



ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(11): 283-288
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www.thepharmajournal.com
Received: 25-09-2019
Accepted: 28-10-2019

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Chlorpyrifos (CPF) induced renal and cardiac alterations in wistar rats - mitigating effect by co-administration of *Withania somnifera* and selenium

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Abstract

The present experiment was carried out to investigate the protective and synergistic effect of *Withania somnifera* and selenium on chlorpyrifos (CPF) induced alterations in biochemical parameters and histology of kidney and heart in rats. Total of forty two male albino *Wistar* rats were procured and divided into 7 groups consisting of 6 in each. Group 1- Normal Control. Group 2 - Chlorpyrifos (CPF) (@ 12.5 mg/kg body weight/p.o./day). Group 3 – *Withania somnifera* (@ 100 mg/kg body weight/p.o./day). Group 4 – Selenium (@ 10 µg/kg body weight/p.o./day). Group 5 – Chlorpyrifos (CPF) (@ 12.5 mg/kg body weight/p.o./day) + *Withania somnifera* (@ 100 mg/kg body weight/p.o./day). Group 6 – Chlorpyrifos (CPF) (@ 12.5 mg/kg body weight/p.o./day) + Selenium (@ 10 µg/kg body weight/p.o./day). Group 7 – Chlorpyrifos (CPF) (@ 12.5 mg/kg body weight/p.o./day) + *Withania somnifera* (@ 100 mg/kg body weight/p.o./day) + Selenium (@ 10 µg/kg body weight/p.o./day). The experiment was carried out for a period of 28 days. Group 7 rats revealed a significant (P<0.05) decrease in the mean values of blood urea nitrogen (BUN) and creatinine and rejuvenated the histology of kidney and heart when compared to group 2 rats. These results suggested that the co-administration of *Withania somnifera* and selenium offered remarkable synergistic protective action against CPF induced alterations in biochemistry and histology.

Keywords: Chlorpyrifos, *Withania somnifera*, selenium, kidney, heart

Introduction

Chlorpyrifos (CPF), a broad spectrum organophosphate insecticide (OPI), is one of the most extensively used OPIs in domestic and industrial applications all over the world [1]. CPF is known to inhibit acetylcholinesterase activity in target tissues [2]. Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipid bilayers [3], and they may damage membranes by inducing lipid peroxidation (LPO) [4].

The kidney is a vital organ, which plays an essential role in health, disease and overall development and growth. A number of environmental variables including certain xenobiotics influence the functions of kidney [5]. The susceptibility of kidney tissues to the stress due to exposure to pesticides is a function of the overall balance between the degree of oxidative stress and the antioxidant capability [6]. After entering the body of organism chlorpyrifos is eliminated primarily through kidneys in urine. In rats, following oral intake of chlorpyrifos, about 90% is removed in urine and 10% is excreted in faeces [7]. Chlorpyrifos causes functional heart disorders, cardiac autonomic input imbalance, vascular wall damage and inhibits the nitric oxide synthase (NOS) activity [8]. Histopathological studies are useful to evaluate the pollution potential of pesticides since trace levels of pesticides, which do not cause animal mortality over a given period, are capable of producing considerable organ damage [9].

Withania somnifera, also known as Indian ginseng, is a herb which contains alkaloids, fatty acids, beta-sitosterol, polyphenols, phytosterols and steroidal lactones called withanolides, and is employed as an adaptogen in Ayurvedic traditional medicine [10]. *Withania somnifera* significantly increases the levels of natural antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) [11] and inhibits lipid peroxidation and generation of free radicals [12], thus providing protection against nephrotoxicity and heart diseases. The presence of high selenium as antioxidant selenoenzymes {glutathione peroxidase (GPx) and thioredoxin reductase (TrxR)} and selenoproteins, protects cells from oxidative stress and may help to reduce the production of oxidised low-density lipoprotein (LDL) and, therefore, would reduce the incidence of heart diseases [13].

There is scanty literature available on synergistic action of microelement and herbal combination for their greater antioxidant potentiality. The aim of this experiment was to investigate the protective effect of *Withania somnifera* and selenium given in combination on biochemical and histopathological parameters after repeated exposure to CPF in *Wistar* rats.

