Popular Article

Trends in Agriculutre Science ISSN:2583-7850

Vol.2 Issue 7 July 2023, Page: 616-625

Analysis of serum biochemical parameters in imidacloprid toxicity and assessment of effect of *Withania Somnifera* against it in female rats

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https://doi.org/10.5281/zenodo.8370761

Abstract

48 female *Wistar* rats were used in present experimental study which was conducted for a duration of 30 days to analyze the biochemical changes in serum due to imidacloprid induced toxicity and also to evaluate the ameliorative role of *Withania Somnifera* against it. The rats were divided into equal number of following groups. Group 1 was control, daily group 2 rats were orally gavaged with imidacloprid (30 mg/kg body weight), for group 3 rats the *Withania Somnifera* was mixed in feed (1g/kg feed) and group 4 rats were gavaged with imidacloprid and also given with *Withania Somnifera* in feed (dose as above). On 16th day of experiment, blood samples were collected from half of the rats in each group and from remaining rats blood was collected on 31st day from retro orbital plexus of rat eye. Serum samples were separated and analyzed for various parameters. There was a significant ($P<0.05$) decrease in total protein, AChE levels and a significant ($P<0.05$) increase in ALT, AST, ALP, BUN, creatinine levels in imidacloprid treated rats when compared to control rats. Whereas group 4 revealed a significant ($P<0.05$) decrease in all the above parameters except TP and AChE which were significantly ($P<0.05$) increased in comparison to group 2 rats on 16th and 31st day of experiment. The simultaneous treatment of rats with both i.e. *Withania Somnifera* and imidacloprid provided mild to moderate improvement in serum biochemical parameters which indicates that *Withania Somnifera* acts as a good ameliorating agent against the toxic effects of imidacloprid.

Key words: AChE, ALT, AST, BUN, Imidacloprid, *Withania somnifera*

Introduction

Imidacloprid (IMI) belongs to an insecticide group called neonicotinoid and is widely used in agriculture to protect the various crops from insects. IMI is similar to nicotine and it activates the α subunits of nicotinic acetylcholine receptors (nAChRs) in insects[1]. IMI is highly efficient for soil application due to its mobility from the roots to the upper parts of the plants[2].

Due to excessive usage of imidacloprid on crops it leads to its persistence in the environment and produce adverse effects in animals and humans.

Withania somnifera (WS) is a potential medicinal Indian herb used in ayurveda for more than 3000 years. WS belongs to Solanaceae family, also called as ashwagandha, Indian ginseng and winter cherry[3]. The WS is used to treat many pathological conditions in human being such as joint inflammation, reproductive failure, amnesia, anxiety, tumours, cardiovascular and neurodegenerative disorders[4].

In current experimental work, the alterations in serum biochemical parameters induced due to toxic action of IMI when it was given through oral gavage in female *Wistar* rats were studied and it's amelioration by WS was also analyzed.

Material and methods

In the present study approximately 200-250 gm weighing female albino *Wistar* rats in forty- eight number were brought from Sanzyme Private Limited (Pvt. Ltd.), Gagan Pahad, Hyderabad. They were kept in polypropylene cages. Sterile rice husk was used as a bedding material in cages. In lab animal house, the room temperature was maintained at 20-22^{0}C, the rats were given with standard pellet diet and sterile water adequately throughout the experimental period. The rats were allowed to acclimatize for one week before the start of experiment. The rats were divided into four groups each containing 12 animals. Group 1 was control, group 2 was imidacloprid control (orally gavaged with imidacloprid at the rate of 30 mg/kg body weight/day), group 3 was *Withania somnifera* control (treated with *Withania somnifera* at the rate of 1gr/kg feed), group 4 was administered with both imidacloprid and *Withania somnifera* (dose as above) for a period of 30 days. All the rats were kept under observation for occurrence of any clinical signs and deaths during the entire experimental duration. Institutional Animal Ethics Committee was approved the current study (IAEC-No.7/22/C.V.Sc., Hyd. /IAEC) and all the regulations of IAEC were strictly followed during the experiment.

