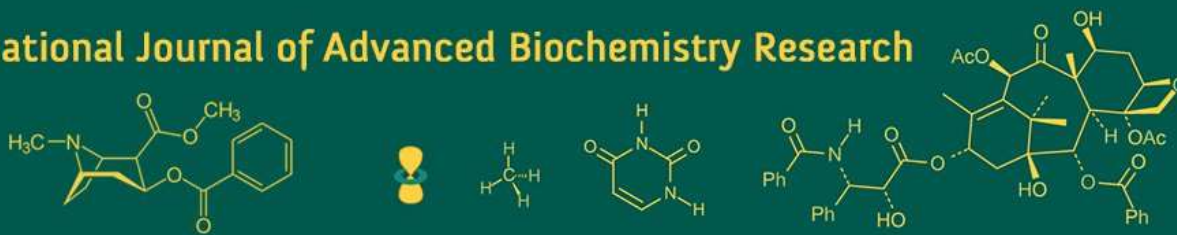


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Imidacloprid induced histopathological changes in kidney and ameliorative role of *Withania somnifera* in female rats

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Abstract

In present experiment, the histopathological alterations in kidney caused due to oral administration of imidacloprid were studied and role of *Withania somnifera* in alleviating the renal damage was estimated. The female rats were used in the study were forty eight and they were grouped into 4 categories. Group 1 was control, Group 2 was imidacloprid control (30mg/Kg Body weight/Day/Oral), Group 3 was *Withania somnifera* control (1g/Kg feed) and Group 4 was treated with both imidacloprid and *Withania somnifera*. The kidney tissue samples were collected at the end of experiment to analyze the renal function. A significant ($p < 0.05$) reduction in activities of glutathione, super oxide dismutase and a significant ($p < 0.05$) elevation in thio barbituric acid reactive substances levels were observed in kidneys of imidacloprid treated rats. Microscopically, significant pathological changes were noticed in renal sections of group 2 rats whereas *Withania somnifera* as a nephroprotective molecule significantly reduced the renal damage and restored the renal function on imidacloprid induced nephrotoxicity.

Keywords: Histopathology, imidacloprid, oxidative stress, renal toxicity, *Withania somnifera*

Introduction

Neonicotinoids are the most widely used newer class of insecticides due to its lower mammalian toxicity and highly selective insecticidal activity. Neonicotinoids are broad spectrum and systemic insecticides with rapid action. They are applied as sprays to treat the seed and soil (Tomizawa and Yamamoto, 1993) [25]. Imidacloprid (IMI) is one of the neonicotinoid insecticide having high insecticidal activity at very low application rate (Kidd and James, 1994) [14]. It is used either through oral or through dermal route to effectively control sucking insects (Cordone and Durkin, 2005) [8]. IMI inhibits the nicotinic acetylcholine receptors in the nervous system resulting in impairment of normal nerve function (Liu and Casida, 1993; Zwart *et al.*, 1994) [16, 29]. IMI is having higher binding strength to insect nicotinic acetylcholine receptors than mammalian receptors which indicates the selective toxicity of it towards insects (Zwart *et al.*, 1994) [16].

Withania somnifera (WS) is a potential medicinal Indian herb used in ayurveda for more than 3000 years. Various experimental studies have shown the ameliorative role of WS against the renal toxicity induced by gentamycin (Jeyanthi *et al.*, 2014; Prem Kumar Govindappa *et al.*, 2019) [12, 23] due to presence of its bioactive compounds. The present study was designed to understand the nephrocurative role of WS against the IMI induced nephro toxicity.

Materials and Methods

In the present study, forty-eight (48) female albino rats (Wistar strain) weighing 200-250 g were procured from Sanzyme Private Limited (Pvt. Ltd.), Gagan Pahad, Hyderabad. The rats were housed in solid bottom polypropylene cages at lab animal house, College of Veterinary Science, Rajendranagar, Hyderabad and were maintained in controlled environment (Temperature 20- 22 °C) throughout the course of the experiment. Sterile rice husk was used as a bedding material. All the rats were provided with standard pellet diet (procured from Vyas Labs, Uppal, Hyderabad) and deionized water ad libitum throughout the experimental period.

