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Biological control of Malathion induced toxicity through dietary supplementation of *Spirulina platensis* in white Swiss albino mice

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Organophosphates like malathion are extensively being used in crops, gardens and domestic places to control flies and mosquitoes. Malathion is being used as a potent insecticide to crops since half century and its extensive use has severely contaminated the environment of non-targeted species like humans, animals, birds and aquatic ecosystems. *Spirulina platensis* (SP) is a blue green alga which has high nutritious value and is commercially being cultivated for use as food. This experiment was aimed to examine the mitigative potency of SP against malathion induced oxidative stress and toxicopathological alterations in Swiss albino mice. Forty-eight mice were divided into six equal groups and fed varying combinations of malathion (40 and 60 mg/kg body weight) and SP (1% of feed) for 28 days. Parameters studied were feed intake, body weight, relative organ weights, hematological, serum biochemical, gross and histopathological alterations along with serum total antioxidant capacity and antioxidant status. Results of the experiment revealed that Malathion administration severely affected feed intake and body weight gain along with altered hematological and serum biochemical indices in mice which were efficiently restored by the dietary supplementation of SP. Additionally this restoration in the parameters was partial against higher doses malathion (60 mg/kg BW) while amelioration was completely observed at lower malathion doses (40 mg/kg BW). However, the actual ratio of malathion and SP to deliver such mitigation is yet to be evaluated and requires further research in this regard.

Keywords: Malathion, *Spirulina platensis*; Swiss albino mice; serum biochemical; oxidative stress; toxico pathological, Haematological

INTRODUCTION

Pesticides/insecticides belonging to the group of organophosphates are extensively being used at domestic places including gardens for the control of mosquitoes and flies (Mahmood et al. 2021; Al-Saeed et al. 2023). Along with this their topical use is also a normal practice in order to control head lice and are considered as a serious environmental pollutant due to their deleterious effects on certain non-targeted species including humans and other animals. The residues of such pesticides also contaminate certain food commodities such as grains, animal tissues and vegetables posing their indirect effects in the organisms consuming such food products (Zahoor et al. 2022). Animals exposed to insecticides may show different signs of illness depending upon the concentration, chemical nature and duration of insecticide's exposure however animal species, its health & age along with its nutritional status are also being considered to be the important pre-disposing factors in this regard. Insecticides act by inhibiting acetylcholinestrase (AChE) which hydrolyses acetylcholine in cholinergic synapse and neuromuscular junctions. This inhibition of acetylcholinestrase results in the accretion of acetylcholine ultimately causing the activation of cholinergic nicotinic and muscurinic receptors (Rezg et al. 2008). Malathion (1, 2- dicarbethoxyethyl), an important organophosphate, is extensively being used for the control of pests upon certain field crops and equally considered effective for the control of ectoparasites in domesticated animals. The residues of Malathion have been detected in water, certain types of animal tissues, vegetables and grains. Such contaminated food grains, animal tissues, grass, water

and vegetables when consumed by humans, animals and birds may act as a possible source of malathion associated toxicities in these organisms (Naraharisetti et al. 2009). It affects male reproductive system & spermatogenesis in animals and also blocks steroidogenesis and/or acetylcholinestrase activity along with reduction in testosterone levels ultimately causing a reduced reproductive activity of male mice. Apart from these, malathion is also responsible for lipid peroxidation through its extended ability of inducing oxidative stress by depletion of endogenous antioxidant enzyme system like glutathione peroxidase (GPx), superoxide dismutase (SOD) etc (Selmi et al. 2015).

