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Abamectin induced toxicity and its amelioration by *Aegle marmelos* in rats

Dishant Aggarwal, Vinod Dumka, Simratpal Saini and Manjinder Sharma

Abstract
Among Pesticides, Abamectin are used worldwide as antiparasitic drug in agriculture as well as veterinary medicines and also shows potential harmful effects. Therefore, the present study was conducted to seen the undesirable effects of exposure to sub-acute toxicity of abamectin (2 mg/kg bw) in female rats for 28 days on neurobehavioral, biochemical and antioxidant activity changes. A hydro-alcoholic extract of *Aegle marmelos* leaves were also tested for its potential to reduce pesticide poisoning. Results showed that marked and significant anxiolytic activity is seen by leaf extract against abamectin induced rats which showed anxiogenic activity. The serum levels of Alanine and Aspartate aminotransferase, Blood urea nitrogen and creatinine is significantly reduced as compared to Abamectin-induced rats, while elevation in total protein was observed. Exposure to abamectin significantly decrease the Superoxide dismutase activity, while show no significantly effect on catalase, reduced glutathione and lipid peroxidation antioxidant activity. *Aegle marmelos* leaves treated rats with abamectin showed marked improvements in antioxidant activity, indicating that *Aegle marmelos* extract bear a potent anxiolytic, hepatoprotective, nephroprotective and antioxidant activity. As a result, the subsequent leaf extract of *Aegle marmelos* as herbal medicine may be advantageous for individuals with liver and kidney impairment, as well as for reversing the damage caused by abamectin exposure.

Keywords: abamectin, *Aegle marmelos,* anxiolytic, hepato-protective, nephro-protective

1. Introduction
Pesticides and agricultural chemicals are the compounds which are used mostly now days all over the world and showed enormous benefits to humanity by reducing pests, increasing food production, and improving health. However, their wide spread and indiscriminate use in crop protection, food preservation, and insect pest management has resulted in acute and chronic poisoning in humans, domestic animals, and wild life, as well as broad environmental harm. Abamectin is an example of a recently produced, widely used pesticide with a high toxicity that is used to control a variety of insects, ants, and plant infections (Zanoli *et al*. 2012) [51]. Abamectin (ABM), an isomer of iverrmectin, is derived from the soil bacterium Streptomyces avermitilis (Campbell *et al*., 1983; Agarwal, 1998), consisting of avermectins mixture having B1a (more that 80%) and B1b (less than 20%) (Meister, 1992; Zeng *et al*., 1996; Agarwal, 1998) [52, 34, 5]. ABM is a macrocyclic lactone disaccharide, widely used as an antiparasitic drug in agricultural and domestic animals (Kolar *et al*., 2008) [28]. Abamectin was used to manage insects and mites in a variety of agricultural goods, including fruits, vegetables, and decorative crops (Campbell, 2012) [15].
A large number of phytochemical constituents present in plants which act as phyto-medicinal agents and proven to cure many diseases (Huang *et al*., 2012; Abdel Rasoul and Marei, 2016) [22, 1]. *Aegle marmelos* (L.) (AM) Corr. belonging to the family Rutaceae, commonly known as Bael, is a Sub-tropical plant with short and thick trunk, soft and flaking bark, that can grown up to an altitude of 1200 m and native to indo-Malayan region (Hooker, 1975) [21] mainly India, Pakistan, Bangladesh, Sri Lanka, Burma, and Thailand (Islam *et al*., 1995) [23]. This plant has also been mentioned in ancient Indian scriptures like Yajurveda and Mahabharata (Asha & Krishan, 2016) [10]. The bael leaves are used to cure wound, leucorrhoea, conjunctivitis, jaundice, deafness, ulcer, cholera, hypoglycemia, asthma, hepatitis and the use of fruit as carminative, astringent, and has good utility in thyroid disorder (Patel *et al*., 2012) [38].
The Bael (AM) has vast therapeutics properties due to presence of mainly alkaloids, cardiac glycoside, saponin, steroids, coumarins (marmelosin, marmesin, marmin, imperatorin,