All the experimental animals were observed thrice daily for clinical signs and mortality, if any, during the entire period of study. The experiment was conducted according to the guidelines and with prior approval of the Institutional Animal Ethics Committee (IAEC-No.7/22/C.V.Sc., Hyd./IAEC).

Rats were categorized into 4 groups. First group is control given with normal diet, the second group rats were orally gavaged with imidacloprid @ 30mg/kg body weight/day, for third group rats *Withania somnifera* was mixed in feed @ 1 g/kg feed and fourth group rats were maintained on both IMI and WS. Experiment was done for 30 days. On 16th day and at the end of experiment six rats from each group were sacrificed by cervical dislocation and detailed postmortem examination was carried out as per standard procedure suggested by Feinstein (2000). The gross lesions were recorded and kidney weights were recorded by using electronic balance to study the relative organ weights. Relative organ weight was expressed as per cent (%) of body weight in relation to body weight.

Calculation

Relative organ weight = Organ weight / Body weight x 100
The kidney tissue samples were collected to assess oxidative stress in the kidney and also analyzed for histopathology. One gram of tissue sample with 10 mL of 0.2 M Tris HCl buffer (pH 7.2) was taken into a tissue homogenizer to get 10 per cent homogenate to carry out all the tissue antioxidant parameters. The tissue oxidation was measured by reduced glutathione (GSH) (Moron *et al.*, 1979) [20], the reaction of the lipid peroxidation end products like malondialdehyde (MDA) with thiobarbituric acid reactive substances (TBARS) (Balasubramanian *et al.*, 1988) [19] and activity of superoxide dismutase (SOD) (Madesh and Balasubramanian, 1998) [19] to know the antioxidant status. Tissues were fixed in 10 per cent neutral buffered formalin (NBF) soon after necropsy. The samples were processed, sectioned (5µm) and stained with Hematoxylin and Eosin (H&E) for histopathological examination as per the standard procedure (Luna, 1968) [18].

Statistical Analysis

The data obtained were subjected to statistical analysis by applying one way ANOVA using Statistical Package for Social Sciences (SPSS) version 16.0. Differences between the means was tested by using Duncan's multiple comparison test and significance level was set at $p < 0.05$ (Snedecor and Cochran, 1994) [24].

Results

Gross pathology: Necrotic foci were observed on kidneys of group 2 rats on day 16 (Fig. 1a) and severe congestion of kidneys was noticed on day 31 (Fig. 1b). Kidneys revealed a normal gross appearance in groups 1, 3 and 4.

Relative kidney weights (% of body weight): Significantly ($p < 0.05$) decreased mean values of relative kidney weights (% of body weight) were recorded in group 2 when compared with group 1 on 16th and 31st day of experiment. There was no significant difference in mean values of relative kidney weights between groups 1, 3 and 4 on 16th and 31st day of experiment (Table 1).

Reduced Glutathione Concentration: GSH (nmol/mg protein): The mean values of GSH concentrations (nmol/mg protein) in renal tissues in group 2 and group 4 were significantly ($p < 0.05$) reduced when compared with group 1 and group 3 on 16th and 31st day of experiment. In group 4, there was a significant ($p < 0.05$) increase in renal GSH concentration in comparison to group 2 and group 3 value was insignificant from control (Table 2).

Superoxide Dismutase Activity: SOD (U/mg protein): The mean value of SOD activity (U/mg protein) in renal tissue in group 2 and group 4 were significantly ($p < 0.05$) decreased when compared with group 1 and group 3 on 16th and 31st day of experiment. In group 4, a significant ($p < 0.05$) increase in renal SOD activity was recorded in comparison to group 2 and group 3 value was insignificant from control (Table 2).