Imidacloprid was purchased from Tropical Agrosystem India Pvt. Ltd., Chennai, Tamilnadu and *Withania somnifera* was brought from Herboleaf Organic, Haryana. Other chemicals were procured from Qualigens Pvt. Ltd., and Sisco Research Laboratory (SRL) Pvt. Ltd., Mumbai. Blood samples were collected from six rats in each group on 16th day and from remaining six rats on 31st day of experiment. From the retro orbital plexus of rat eye, blood was collected by using capillary tubes. Approximately 2 mL of blood was collected from each rat into serum vaccutainers{(Vit K- coated- clot activator tube-plain 13mm x 75mm, 5mL) (Rapid Diagnostics Pvt. Ltd., Delhi)} and allowed to clot for 3 to 4 hours, later centrifuged

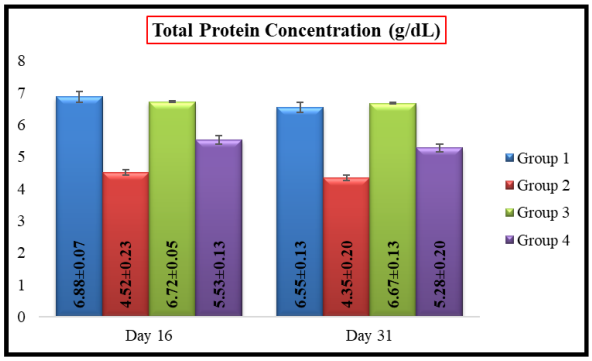
(Sigma 1-13- bench top laboratory centrifuge, USA) at 2000 rpm for 10 minutes; serum was separated into Eppendorf tubes and stored at -20^0 C. Serum biochemical analysis was done in auto biochemistry analyzer (Prietest touch– Robonik India Pvt. Ltd., Navi Mumbai) by using Transasia biochemical kits (Transasia biomedical Ltd., Solan, Himachal Pradesh). Standard Biuret procedure was used to estimate the total protein (TP)[5]. International Federation of Clinical Chemistry (IFCC) method was carried out for analysis of Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP)[6]. Evaluation of Blood urea nitrogen (BUN) was done as per the Glutamate dehydrogenase (GLDH) – Urease method[7]. Alkaline picrate technique[8] by using modified Jaffes reaction was employed to determine the levels of serum creatinine. Acetyl cholinesterase (AChE) was estimated by using chemical method[9]. Statistical analysis of the data was done by using Statistical Package for Social Sciences (SPSS) version 20.0 by applying one way ANOVA. Differences between the means was tested by using Duncan's multiple comparison test and significance level was set at $P<0.05$.

Results

Clinical signs and mortality; The rats of groups 1 and 3 remained healthy throughout the experiment. Group 4 rats also did not manifest any clinical signs. The rats of group 2 showed clinical signs like dullness, restlessness, reduced feed and water intake, salivation and mild diarrhoea during the 3rd and 4th week of experiment. No mortality was noticed in all the experimental groups during the entire period of study.

Total Protein Concentration (g/dL): The mean values of serum TP concentration (g/dL) were significantly ($P<0.05$) low in group 2 (4.52 ± 0.23 and 4.35 ± 0.20) and group 4 (5.53 ± 0.13 and 5.28 ± 0.20) when compared with group 1 (6.88 ± 0.07 and 6.55 ± 0.13) and group 3 (6.72 ± 0.05 and 6.67 ± 0.13) on 16th and 31st day of experiment respectively. The mean values of serum total protein were significantly ($P<0.05$) high in group 4 when compared with group 2 and there was no significant difference between groups 1 and 3 (Fig. 1).

Fig 1. Total Protein Concentration (g/dL) in different groups.

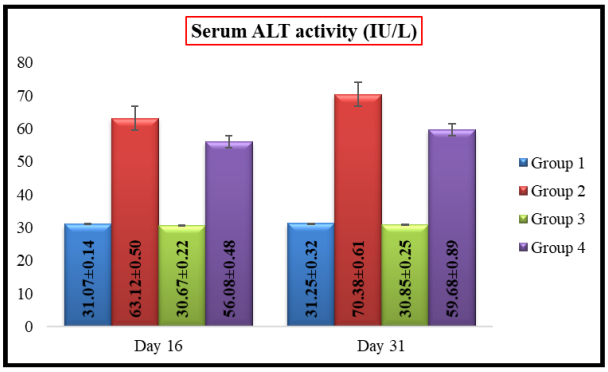


Values are Mean + SE (n = 6); One way ANOVA

Means with different superscripts in a column differ significantly at P<0.05.