scopoletin), Limonene, terpenoids (α-Phellandrene) (Asha and Krishan, 2016) [10], phenylpropanoids, tannins (skimmianine), polysaccharides, flavonoids (rutin flavon and flavonols) (Neeraj and Johar, 2017) [36] and α-glucosidase inhibitors (anhydromarmeline, aegelinosides A and B) (Tuticorin and Manakkal, 1983) [48]. Aeglin, rutin, γ-sitosterol, lupeol, marmesinin, β-sitosterol, flavone, glycoside, and phenylethyl cinnamamides are all found in the leaves of *Aegle marmelos*. Shahidine, an alkaloid with an oxazoline core that has been extracted as a main constituent from the fresh leaves of *Aegle marmelos* and exhibited efficacy against a few Gram-positive bacteria (Asha & Krishan, 2016) [10]. Hence the present study was undertaken to evaluate the ameliorative effects of leaf hydro-alcoholic extract of AM on abamectin induced toxicity to female rats.

2. Material and Methods

2.1 Chemicals

The commercial formulation of ABM (Pestanal) was obtained from Sigma-Aldrich chemical Pvt. Ltd., Banglore, India. All other chemicals used were of highest purity grade available from Sigma and Merck Chemical companies.

2.2. Preparation of the hydro-alcoholic leaf extract

Fresh mature leaves of AM were collected from Botanical Garden, GADVASU, Ludhiana, Punjab, India. Immediately after collection, leaves were washed, shade dried at room temperature and grounded into fine powder. 100 gram of powdered leaves was soaked in 1 litre of ethanol (70%) and distilled water (30%) at room temperature for 48-72 h and stirred at frequent time intervals. After maceration, the extracts were first filtered through muslin cloth and then through Whatmann filter paper No. 1 again. The filtrate obtained was evaporated in oven at a temperature of 40 °C. The residue (13.6 ± 0.11 g) obtained was lyophilized and kept at 4 °C in air tight bottles until use.

2.3. Experimental procedure

Adult Female albino rats (Sprague-Dawely), *Rattus norvegicus albinus*, weighting 150-200 g were obtained from IMTECH Centre for Animal Resources and Experimentation (I-care), CSIR-IMTECH, Chandigarh India. Animals were housed in clean transparent plastic cages (28 X 45 X 25 cm) at 25±2 °C with 12 h dark/light photoperiod and 70±10% humidity. Before the studies, the animals were acclimatized to the laboratory conditions for two weeks. The animals were given a balanced commercial diet and water *ad libitum*. Handling of the experimental animals was consistent with the international ethics of the care and use of experimental animals (National Research Council, 2011). Rats were divided into 6 groups of 6 adult females each. Group I received carboxy methyl cellulose (CMC) 1% solution, used as control. Group II was given the hydro-alcoholic leaf extract of AM as the sole drinking fluid at a dose of 200 mg/kg bw and in group III ABM, rats were administered 1/5 LD_{50} (2 mg/kg bw) of ABM (LD_{50} = 10 mg/kg bw, Tomlin, 2004). Group IV (ABM and AM) of rats were given 1/5 LD_{50} of ABM and 200 mg/kg of hydro-alcoholic leaf extracts of AM. Rats were orally administered the doses for 4 weeks. The body weights of rats were recorded initially and finally to calculate relative weight changes according to Chapman *et al*. (1959) [16]. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) vide reference no. GADVASU/2020/IAEC/55/20 dated 31.10.2020 and was conducted in accordance with ethical committee guidelines.

2.4. Neurobehavioural activity

Elevated plus maze is the simplest apparatus to study neuroprotective effect (Latha *et al*., 2015) [31] and anxiolytic response produced by test drug. Rodents dislike high and open spaces and prefer closed arm environment, therefore they spend more time in the closed arm. Animals freeze, become immobile, defecate, and make fear-like gestures when they enter open arm (Pellow *et al*., 1985) [39]. In the EPM, anxiety was expressed by the animal spending more time in the enclosed arm. Time spent in open and closed arms was automatically recorded by the camera attached to the computer through software.