Materials and Methods

In the present study, a total of 42 male albino *Wistar* rats weighing 150-180 grams were procured from Jeeva Life Sciences Pvt. Limited, Hyderabad. The rats were housed in solid bottom polypropylene cages at the lab animal house, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Hyderabad and were maintained at ambient temperature (20-22 °C) throughout the course of experiment. Sterile husk was used as standard bedding material. All the rats were provided with standard pellet diet obtained from National Institute of Nutrition (NIN), Hyderabad and *ad libitum* water throughout the experimental period. Rats were randomly divided into 7 groups consisting of 6 in each group. Group 1 served as normal control whereas group 2 served as CPF toxic control (@ 12.5 mg/kg b.w./p.o./day). Groups 3 and 4 were administered with *Withania somnifera* @ 100 mg/kg body weight/p.o./day and Selenium @ 10µg/kg body weight/p.o./day, respectively and served as non-toxic controls. Group 5 was administered with CPF @ 12.5 mg/kg body weight/p.o./day + *Withania somnifera* @ 100 mg/kg body weight/p.o./day. Group 6 was administered with CPF @ 12.5 mg/kg body weight/p.o./day + Selenium @ 10µg/kg body weight/p.o./day. Group 7 was administered with CPF @ 12.5 mg/kg body weight/p.o./day + *Withania somnifera* @ 100 mg/kg body weight/p.o./day + Selenium @ 10µg/kg body weight/p.o./day. The experiment was carried out according to the guidelines and prior approval of Institutional Animal Ethics Committee (IAEC-No. III/2019-05/IAEC/C.V.Sc., Hyd, Dated 17/04/2019).

Drugs and chemicals

Chlorpyrifos (Chlorpyrifos 20% EC) was procured from Insecticides India Ltd. Root powder of *Withania somnifera* was obtained from KSM-66 Ashwagandha. Selenium as sodium selenite was procured from Sisco Research Laboratories Pvt. Ltd., Taloja, Maharashtra, India. Stains for the histopathological study of kidney and heart were obtained from Qualigens Pvt. Ltd., Mumbai.

Biochemistry

Prior to blood collection, the selected experimental rats were put to fast for 12 hours. Blood collection was carried out at fortnight intervals for sero-biochemical analysis after initiation of the drug administration till the end of experiment. Approximately, 2-3ml of blood was collected through retro-orbital plexus with the help of capillary tube into serum vacutainers to analyse biochemical parameters. Blood was centrifuged at 3000 RPM for 15 min, and serum was separated and stored at -80 °C till analysis. Kits for BUN and creatinine estimation were procured from ERBA diagnostics Ltd, Surat, India.

Histopathology

On the 28th day, after blood collection, rats were euthanized by carbon dioxide exposure and tissue pieces of kidney and

heart were collected from the rats and fixed in 10% neutral buffered formalin (NBF) for histopathology. The small representative pieces of fixed tissues were cut and subjected to overnight washing under running tap water. The tissues were then dehydrated in ascending grades of alcohol, cleared in xylol and embedded in paraffin at 55-56°C. The paraffin blocks were cut into thin sections of 5 micron thickness by microtome. The cut sections were lifted on grease free glass slides precoated with Mayer's egg albumin and were kept in incubator overnight at 37 °C for drying. The slides were stained with routine Haematoxylin and Eosin (H and E) stain [14-15] and the stained sections were mounted with DPX mountant and kept ready for microscopic examination.

Data obtained (biochemical parameters) was subjected to statistical analysis by applying one-way ANOVA and using statistical package for social sciences (SPSS) version 25.0. Differences between the means were tested by using Duncan's multiple comparison tests and significance level was set at P<0.05 [16].

Results

Blood urea nitrogen (BUN)

The concentration of BUN (mg/dl) in group 2 (40.57±0.82 and 41.74±1.14, respectively) was significantly (p<0.05) higher when compared to group 1 (32.45±1.56 and 32.78±1.50, respectively) on 14th and 28th day, while treatment groups 5 (35.92±1.05 and 35.81±1.28, respectively), 6 (36.09±0.95 and 35.77±1.31, respectively) and 7 (33.84±1.09 and 33.81±1.26, respectively) showed significantly (p<0.05) lower values in comparison to group 2. The values of treatment groups 5, 6 and 7 were comparable to group 1 without any significant difference (Table 1).