Alanine Transaminase (IU/L): Significantly (P<0.05) increased mean values of serum ALT activity (IU/L) was recorded in group 2 (63.12 ± 0.50 and 70.38 ± 0.61) and group 4 (56.08 ± 0.48 and 59.68 ± 0.89) when compared with group 1 (31.07 ± 0.14 and 31.25 ± 0.32) and group 3 (30.67 ± 0.22 and 30.85 ± 0.25) on 16th and 31st day of experiment respectively. The mean value of ALT activity was significantly (P<0.05) decreased in group 4 when compared with group 2 and there was no significant difference between groups 1 and 3 (Fig. 2).

Fig 2. Serum ALT activity (IU/L) in different groups.

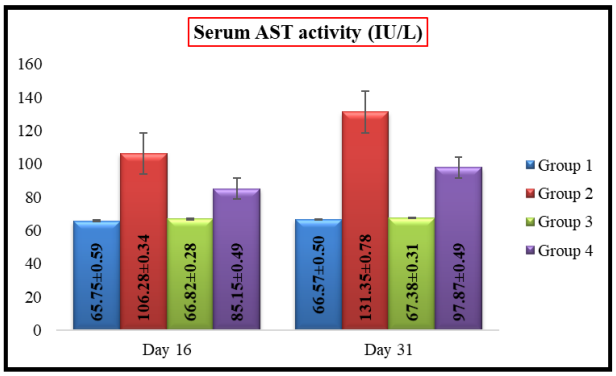


Values are Mean + SE (n = 6); One way ANOVA

Means with different superscripts in a column differ significantly at P<0.05.

Aspartate Transaminase (IU/L): The mean values of serum AST activities (IU/L) in group 2 (106.28 ± 0.34 and 131.35 ± 0.78) and group 4 (85.15 ± 0.49 and 97.87 ± 0.49) were significantly (P<0.05) increased when compared with group 1 (65.75 ± 0.59 and 66.57 ± 0.50) and group 3 (66.82 ± 0.28 and 67.38 ± 0.31) on 16th and 31st day of experiment respectively. In group 4, there was a significant (P<0.05) decrease in AST activity in comparison to group 2 and group 3 value was insignificant from control (Fig. 3).

Fig 3. Serum AST activity (IU/L) in different groups.

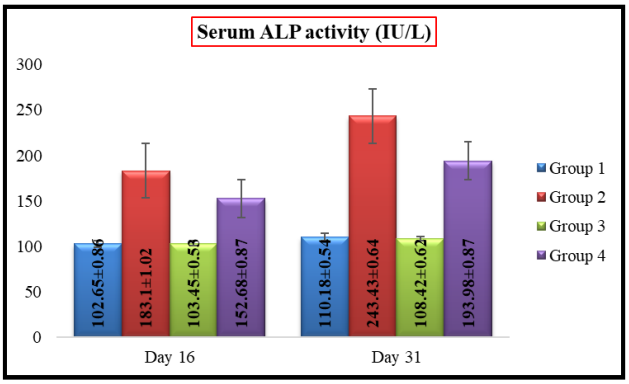


Values are Mean + SE (n = 6); One way ANOVA

Means with different superscripts in a column differ significantly at P<0.05.

Alkaline Phosphatase (IU/L): The mean values of serum ALP activities were significantly (P<0.05) high in group 2 (183.10 ± 1.02 and 243.43 ± 0.64) and group 4 (152.68 ± 0.87 and 193.98 ± 0.87) when compared with group 1 (102.65 ± 0.86 and 110.18 ± 0.54) and group 3 (103.45 ± 0.53 and 108.42 ± 0.62) on 16th and 31st day of experiment respectively. The mean value of ALP activity was significantly (P<0.05) low in group 4 when compared with group 2 and there was no significant difference between groups 1 and 3 (Fig. 4).

Fig 4. Serum ALP activity (IU/L) in different groups.