2.5. Blood sampling

Experimental rats were sacrificed using mild diethyl ether anaesthesia 24 hrs after the last treatment/dose. Blood samples were collected directly from the posterior vena cava and heart in tubes containing heparin from all the rats. A measured volume of blood about 1 ml was taken for biochemical parameters and rest of the blood was used for separation of plasma. Heparinized blood samples were centrifuged at 3500 rpm for 15 min and plasma was collected from the top most layers. The extracted plasma samples were then stored at -20 ºC till further analysis.

2.6. Preparation of RBC lysate

The blood was collected in heparinized vials and gently mixed to avoid any clot formation. The blood samples were centrifuged at 3000 rpm for 15 min. Plasma and buffy coat were removed. The resulting erythrocyte pellet was washed thrice with PBS (pH 7.4). The washed erythrocyte pellet was re-suspended in DW; and kept at -20 °C until further analysis. The 1:10 dilution of erythrocyte lysate (10%) was used for estimation of oxidative stress indicators and membrane enzymes. PBS (pH 7.4) was prepared by dissolving NaCl (8 g), KCl (0.2 g), KH_2PO_4 (0.2 g) and Na_2HPO_4 (0.94 g) in about 800 ml of distilled water and the volume was made to 1 litre with distilled water.

2.7. Biochemical analysis

The biochemical assays of enzymes of the serum samples were done using ERBA autopak kits. ALT and AST activities were determined according to Tietz (1986) [45] and Bradley *et al*. (1972) [13], while BUN and creatinine were measured according to methods of Tietz (1976) [44] and Jaffe's detection method. The concentration of total protein was measured using Biuret End Point method on Auto analyzer.

2.8. Assessment of antioxidant enzyme activities

Catalase activity was measured according to the method of Aebi with hydrogen peroxide (30 mM) as the substrate (Aebi, 1984) [3]. The micromoles of H_2O_2 consumed per mg of protein sample were used to determine one unit of catalase activity. Superoxide dismutase (SOD) activity in RBC lysate was determined by the method of Madesh and Balasubramanian (1998) [33]. In a colorimetric assay, the SOD activity was measured at 570 nm. The amount of enzyme required to inhibit the rate of MTT decrease by 50% was defined as one unit of SOD. The results are expressed as unit per mg Hemoglobin.

Reduced Glutathione (GSH) content as a non enzymatic antioxidant was determined according to the method of

Beutler & Kelly (1963) [11]. This method involves the reduction of glutathione with 5, 5' dithiobis (2- nitrobenzoic acid) (DTNB) to produce a yellow compound at 412 nm OD, the reduced chromogen directly proportional to GSH content. The amount of GSH was expressed as micromole glutathione oxidized/ml blood. Lipid peroxidation (LPO) in erythrocyte lysate was performed by the method of Stocks and Dormandy (1971) [42]. MDA, which reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive material to generate a red color complex with a peak absorbance at 532 nm, was used to test lipid peroxidation of RBC homogenate. The values were expressed for lysates as nmoles of MDA produced/g Hb/h using a molar extinction coefficient of pure MDA as 1.56 X 10^5 (Esterbauer *et al*., 1982) [19]. For RBC lysate, the amount of LPO was expressed as nanomoles of MDA produced/g Hb/h

$$\text{LPO (nm MDA gm}^{-1}\text{)} = \frac{\text{OD}}{\text{EC}} \times \frac{\text{Total volume of reaction mixture}}{\text{Amount of sample taken}} \times 10^9$$

Where OD: Optical Density, EC: Extinction coefficient.

3. Results

3.1. Neurobehavioral activity

The results demonstrating the effect of hydro-alcoholic extracts of *A. marmelos* on neurobehavioral activity of rats are given in the Tables 1.

Table 1: Effect on neurobehavioral activity

Treatment	Time spent in open arms(s)	Time spent in closed arms(s)
Group I (CMC)	14.83±2.47^a	285.17±2.47^b
Group II (ABM)	25±2.69^b	275±2.70^a
Group III (AM)	12.5±2.71^a	287.5±2.72^b
Group IV (AM and ABM)	10±1.73^a	290±1.73^b

Values lacking a common superscript in given column differ significantly from each other (p<0.05). Where ABM: Abamectin, AM: *Aegle marmelos* extract

3.2 Body weight

During the study period, the rats did not show any clinical signs of toxicity. Body weights of rats treated with plant extract did not differ significantly control values (Table 2). However, when rats treated with ABM and their combinations, also showed no significant change (p<0.05) in body weight were observed.