Serum creatinine

The concentration of serum creatinine (mg/dl) in group 2 (1.02±0.04 and 1.04±0.03, respectively) was significantly (p<0.05) higher when compared to group 1 (0.74±0.02 and 0.74±0.01, respectively) on day 14th and 28th. Treatment groups 5 (0.86±0.02 and 0.85±0.03, respectively), 6 (0.87±0.02 and 0.86±0.01, respectively) and 7 (0.81±0.03 and 0.80±0.02, respectively) had a significantly (p<0.05) lower values when compared to group 2. The values of groups 5, 6 and 7 were comparable without any significant difference (Table 1).

Table 1: Serum blood urea nitrogen (BUN) concentration (mg/dl) and serum creatinine concentration (mg/dl) in different groups of rats

Group	Bun (mg/dl)		Creatinine (mg/dl)	
	Day 14	Day 28	Day 14	Day 28
G1	32.45±1.56 ^a	32.78±1.50 ^a	0.74±0.02 ^a	0.74±0.01 ^a
G2	40.57±0.82 ^b	41.74±1.14 ^b	1.02±0.04 ^c	1.04±0.03 ^c
G3	32.26±1.27 ^a	31.93±1.71 ^a	0.74±0.03 ^a	0.73±0.01 ^a
G4	32.28±1.60 ^a	31.94±1.64 ^a	0.74±0.04 ^a	0.73±0.02 ^a
G5	35.92±1.05 ^a	35.81±1.28 ^a	0.86±0.02 ^b	0.85±0.03 ^b
G6	36.09±0.95 ^a	35.77±1.31 ^a	0.87±0.02 ^b	0.86±0.01 ^b
G7	33.84±1.09 ^a	33.81±1.26 ^a	0.81±0.03 ^{ab}	0.80±0.02 ^{ab}

Values are Mean±SE (n=6); One-way ANOVA Means with different superscripts in a column differ significantly at P<0.05(*).

Histopathology

Kidney

The sections of the kidney from the CPF control group (2) showed swollen and shrunken glomeruli with vacuolations, dilatation of tubules and degeneration of epithelial cells (Figure 2), mild to moderate interstitial fibrosis and mild

proliferation of inflammatory cells (Figure 3) and bowmann's capsule devoid of glomerulus (Figure 4), whereas upon treatment there were few atrophied glomeruli, presence of casts in tubular lumen and cystic dilatations of intertubular spaces (Figure 9) in group 7.

Heart

The sections of the heart from the CPF control group (2) showed disorganized muscle fibers, increased interfiber space with haemorrhages, vacuolations and degeneration in muscle fibers (Figure 11), whereas the treatment groups showed capillary dilation and congestion with increased interfiber space (Figure 14) in group 5, disorganized muscle fibers (Figure 15) in group 6 and mild disorganization and focal degeneration of muscle fibers (Figure 17) in group 7.

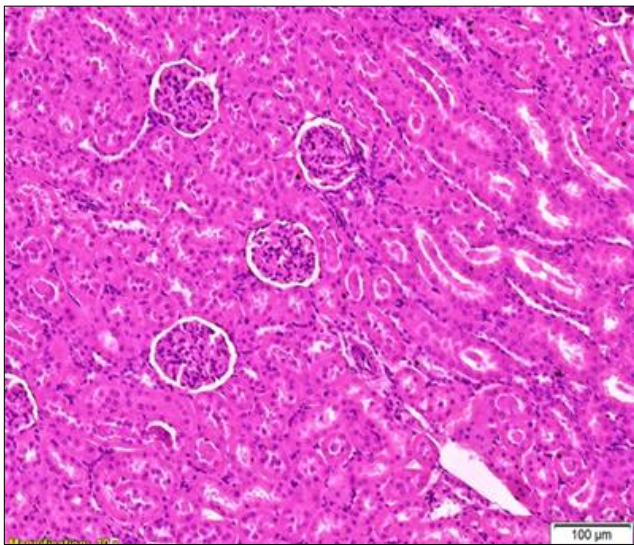


Fig 1: Microphotograph of kidney showing normal structure of glomeruli with uniform urinary space and normal tubules. (Group 1). H&E X 100µm