Thiobarbituric Acid Reactive Substance Concentration: TBARS (nmol MDA/g tissue): Significantly ($p < 0.05$) increased mean values of TBARS concentrations (nmol MDA/g tissue) in renal tissues were recorded in group 2 and group 4 when compared with group 1 and group 3 on 16th and 31st day of experiment. The mean values of renal TBARS were significantly ($p < 0.05$) decreased in group 4 when compared with group 2 and there was no significant difference between groups 1 and 3 (Table 2).

Histopathology: Histological sections of kidneys of group 2 rats on 16th day of experiment showed shrunken glomeruli with increased Bowman's space. Moderate inter tubular haemorrhage, vacuolar degeneration of the tubular epithelium, Bowman's space and interstitium were noticed (Fig. 2a, b, c and d). Some other sections showed focal lymphoid aggregates, moderate dilation and congestion in the interstitial spaces, hyaline casts in the tubular lumen and degeneration of tubular epithelial cells (Fig. 2e, f and g). On 31st day, degenerated and atropic glomeruli with increase in the Bowman's space, severe periglomerular and interstitial congestion and focal infiltration of MNCs around the degenerating glomeruli (Fig. 3a, b and c) were noticed. Diffuse inter tubular haemorrhage, lymphoid aggregates and focal necrosis in the interstitium (Fig. 3d, e and f) were observed. Degeneration and necrosis of tubular epithelial cells, proteinaceous eosinophilic casts and desquamated tubular epithelial cells in the tubular lumen (Fig. 3g, h and i) were also observed.

Kidney sections of group 4 rats showed cloudy swelling of the tubules with decreased tubular lumen, mild degeneration of tubular and glomerular epithelium, mild infiltration of inflammatory cells and congestion in the interstitial space (Fig. 4a, b and c) on 16th day of experiment. Cloudy swelling of the tubular epithelial cells with decreased tubular lumen, vacuolar degeneration towards tubular lumen, pyknotic nuclei of tubular epithelial cells, formation of cystic spaces and mild infiltration of inflammatory cells in the interstitium (Fig. 4d, e and f) were noticed on 31st day.

Discussion

Gross changes in kidneys of group 2 rats suggest the toxic effect of IMI on kidneys. Renal congestion might be due to vascular changes. Contrary to this, Palkhade *et al.* (2018) [21] reported non-significant gross lesions in kidney of rats treated with IMI.

The changes in relative kidney weight in current experiment are in agreement with the observations of Al-Dabbagh *et al.* (2015) [1]. Whereas, Bhardwaj *et al.* (2010) [7], Arfat *et al.* (2014) [3] and Lohiya *et al.* (2018) [17] reported a significant increase in relative kidney weights. And no significant difference in relative kidney weights was documented by previous workers (Bagri *et al.*, 2013; Preeti *et al.*, 2014; Vohra *et al.*, 2014; Vohra and Khera, 2015; Palkhade *et al.*, 2018) [5, 22, 26, 27 21]. The reduced relative kidney weights in IMI treated rats could be due to degeneration and necrosis of renal parenchyma in kidneys of group 2 rats. In group 4 rats, the relative kidney weights were significantly ($p<0.05$) increased in comparison to group 2. This might be due to nephro-protective and antioxidant nature of WS.

The observations of significantly ($p<0.05$) decreased GSH and SOD and significantly ($p<0.05$) increased TBARS levels in kidneys of group 2 rats when compared to group 1 rats in current study are in accordance with the findings of Lohiya *et al.* (2018) [17]. These findings indicate that IMI might have been induced oxidative stress in tissues resulted in increased lipid peroxidation of cell membranes, led to more production of TBARS and to neutralize them the GSH and SOD were utilized which are the common antioxidants in tissues and resulted in decreased levels of GSH and SOD. El-Feki *et al.* (2008) [9], Bhardwaj *et al.* (2010) [7], Vohra and Khera (2015) [27], Lohiya *et al.* (2018) [17] and Hassan *et al.* (2019) [11] observed the similar histopathological findings in IMI treated rats. Arfat *et al.* (2014) [3] and Al-Dabbagh and Al-Bahadyli (2016) [2] also found similar changes in