Table 2: Effect of abamectin on body weights of rats and the modulation by *A. marmelos*

Treatment	Dose	0 day	28 day	B.w. gain	% wt. gain
Group I (Control)	---------	173±6.73^a	209.5±7.91^a	36.5±11.15^a	16.74±4.53^a
Group II (ABM)	2 mg/kg	188.83±11.67^a	217±10.81^a	28.17±5.09^a	13.10±2.38^a
Group III (AM)	200 mg/kg	185.5±10.46^a	227.17±7.81^a	41.67±4.76^a	18.39±2.26^a
Group IV (AM+ABM)	200 mg/kg + 2 mg/kg	182.67±6.07^a	212.83±5.26^a	30.17±2.17^a	14.24±1.19^b

Each value is a mean of 6 rats ± S.E.; Where ABM: Abamectin, AM: *Aegle marmelos* extract
Means having the same letters are not significant differ from each other, p<0.05;
% of body weight change/week = [(final b.wt.- initial b.wt.)/ initial b.wt.]/ no. of weeks X 100.

3.3. Biomarkers of liver and kidney dysfunctions

The administration of AM extract cause changes in all the determined biochemical parameters compared to untreated rats (Table 3 and Figure 1). The activity of ALT and AST enzymes were utilized as hepatotoxic indicators to determine the damage caused by ABM and the protective impact of AM. Significant increases in liver enzyme activity were seen after 4 weeks of treatment with the tested pesticides, indicating the presence of hepatic damage. When compared to the control group, total protein was considerably lower (P<0.05). However, combining AM extract with ABA enhanced the majority of the biochemical parameters assessed. It was, also, noticed that the BUN and creatinine content was enhanced as a result of administration of ABM (Table 3 and Figure 1) while When AM hydro-alcoholic extract was administrated with ABM to the treated rats, a marked improvement in BUN and creatinine content was observed.

3.4 Antioxidant activities

The antioxidant parameters such as CAT, SOD, GSH and LPO were measured after administration of ABM for 4 weeks (Table 4 and Figure 2).

Table 3: Effect of abamectin on serum biochemical parameters of rats and the modulation by *Aegle marmelos*

Treatment	ALT (U/L)	AST (U/L)	Total proteins (g/dl)	BUN (mg/dl)	Creatinine (mg/dl)
Group I (Control)	37.67±1.89^a	146.5±3.13^a	5.3±0.09^b	17±0.73^a	0.31±0.02^a
Group II (ABM)	46.17±2.00^b	179±3.40^b	4.88±0.94^a	25.17±1.19^c	0.43±0.02^b
Group III (AM)	37±.58^a	145.17±2.12^a	4.97±0.11^a	17.5±0.89^a	0.32±0.02^a
Group IV (AM + ABM)	42.16±0.91^b	151.5±2.53^a	4.93±0.07^a	20.83±0.60^b	0.38±0.03^{ab}

Each value is a mean of 6 rats ± S.E.M; Where ABM: Abamectin, AM: *Aegle marmelos* extract
Means having the same letters are not significantly differ from each other, p<0.05.

Table 4: Effect of abamectin on antioxidant parameters of rats and the modulation by *Aegle marmelos*

Treatment	Catalase (µm of H_2O_2 decomposed/min/mg protein)	SOD (EU/mg Hb)	GSH (µmole/ml)	LPO (nmol MDA/g Hb)
Group I (Control)	19.74±11.35[a]	111.60±2.94[b]	144.67±15.31[a]	1.89±0.33[a]
Group II (ABM)	4.90±1.98[a]	90.63±6.70[a]	98.89±9.67[a]	2.63±0.69[a]
Group III (AM)	11.32±6.15[a]	104.60±6.24[b]	126±21.16[a]	2.06±0.28[a]
Group IV (AM + ABM)	6.12±3.15[a]	98.89±1.80[ab]	111.33±16.43[a]	2.56±0.25[a]