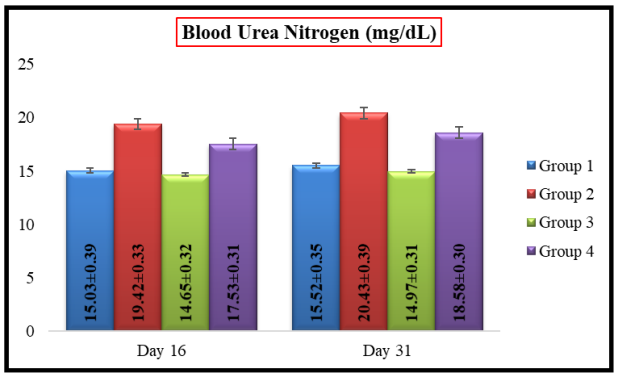


Values are Mean + SE (n = 6); One way ANOVA

Means with different superscripts in a column differ significantly at P<0.05.

Blood Urea Nitrogen (mg/dL): Significantly (P<0.05) increased mean values of BUN (mg/dL) was recorded in group 2 (19.42 ± 0.33 and 20.43 ± 0.39) and group 4 (17.53 ± 0.31and 18.58 ± 0.30) when compared with group 1 (15.03 ± 0.39 and 15.52 ± 0.35) and group 3 (14.65 ± 0.32 and 14.97 ± 0.31) on 16th and 31st day of experiment respectively. The mean value of BUN was significantly (P<0.05) decreased in group 4 when compared with group 2 and there was no significant difference between groups 1 and 3 (Fig. 5).

Fig 5. Blood Urea Nitrogen (mg/dL) in different groups.



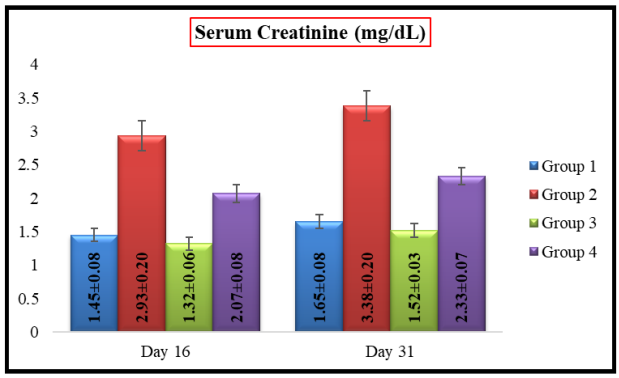
Values are Mean + SE (n = 6); One way ANOVA

Means with different superscripts in a column differ significantly at P<0.05.

Serum Creatinine (mg/dL): The mean values of serum creatinine (mg/dL) in group 2 (2.93 ± 0.20 and 3.38 ± 0.20) and group 4 (2.07 ± 0.08 and 2.33 ± 0.07) were significantly (P<0.05)

increased when compared with group 1 (1.45 ± 0.08 and 1.65 ± 0.08) and group 3 (1.32 ± 0.06 and 1.52 ± 0.03) on 16th and 31st day of experiment respectively. In group 4, there was a significant (P<0.05) decrease in serum creatinine in comparison to group 2 and group 3 value was insignificant from control (Fig. 6).

Fig 6. Serum Creatinine (mg/dL) in different groups.

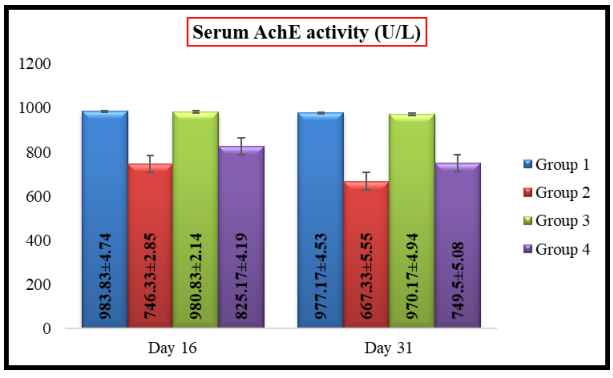


Values are Mean + SE (n = 6); One way ANOVA

Means with different superscripts in a column differ significantly at P<0.05.

Serum Acetyl cholinesterase AchE (U/L): The mean value of serum acetyl cholinesterase activity (U/L) was significantly (P<0.05) low in group 2 (746.33 ± 2.85 and 667.33 ± 5.55) and group 4 (825.17 ± 4.19 and 749.50 ± 5.08) when compared with group 1 (983.83 ± 4.74 and 977.17 ± 4.53) and group 3 (980.83 ± 2.14 and 970.17 ± 4.94) on 16th and 31st day of experiment respectively. The mean value of serum acetyl cholinesterase activity was significantly (P<0.05) high in group 4 when compared with group 2 and there was no significant difference between groups 1 and 3 (Fig. 7).