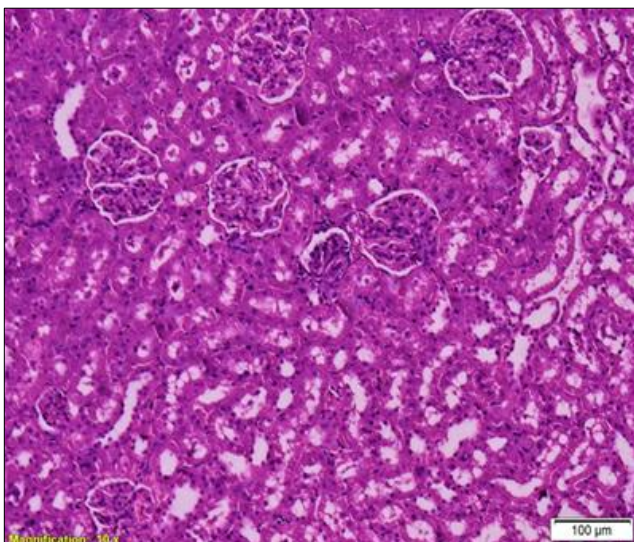


Fig 2: Microphotograph of kidney showing swollen and shrunken glomeruli with vacuolations, dilatation of tubules and degeneration of epithelial cells (Group 2). H&E X 100µm

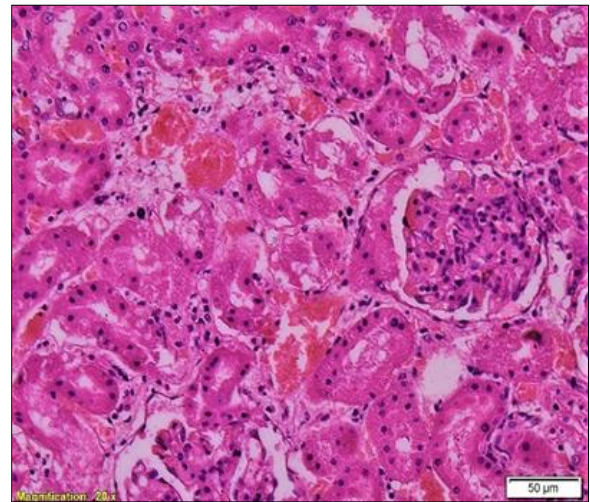


Fig 3: Microphotograph of kidney showing swollen and normal glomerulus with vacuolations, dilated blood vessels with intertubular haemorrhages, mild to moderate interstitial fibrosis, degeneration of tubular epithelium and mild proliferation of inflammatory cells (Group 2). H&E X 200µm

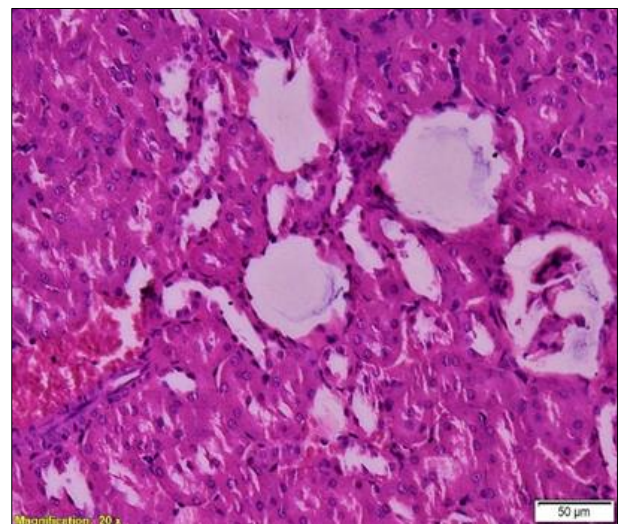


Fig 4: Microphotograph of kidney showing bowmann's capsule devoid of glomerulus, degenerated glomerulus, dilation and congestion of blood vessel and degeneration of epithelial cells (Group 2). H&E X 200µm