Each value is a mean of 6 rats ± S.E.M; Means having the same letters are not significantly differ from each other, $p<0.05$. Where ABM: Abamectin, AM: *Aegle marmelos* extract

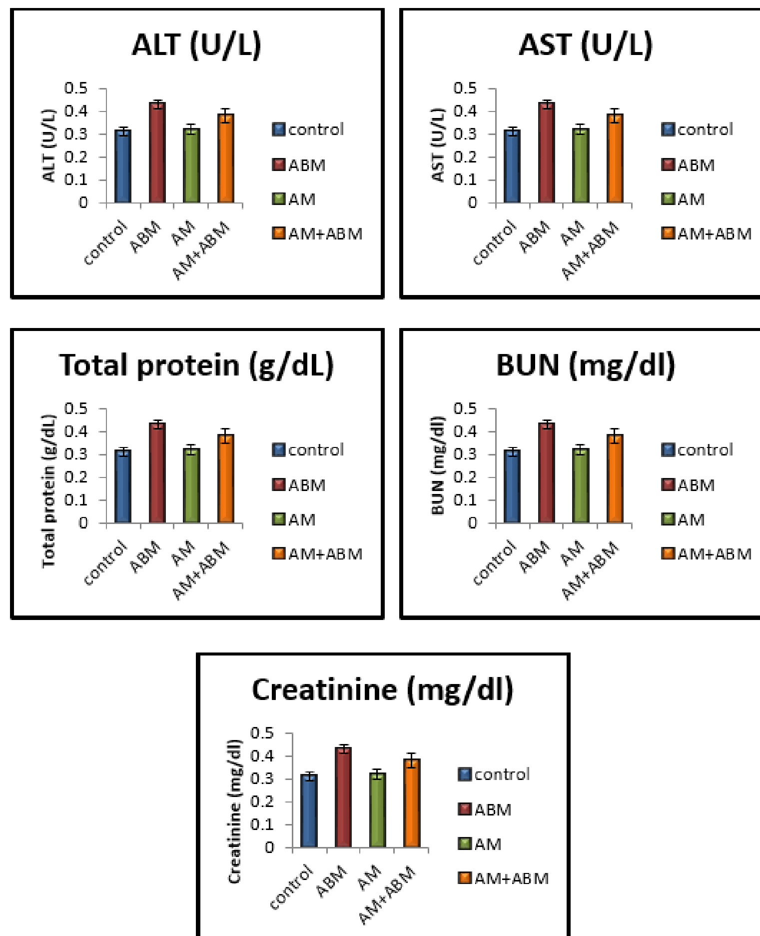

Fig 1: Effect of leaf extract, abamectin and their combination in rats on serum biochemical parameters.

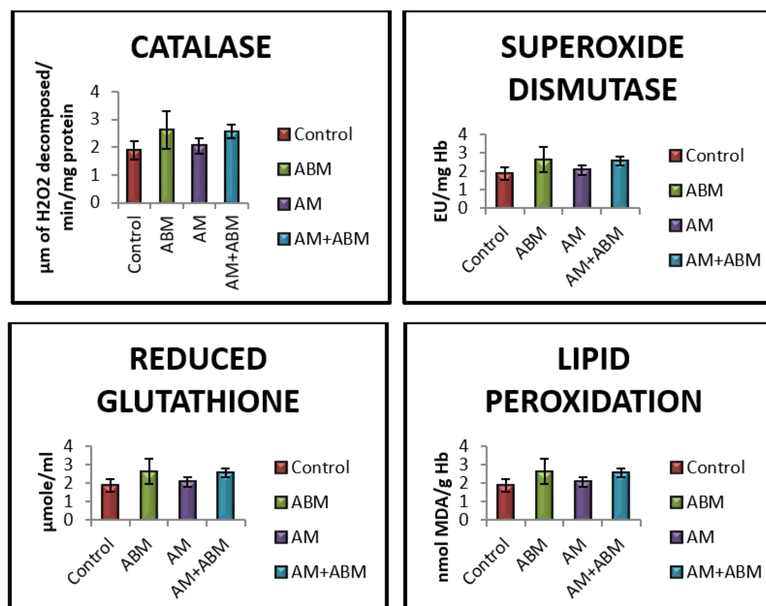

Fig 2: Effect of leaf extract, abamectin and their combination in rats on antioxidant parameter