Fig 7. Serum AchE activity (U/L) in different groups.



Values are Mean + SE (n = 6); One way ANOVA

Means with different superscripts in a column differ significantly at P<0.05.

Discussion

Toxicity of various xenobiotics can be routinely assessed by estimation of TP in serum. A significant (P<0.05) reduction in TP levels were observed in IMI treated rats when compared with group 1 on 16th and 31st day of experiment. These findings are in conformity with the

findings of[10-16]. Contrary to this, a significant augmentation in TP levels were reported by[17-21], whereas no significant change in TP values were recorded by[22,23]. The reduction in TP levels may be due to the decreased intra cellular protein (albumin) synthesis in liver due to IMI induced hepato cellular damage. Hypothesis for decrease in serum total proteins may also be due to diversion of majority of globulins for the synthesis of antibodies against IMI. In addition, the IMI induced anorexia and renal damage might have resulted in production loss and excretory loss of proteins in group 2 rats.

The serum ALT, AST and ALP levels were significantly ($P<0.05$) increased in group 2 rats on 16th and 31st day of experiment when compared to control group rats. They were similar to that of previous authors findings[24-28]. Detoxification of xenobiotics is mainly done in liver and serum ALT and AST are considered as the most sensitive biomarkers to assess the liver function[29]. The increased levels of hepatic enzymes (ALT, AST and ALP) could be due to enhanced production of reactive oxygen species (ROS) due to IMI toxicity led to degeneration and necrosis of hepatocytes, might have resulted in loss of integrity of hepatic cell membranes and lead to release of these enzymes from cytoplasm of hepatocytes into the blood stream. The increased ALP activity may be attributed to biliary damage and degeneration of bile duct epithelial cells which might have synthesized more amount of ALP thereby released into circulation. Group 4 rats showed a significant ($P<0.05$) increase in serum TP and decrease in AST, ALT and ALP activities than the group 2 rats, which might be due to the initiation of repair and regeneration mechanism of hepatocytes by the potential alleviating inflammatory action of WS. These findings are inaccordance with[30] in layer chicken.

Blood urea nitrogen (BUN) and creatinine are known bio-markers of renal function and their alterations in serum indicates the impairment of kidney. A significant ($P<0.05$) increase in BUN and serum creatinine levels were observed in group 2 rats in comparison to control rats suggests the nephrotoxicity due to excess production of ROS by IMI. Kidneys are rich in polyunsaturated fatty acids, they are more prone to membrane lipid peroxidation by IMI may be resulted in degeneration of glomeruli and tubules and lead to decreased glomerular filtration rate. These findings are supported by the findings of[11,16,17,21,25]. Contrary to this[23] noticed insignificant change in concentrations of BUN and serum creatinine in IMI toxic animals. In group 4 rats, a significant ($P<0.05$) decrease in BUN and serum creatinine levels were observed in comparison to group 2. This significant change might be due to marked regeneration of functional units of kidneys due to natural antioxidant and free radical quenching activity of WS. Similar findings were observed by[30] in layer chicken.

The serum acetyl cholinesterase (AChE) activity was significantly ($P<0.05$) reduced in group 2 on 16th and 31st day of experiment when compared to group 1 rats. These results are in

agreement with previous findings[11,13,17,18,25]. Contrary to this[22] reported no significant difference in plasma AChE activity in IMI treated layer chicken. Liver is the major site for production of plasma AChE and the decrease in plasma AChE activity could be due to IMI induced hepatotoxicity. In comparison with group 2, a significant ($P < 0.05$) increase in serum AChE levels were observed in group 4 and it might be due to WS protective action against ROS and inflammatory activity of IMI.

Conclusion

In present study treatment with IMI in rats resulted in marked alterations in biochemical parameters in serum. It could be due to IMI induced hepato and renal toxicity. Whereas, mild to moderate amelioration was noticed in all the above parameters due to co-administration of WS along with the IMI which indicates the protective role of WS against the toxic effects of IMI.

Acknowledgement

We are thankful to the Dean, Faculty of Veterinary Science, P. V. Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, Telangana, INDIA providing the facilities to do the research work

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