mice. Similar lesions were also published by Kammon *et al.* (2010) [13] and Komal *et al.* (2016) [15] in chicken and by Wankhede *et al.* (2017) [28] in quails. These changes could be due to IMI induced oxidative damage. Kidneys are the major excretory organs for many of the xenobiotics and are frequently susceptible to nephrotoxicity. Degeneration of the glomeruli and tubules may be resulting from collection of albuminous material during excretion of high concentration of the toxin in the urine (El-Feki *et al.*, 2008) [9]. These observations are further corroborated by increased level of TBARS and decreased levels of GSH and SOD in kidneys of IMI treated rats in the present experiment. Group 4 kidney sections revealed mild histopathological changes in kidneys. These are in accordance with the observations of Babu *et al.* (2015) [4] in chicken. These changes might be due to the antioxidant or free radical scavenging action of WS.

Table 1: Relative kidney weights (% body weight) in different groups.

Group	Day 16	Day 31
Group- 1	0.77±0.02 ^a	0.75±0.04 ^a
Group- 2	0.61±0.04 ^b	0.54±0.03 ^b
Group- 3	0.76±0.02 ^a	0.74±0.03 ^a
Group- 4	0.73±0.04 ^a	0.69±0.03 ^a

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

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

Table 2: Kidney anti oxidant profile in different groups.

Group	GSH (n mol/mg tissue)		TBARS (n mol/g tissue)		SOD (U/mg protein)	
	Day 16	Day 31	Day 16	Day 31	Day 16	Day 31
Group 1	120.10±0.82 ^a	118.55±0.91 ^a	177.92±0.99 ^c	178.25±0.57 ^c	7.35±0.26 ^a	6.83±0.28 ^a
Group 2	78.70±0.79 ^c	63.00±0.74 ^c	186.42±0.69 ^a	190.77±0.74 ^a	4.87±0.20 ^c	4.45±0.18 ^c
Group 3	121.30±0.79 ^a	120.07±0.62 ^a	177.00±0.87 ^c	178.68±0.98 ^c	7.33±0.38 ^a	6.93±0.23 ^a
Group 4	100.60±0.57 ^b	90.78±0.75 ^b	183.43±0.43 ^b	184.15±0.64 ^b	6.22±0.18 ^b	5.93±0.23 ^b

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

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

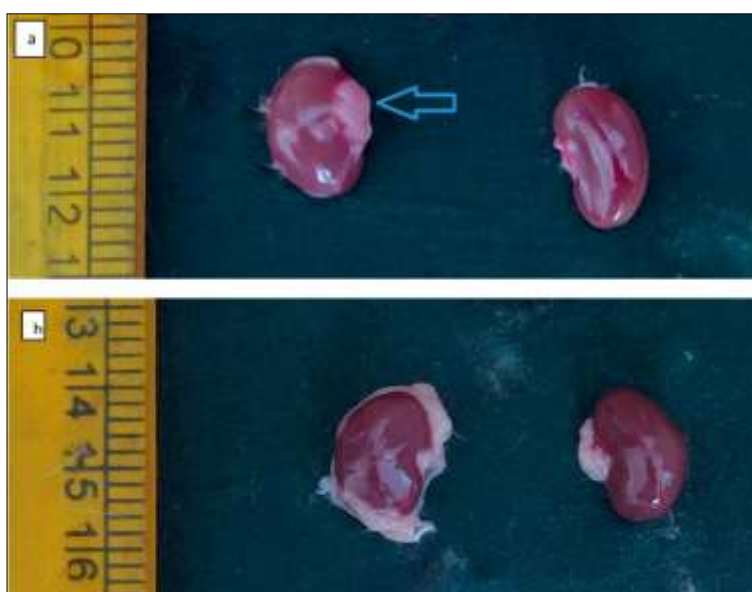


Fig 1: Photomicrograph showing a. necrotic foci on kidneys on day 16 and b. severe congestion of kidneys on day 31(group 2).