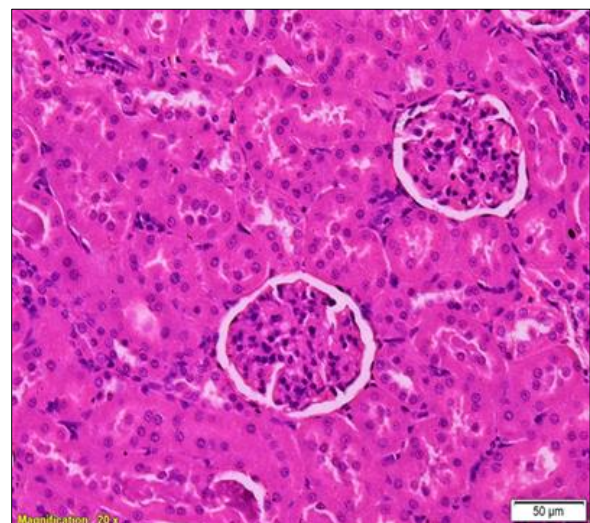


Fig 5: Microphotograph of kidney showing normal glomerulus and tubules (Group 3). H&E X 200µm.

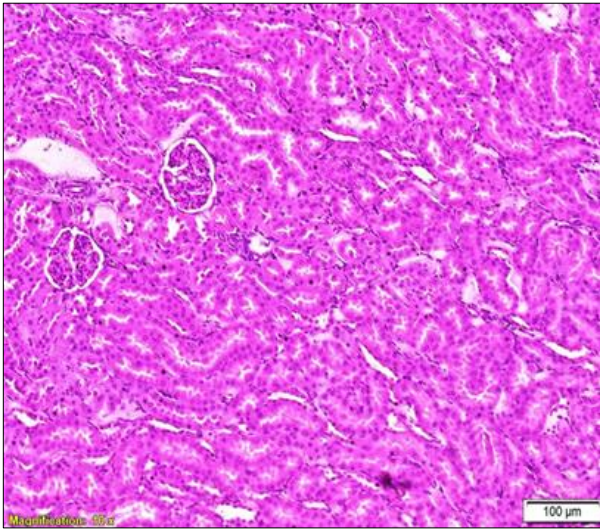


Fig 6: Microphotograph of kidney showing normal structure of glomerulus and tubules (Group 4). H&E X 100µm

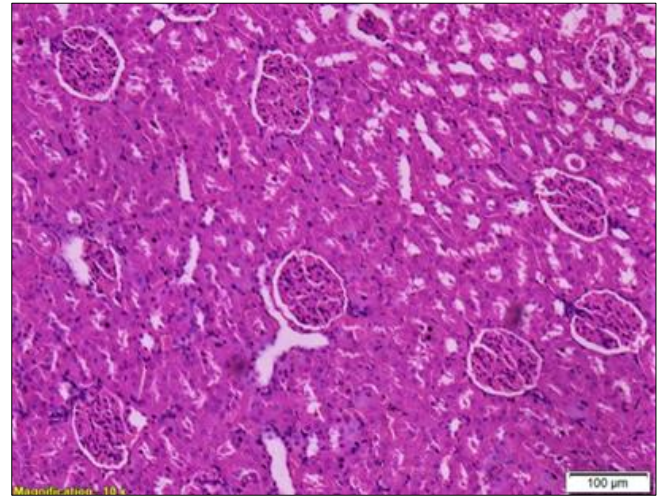


Fig 9: Microphotograph of kidney showing few atrophied glomeruli, presence of casts in tubular lumen and cystic dilatations of intertubular spaces (Group 7). H&E X 100 µm

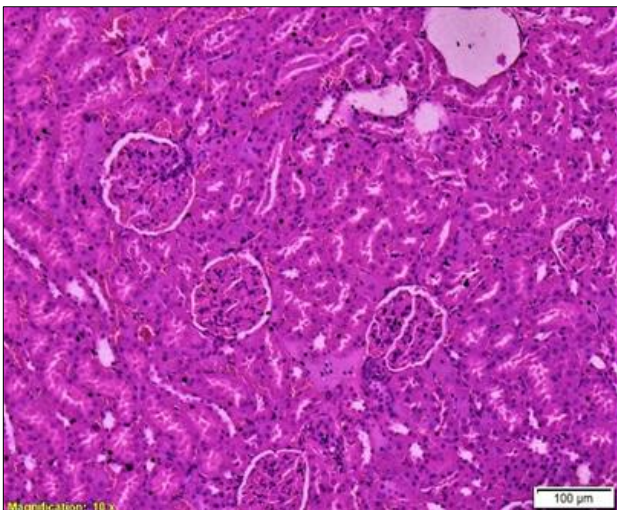


Fig 7: Microphotograph of kidney showing swollen, normal, shrunken glomerulus, bowmann's capsule without glomerulus, intertubular haemorrhages, cystic dilatation of intertubular spaces, intertubular oedema (Group 5) H&E X 100 µm