4. Discussion

4.1. Neurobehavioral activity

The elevated plus maze has been characterized as a simple tool for evaluating rodent anxiety responses by Pellow *et al.* (1985) [39]. The elevated plus maze relies on rats' proclivity toward enclosed spaces (approach) and an unconditioned fear of heights/open spaces (avoidance), also known as thigmotaxis, unlike other behavioral assays used to assess anxiety responses, which rely on noxious stimuli that typically produce a conditioned response.

In the present study, group I served as control. Group II was given ABM @ 2 mg/kg orally and group III was given hydro-alcoholic leaf extract of AM @ 200 mg/kg orally. Group IV was given combination of ABM and hydro-alcoholic leaf extract @ 2 mg/kg and 200 mg/kg orally. Time spent in open and closed arms was recorded automatically with the help of inbuilt overhead camera and software. It was observed that in ABM-induced group, there was a significant change in the time spent in open or closed arms as compared to control group whereas the group which is treated with ABM and hydro-alcoholic leaf extract showed a significant activity as compared to abamectin-induced group which revealed that AM leaf extract has ability to modify the normal thigmotaxic behavior of rodents on sub-acute toxicity with abamectin. In contrary to present study Kothari *et al.* (2009) [29] showed that *A. marmelos* attenuated anxiety parameters in the EPM test. A. marmelos administration at the dose (150 and 300 mg/kg) significantly and dose dependently increased open arm activity by increasing time spent on and number of entries into open arms while decreased risk assessment behaviour by decreasing number of stretch attend posture and head dips. It is concluded that *A. marmelos* possess potential anxiogenic and antidepressant activities.

4.2. Body weight

Abamectin treated group showed a non significant effect on body weight gain as compared to control group, plant treated group (Group II) and group IV which is having both ABM and their combination with AM extract (Table 2). The weight gain in rats and other animal species serve as a positive physiological parameter indicating increase in growth rate and good health whereas decrease in body weight, body weight gain and relative organ weight is used to study pathophysiological state of animals under experimental conditions which may indicate toxicity (Palani *et al.* 1999, Aly *et al.* 2009) [37, 8]. Changes in body weight have been utilized as an indicator of adverse effects of drugs and chemicals (Tofovic and Jackson 1999, Al-Shabanah and Raza 2002, Teo *et al.* 2002) [46, 7, 43]. Abdelrasoul (2018) [2] reported that there is gradual decrease in body weight of rats when treated with abamectin (2 mg/kg) as compard to control group. Our results are in agreement with Chauhan *et al.* (2007) [17] who showed that a little increase in body weight of animals with administration of 50 percent ethanolic leaf extract of *A. marmelos* at dose of 200 mg/kg/day for 60 days as compared to control.

4.3. Biomarkers of kidney and liver dysfunction

The administration of AM extract did not cause any changes in all the determined biochemical parameters compared to control group rats (Table 3 and figure 1). Concerning the determination of damage induced by ABM group and the protective effect of AM, the activities of some enzymes (ALT and AST) were used as hepatotoxic biomarkers. Following 4 weeks of tested insecticides administration, marked significant increases in liver enzyme activities were observed, indicating the occurrence of hepatic injuries. Total protein was significantly decreased ($P<0.05$) compared with control group. However, a administration of AM extract in combination with ABM, improved most of the tested biochemical parameters. Changes in serum on ALT, AST, total protein, BUN and creatinine level in various experimental groups are represented in Figure 1. Liver and kidney are important organs in animal body where detoxification and elimination of toxic materials occur. A foreign body in the form of a chemical stress is sufficient enough to cause severe hepatic and renal dysfunction (Waggas 2013) [49].