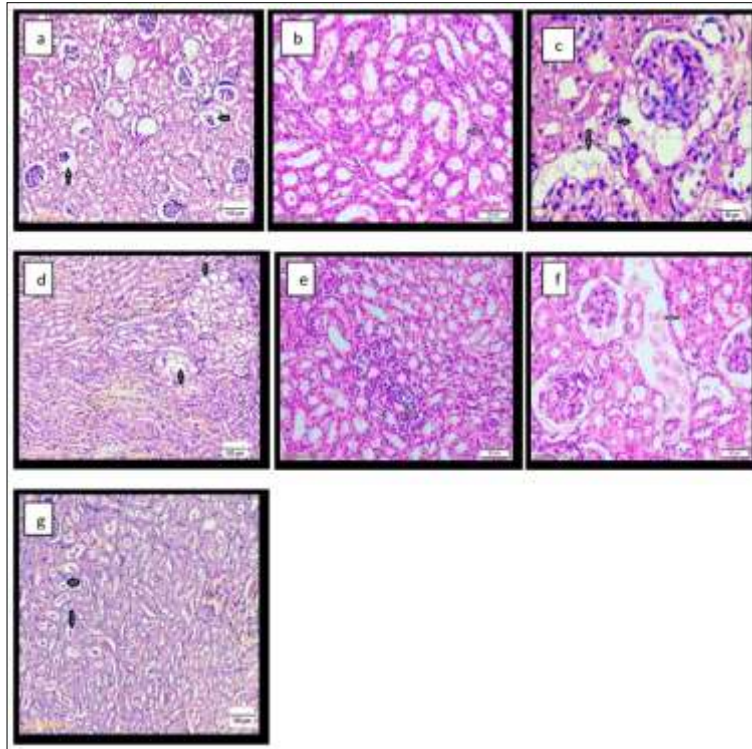


Fig 2: a. Photomicrograph of kidney showing shrunken glomeruli with increased Bowman's space: H&E x100
 b. vacuolar degeneration of the tubular epithelium: H&E x200
 c. vacuolar degeneration in the Bowman's space: H&E x400
 d. vacuolar degeneration in interstitium and moderate inter tubular haemorrhage: H&E x100
 e. focal lymphoid aggregates in the interstitial spaces: H&E x200
 f. moderate interstitial dilation and congestion: H&E x200
 g. hyaline casts in the tubular lumen and degeneration of tubular epithelial cells: H&E x100 (Group 2, Day 16).