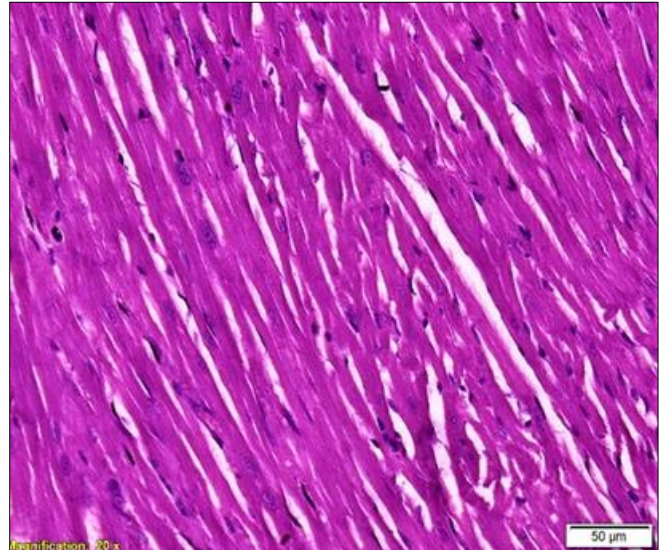


Fig 10: Microphotograph of heart showing normal appearance of muscle fibers (Group 1). H&E X 200 µm

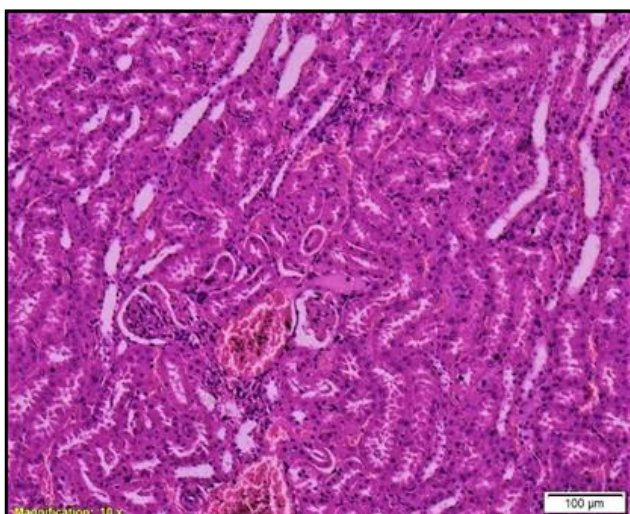


Fig 8: Microphotograph of kidney showing distorted and shrunken glomeruli, dilatation of tubules, intertubular oedema, degeneration of epithelial cells, infiltration of inflammatory cells, dilation and congestion of blood vessels with intertubular haemorrhages (Group 6) H&E X 100 µm



Fig 11: Microphotograph of heart showing disorganized muscle fibers, increased interfiber space with haemorrhages, vacuolations in muscle fibers and degenerated muscle fibers (Group 2). H&E X 200 µm

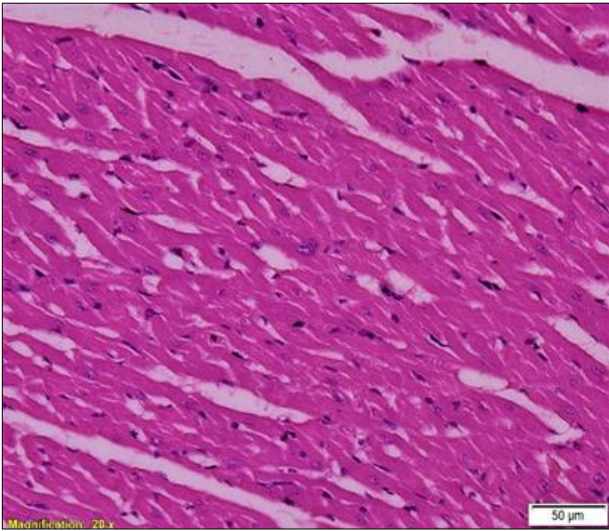


Fig 12: Microphotograph of heart showing normal architecture of muscle fibers (Group 3). H&E X 200 μm