Liver plays an important role in the detoxification process for any foreign substances. Consequently, any injury or disturbance of its function results in hepatotoxicity and leading to health complications. Liver biomarker enzymes are commonly used for liver injury. In the present work, AST and ALT enzymes activities exhibited a significant increment with ABM treated rats (Group II). It is well known that increased or decreased serum levels of biomarker enzymes in this study confirm the destruction of the hepatocytes by allowing these enzymes to leak into the circulation. Cellular leakage and loss of hepatocyte membrane integrity were indicated by Sakr (2007) [40]; Khaldoun- Oularbi *et al.* (2013) [26]. This alteration is likely to affect the membrane permeability and provokes derangement in metabolites transport. Elevated liver enzymes are the cause of the muscle damage, hepatic injury and toxic hepatitis (Farkaset *et al.*, 2004) [20]. The high activities of these enzymes could be referred to destruction of cells and the occurrence of damage to tissues and the accumulation of triglycerides and release enzymes into the blood stream, which have a clear indication of the emergence of necrosis cell of liver tissue (Alhazza, 2007) [6]. The decreased serum level of total proteins due to ABM treated rats reflects the liver toxicity as reported by Mudaraddi *et al.* (2012) [35]. Liver is a major organ of protein synthesis and any disease in the liver can cause damage of hepatocytes with changes in protein and free amino acid metabolism leading to decreases synthesis and increase wasting via catabolism (Wallace, 2007; El- Shafey *et al.*, 2011) [50, 18]. The hydro-alcoholic leaf extract of AM showed a similar activity as compared to the control group, there’s no significance change in the activity. However, the group IV (AM with ABM) showed a significance decrease in activity of enzymes which indicates the plant having protective activity on liver enzymes (Table 3). In agreement to our study, increase in the activity of hepatic markers also reported by Abdelrasoul (2018) [2] who concluded that abamectin has effect on hepatic injuries. Jayachandra and Sivaraman (2011) [24] showed that the extract of *A. marmelos* leaves at the dose of 100 mg/kg significantly decreased the activity of ALT, AST, alkaline phosphatase, bilirubin and uric acid as compared to control group. Eugenol extracted from bael leaves has been found to exhibit the hepatoprotective activity by Sherwood and Toliver-Kinsky (2004) [41].

The level of BUN and creatinine was increased significantly in abamectin treated rats as compared to control group (Table 3, Fig. 1). Urea is the most common end product of protein metabolism, accounting for the majority of the blood's non-protein nitrogen component. Urea is made in the liver and discharged in the urine via the kidneys. Impaired kidney function, liver disorders, and diseases that impair renal

function all have elevated serum urea concentrations. Creatinine is a waste product generated in muscle from creatine phosphate, a high-energy storage molecule. Creatinine production is mostly a function of muscle mass and is rather steady (unlike urea). It is eliminated from plasma by glomerular filtration and then discharged in urine without significant tubule reabsorption. The level of creatinine in the blood is a good measure of renal function. The rats which were treated with leaf extracts showed activity similar to control group but the groups which were treated with plant extracts and abamectin in combination showed significantly decrease effect as compared to abamectin-induced rats which indicates the extracts having a protective activity. Our results are in agreement with Birudu *et al*. (2020) [12] who showed that the high doses of methanolic leaf extract of A. marmelos can be used in the treatment of diabetes and its complications through the biochemical changes such as blood glucose, AST, ALT, total protein, creatinine, serum albumin and ALP. They showed reduction in the level of BUN as compared to control group.

4.4. Antioxidant activities

ABM exposure for 4 weeks intoxicated rats group caused statistically non-significant elevation of LPO ($p \leq 0.05$), significant reduction of SOD, although non-significant reduction ($p \leq 0.05$) in the levels of GSH and CAT were observed in RBC lysate as compared to control group (Fig. 2; Table 4). It was observed that concurrent dose of AM leaf extract (200 mg/kg b.w.) non-significantly reversed ($p \leq 0.05$) the altered LPO, GSH and CAT levels, however significant reversal was observed in the levels of SOD ($p \leq 0.05$) levels in comparison to toxicant group (Fig. 2). The results indicate that pharmacological action of *A. marmelos* dose (200 mg/kg b.w.) was not much effective against ABM insecticide induced oxidative stress.

