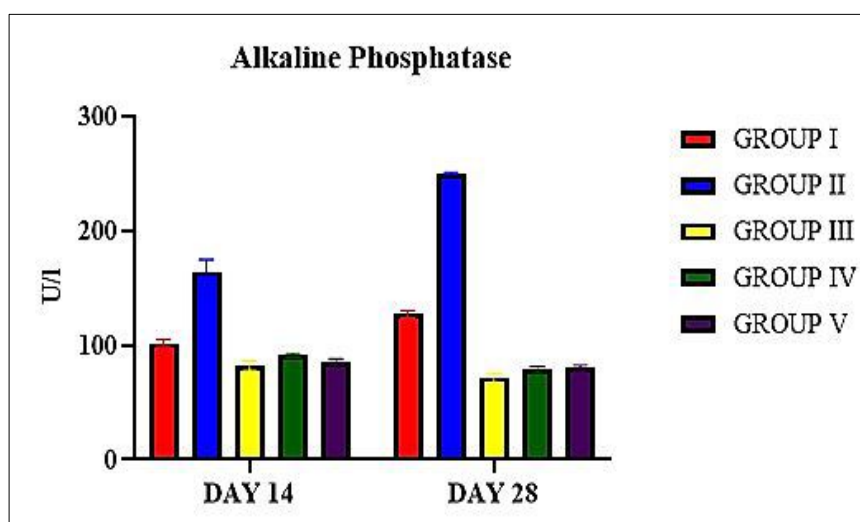


Fig 8: ALP (U/L; Mean $\pm$ SEM) of experimental groups (n=8) of rats

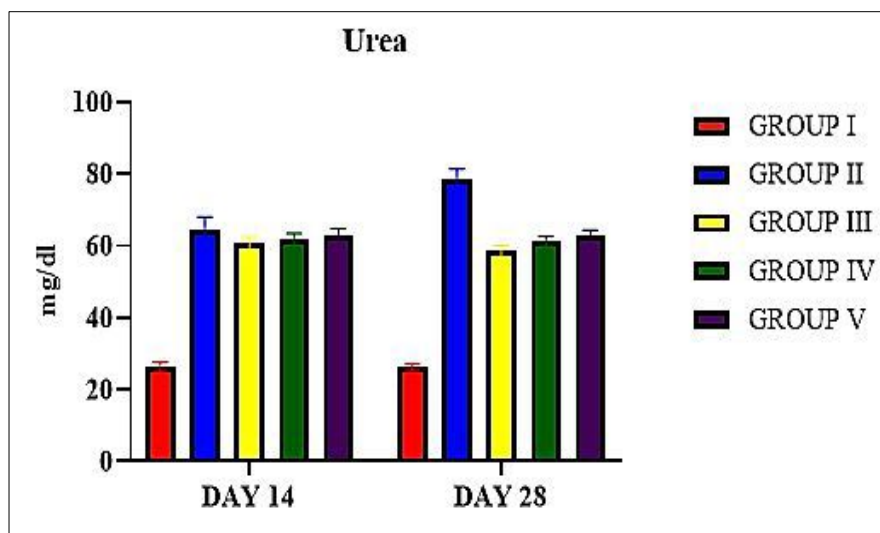


Fig 9: Urea (mg/dl; Mean $\pm$ SEM) of experimental groups (n=8) of rats

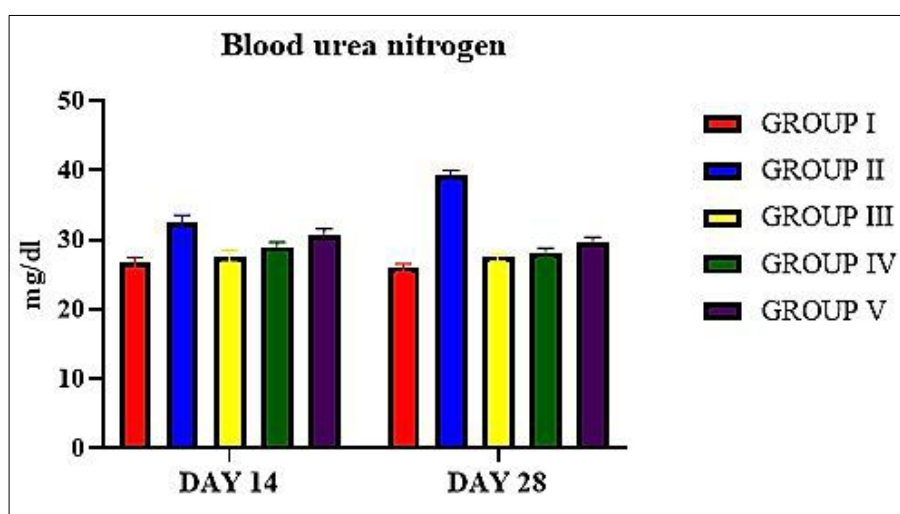


Fig 10: BUN (mg/dl; Mean $\pm$ SEM) of experimental groups (n=8) of rats

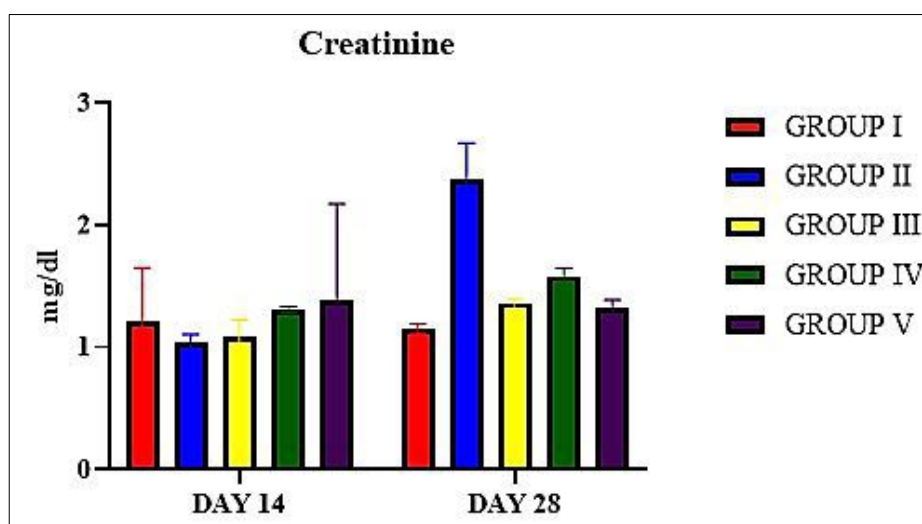


Fig 11: Creatinine (mg/dl; Mean $\pm$ SEM) of experimental groups (n=8) of rats

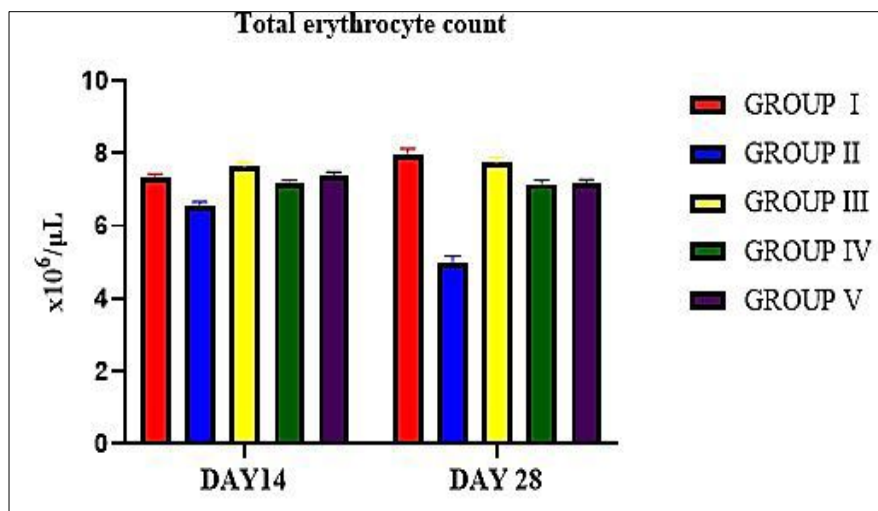


Fig 12: TEC (x10⁶/μL; Mean ± SEM) of experimental groups (n=8) of rats

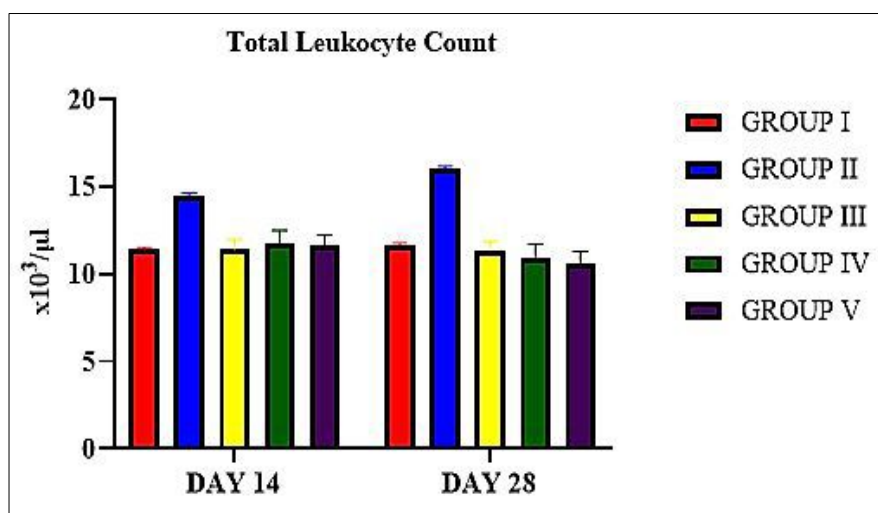