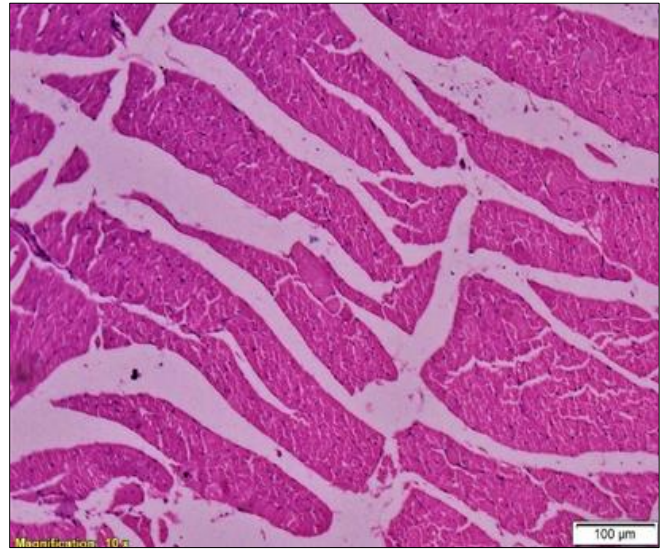


Fig 15: Microphotograph of heart showing disorganized muscle fibers (Group 6). H&E X 100μm

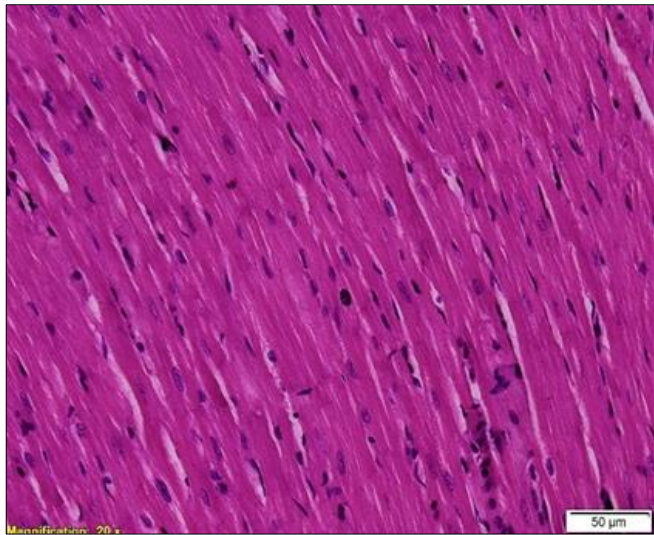


Fig 13: Microphotograph of heart showing normal muscle fibers (Group 4).H&E X 200 μm

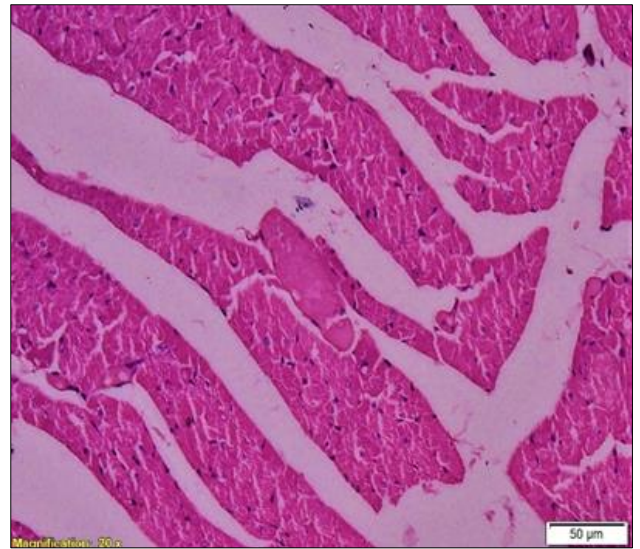


Fig 16: Microphotograph of heart showing oedema in interfiber space, degenerated and disorganized muscle fibers (Group 6). H&E X 200 μm