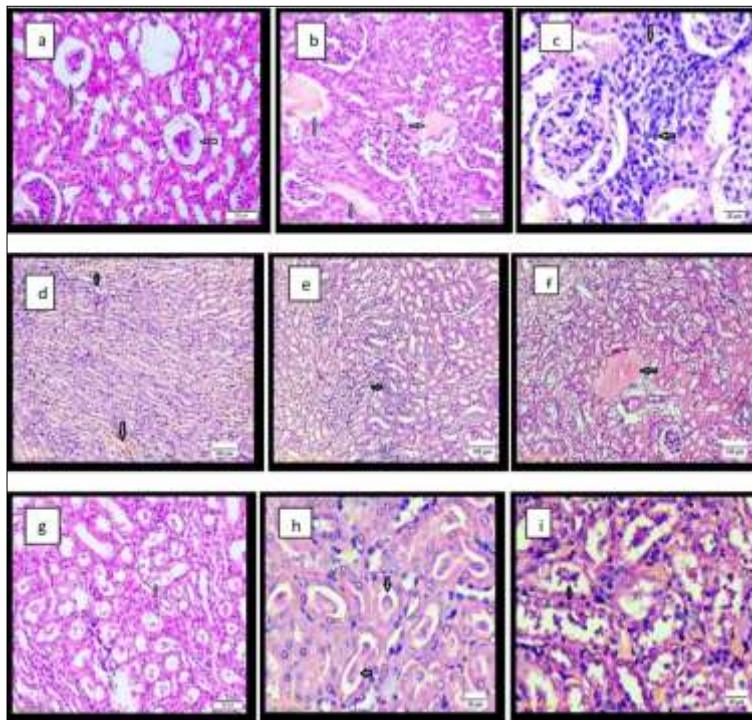


Fig 3: a. Photomicrograph of kidney showing degenerated and atrophic glomeruli with increase in the Bowman's space: H&E x200
 b. severe periglomerular and interstitial congestion: H&E x200
 c. focal infiltration of mononuclear cells around the degenerating glomeruli: H&E x400
 d. diffuse inter tubular haemorrhage: H&E x100
 e. diffuse lymphoid aggregates in the interstitial spaces: H&E x100
 f. focal necrosis in the interstitial space: H&E x100
 g. degeneration and necrosis of tubular epithelial cells and their shedding into lumen: H&E x200
 h. proteinaceous eosinophilic casts in the tubular lumen: H&E x400
 i. desquamated tubular epithelial cell debris in lumen: H&E x400 (Group 2, Day 31).

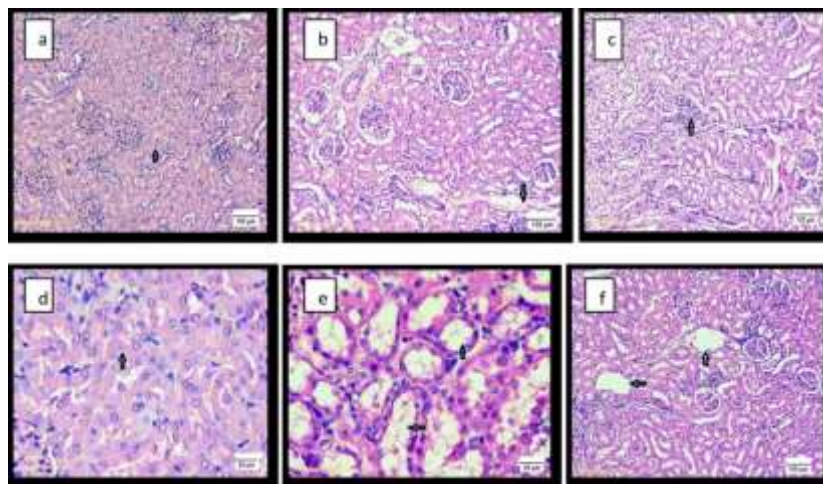


Fig 4: a. Photomicrograph of kidney showing cloudy swelling of the tubules with decreased tubular lumen: H&E x100
 b. mild interstitial congestion and mild degeneration of tubular and glomerular epithelium: H&E x100
 c. mild infiltration of inflammatory cells in the interstitial space: H&E x100 (Group 4, Day 16).
 d. cloudy swelling of the tubular epithelial cells with decreased tubular lumen: H&E x400
 e. vacuolar degeneration towards lumen and pyknotic nuclei of tubular epithelial cells: H&E x400
 f. formation of cystic spaces and mild infiltration of inflammatory cells in the interstitium: H&E x100 (Group 4, Day 31).

Conclusion

Present study concludes that a significant damage caused to kidneys due to imidacloprid administration in rats which was indicated by gross & histopathological lesions and change in relative organ weight. It might be due to free radical production resulting in oxidative stress and lipid peroxidation of tissue which was correlated by changes in tissue antioxidant parameters like TBARS, GSH and SOD. The free radical scavenging and anti-inflammatory role of WS was also observed in current study which indicates the nephroprotective action of WS against the IMI induced nephrotoxicity. Further WS may be used therapeutically to reduce the renal damage caused by IMI.

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