Metabolism of ABM in liver results in the production of reactive metabolites that cause per-oxidative damage to membrane lipid, oxidation of protein and shift the equilibrium between oxidant and antioxidant towards oxidation. The enhanced level of lipid peroxidation usually shows an indication of the failure in antioxidant defense mechanism. Insecticides may induce oxidative stress which causes generation of free radicals and alteration in antioxidants, oxygen free radicals, the scavenging enzyme system and lipid peroxidation and contributes to the toxicity (Afolabi *et al*., 2019) [4]. Excess production of reactive oxygen species (ROS) leads to alterations in the cellular antioxidant defense system which consequently effect the susceptibility to oxidative stress as one the main mechanisms of the action of many of the insecticides (Lopez *et al*., 2007) [32].

Reduced glutathione is non-enzymatic antioxidant, which neutralize H_2O_2 into the cell. It plays an important role in intracellular protection against toxic compounds, such as reactive oxygen intermediates and other free radicals (Anderson and Luo, 1998) [9]. Continuous intracellular oxidative stress causes depletion of GSH which leads to reactive oxygen species oxidizing and damaging lipids, lipid bilayer, proteins, and DNA (Kaplowitz, 2000) [25]. It acts as a free radical scavenger and can scavenge hazardous metabolites with the help of the GST and CAT-SOD pair system. Superoxide is normally produced as a by-product of oxygen metabolism, but with oxidative stress, it is produced in excess. Superoxide dismutase is an antioxidant enzyme that catalyses the dismutation of superoxide (O^{2-}) into oxygen molecule or hydrogen peroxide. Conversely, catalase (CAT) nullifies the effect of hydrogen peroxide (H_2O_2) by the formation of oxygen and water from H_2O_2. CAT is present in the peroxisomes and mitochondria of the cell as a soluble and membrane bound form. High level of CAT activity indicates the presence of huge amount of peroxisomes in kuppfer cells.

In present study, it was observed that ABM administration for 4 weeks successfully induced hepatotoxicity in rats and pharmacological action of *A. marmelos* at dose of 200 mg/kg b.w. was non-significantly effective against ABM induced these oxidative insults. Hence, oxidative stress in hepatocytes leads to alteration in enzymatic activity and cellular defense mechanism which can be destroyed by the administration of *A. marmelos* along with ABM.

In agreement to present study, Khanna *et al*. (2010) [27] concluded that the methanolic extract of *A. marmelos* at the concentration of 100 and 500 mg/kg for three weeks given orally showed no significant changes in catalase activity as compared to control there by showing its antioxidant activity. Subsequently myocardium injury was induced by subcutaneous administration of isoproterenol (ISO) (150 mg/kg) for two consecutive days and showed high and low doses of *A. marmelos* leaf extract (AMLE) caused significant elevation in SOD and CAT activities compared to ISO control. Hence it is concluded that the low dose of AMLE was less effective than the high dose of AMLE. Also, Kumar and Balasubramanian (2011) [30] studied the hepatoprotective activity of *A. marmelos* against alcohol-induced oxidative damage in rats and showed significant decrease in activity of GPx, GSH, CAT and SOD in ethanol-intoxicated rats whereas in *A. marmelos* extract-induced group showed a increased activity of GPx, GSH, CAT and SOD as compared to ethanol-intoxicated rats

5. Conclusion

The present investigation indicated that *A. marmelos* has a potential role in therapeutic action via the presence of phytochemical compounds and its antioxidant nature. However, further investigations are essential to explicate the accurate molecular mechanism of specific bio-active agents from AM leaves for protection against abamectin induced toxicity and it should be tested against various biologically important markers. Overall, it can be concluded that AM leaves bears anxiolytic, hepatoprotective, nephroprotective and antioxidant activity. Finally, the obtained results confirmed the potential ameliorative effects of AM on ABA-induced toxicity on rats.

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