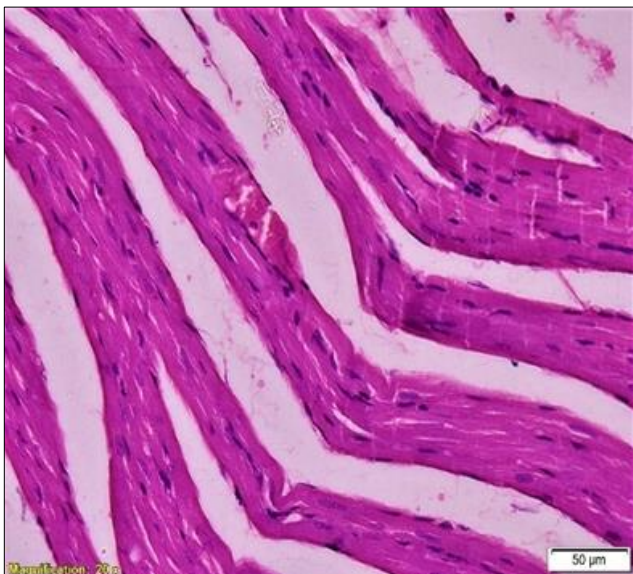


Fig 14: Microphotograph of heart showing capillary dilation and congestion with increased interfiber space (Group 5). H&E X 200 μm

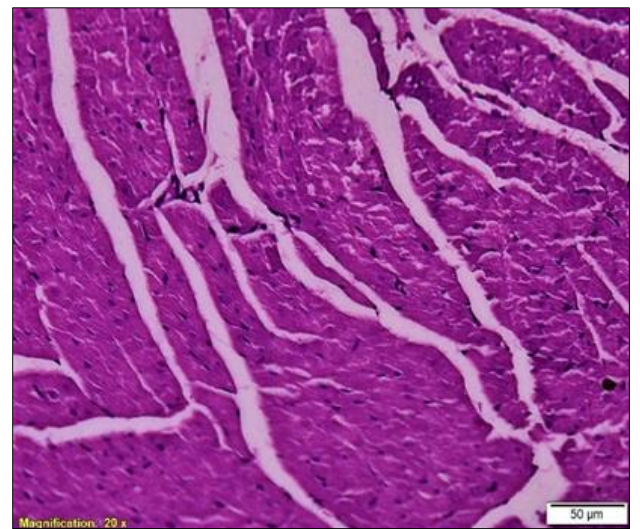


Fig 17: Microphotograph of heart showing mild disorganization and focal degeneration of muscle fibers (Group 7). H&E X 200 μm

Discussion

In the present study, an increase in BUN in group 2 reflects an accelerated rate of protein catabolism and free radical induced oxidative damage by CPF on kidney. The significant rise in the serum creatinine level may be due to the impairment of the glomerular function and tubular damage in the kidneys. These results are in harmony with the earlier studies of Ambali *et al.* (2010) [17] and Heikal *et al.* (2012) [1]. These results are further substantiated by marked histopathological changes in the kidney tissues like swollen and shrunken glomeruli with vacuolations and degeneration of tubular epithelium in group 2. The CPF induced alterations in heart in the present study were evidenced by histopathological changes occurred in heart tissues in group 2.

In the present study, protective effect of *Withania somnifera* could be due to its free radical scavenging and antioxidant property and these results are in harmony with the earlier studies of Mansour and Anis (2014) [10] and Vedi *et al.* (2014) [18]. Selenium alleviated CPF induced organ damage in the present study, which could be due to its antioxidant properties as selenium supplementation might induce the synthesis of GSH-Px in the extrarenal and heart tissues and these findings are in agreement with the previous studies of Tacyildiz *et al.* (2012) [19], Sedighi *et al.* (2014) [20] and Abdel-Rahman *et al.* (2017) [21].

In group 7, a significant decrease in the mean values of BUN and creatinine and restoration of architectural details in kidney and heart tissues were observed when compared to group 2 which might be due to combined antioxidant defense mechanism of *Withania somnifera* and selenium against chlorpyrifos induced oxidative damage of kidney and heart tissues.

In conclusion, the present study clearly demonstrated that both *Withania somnifera* and selenium synergistically attenuate CPF induced biochemical and histopathological alterations in kidney and heart, possibly *via* antioxidant defense mechanism.

Acknowledgements

The authors are thankful to P V Narsimha Rao Telangana Veterinary University for providing support and necessary facilities to carry out the research work.

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