Fig 13: TLC (x10³/μL; Mean ± SEM) of experimental groups (n=8) of rats

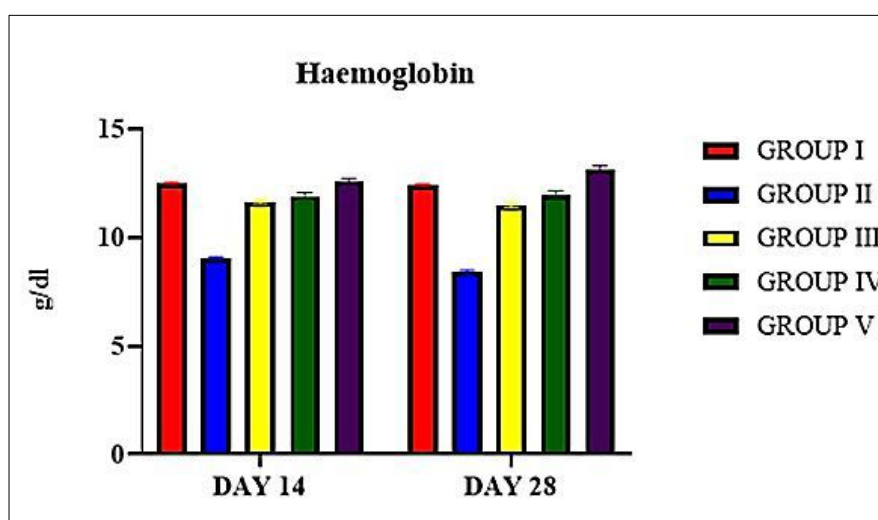


Fig 14: Hb (g/dl; Mean ± SEM) of experimental groups (n=8) of rats

Conclusion

- Sub-acute oral exposure to amitraz caused significant toxic effects in rats, evidenced by reduced body weight gain, altered liver and kidney weights, hematological disturbances (decreased TEC and Hb with increased TLC) with and elevated hepatic and renal biomarkers, indicating hepato- and nephrotoxicity.
- Amitraz exposure resulted in significant increases in serum ALT, AST, ALP, urea, creatinine and BUN levels along with increased relative liver and kidney weights, confirming functional and structural damage to hepatic and renal tissues.
- Co-administration of mangiferin significantly ameliorated amitraz-induced toxicity by improving

body weight gain, normalizing relative organ weights, restoring hematological parameters and reducing elevated liver and kidney biochemical markers.

- The protective effects of mangiferin are likely mediated through its antioxidant and anti-inflammatory properties, including reduction of oxidative stress and inhibition of inflammatory pathways, thereby preserving hepatic, renal and hematological integrity during amitraz exposure.

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