Spirulina platensis (SP), a Cyanobacterium of family *Oscillatoriaceae*, is actually a blue-green algae which is getting great limelight due to its high nutritive and therapeutic properties (Jung et al. 2019). The fact which makes it distinct among all the algae is that it is the only blue-green alga which is commercially being cultivated for use as food and that so because UN-WHO declares it as "the best of tomorrow" in 1996 due to its high nutritive values. SP contains 55-65% protein contents, 2.5-3.29 kcal/gram available energy and 41% phosphorous (Matufi and Choopani, 2020). It also contains all essential along with non-essential amino acids, moreover; certain vitamins like riboflavin, vitamin B_{12}, thiamin, vitamin C, pyridoxine and antioxidants like carotenoids are also abundantly found in it. The active ingredient of *Spirulina platensis* (phycocyanin) has immunomodulatory, anti-inflammatory, neuroprotective, anticancer and hepato-protective activities (Kumar et al. 2022). However, Pereira et al. (2019) also reported the antioxidant activity exhibited by different fractions of *Spirulina platensis*.

There is a scarcity of knowledge regarding the use of S*pirulina platensis* as a protective/ameliorative agent against malathion induced alterations. Based on this discussion and considering the above-mentioned facts, the present study was intended to evaluate the ameliorative potential of *Spirulina platensis* against malathion induced toxicopathological, hematological and serum biochemical alterations in white Swiss albino mice.

MATERIALS AND METHODS

Experimental design

Forty-eight (48) specific pathogen free (SPF) white albino mice were selected from the mice colony of Veterinary Research Institute Lahore Cantt Pakistan irrespective of their gender but having equal weight. These mice were divided into six equal groups containing eight mice each and offered different combinations of malathion (Route® Kanzo Ag containing 57% w/v malathion) and *Spirulina platensis* (Xian DN Biology Co., Ltd, China) as shown in Table 1. The mice were kept in mice boxes under similar housing and managemental conditions. Animals were offered water and feed *ad libitum* during the whole experiment and duration of the experiment was 4 weeks. Feed intake on daily basis while body weight gain for each group was calculated on weekly basis.

At the termination of study, all mice of each group were slaughtered, and different visceral organs were weighed to evaluate relative organ weights. Organs like kidney and liver were then preserved in 10% neutral buffered formalin. Before slaughtering blood samples from each mouse were collected and half of the blood was preserved in EDTA coated tubes for hematological analysis while half the blood was utilized for biochemical analysis after separating serum from them. The study was endorsed by Synopsis Committee of the University (UAF, Pakistan) and strict adherence to the guidelines regarding ethical use of animals were ensured (Dua, 2004).

Table 1: Experimental lay-out

Sr. No	Groups	Treatment
1	Control	Control
2	M1	Malathion (40 mg/kg BW)
3	M2	Malathion (60 mg/kg BW)
4	SP	*Spirulina platensis* (1% of feed)
5	M1SP	Malathion (40 mg/kg BW) + *Spirulina platensis* (1% of feed)
6	M2SP	Malathion (60 mg/kg BW) + *Spirulina platensis* (1% of feed)

Parameters studied

Toxicopathological parameters

Body weight and feed intake

Weekly body weight for each group was recorded while feed intake (in grams per mouse per day) was calculated by subtracting the left-over feed of each group from the feed offered on previous day to that particular group and then dividing it with the number of mice present within the group at that day.

Relative organ weights

Different visceral organs including liver, kidney, intestine and spleen were weighed, divided with body weight and then multiplied with 100 to evaluate relative weights of each organ for each group.

Gross morphology of organs

At termination of experiment, mice from all groups were observed/checked for any sort of gross anomaly/lesions on different organs (intestine, liver, spleen and kidney).

Hematological parameters

Blood samples with EDTA were processed for the evaluation of erythrocytic count, leukocytic count, hematocrit percentage and hemoglobin concentration following the modified method of Benjamin (1978).

Serum biochemical parameters

Separated serum samples of each group were used to evaluate the levels of alanine aminotransferase (ALT), urea and creatinine by commercially available kits (Merck-France, Catalog Nos. 5.17531.0001, 5.17611.0001 and 5.17551.0001 respectively) while levels of albumin and total proteins were estimated following the biuret, and bromocresol green dye binding method respectively (Anonymous, 1984) while globulin concentration was estimated by subtracting values of serum albumin from the values of total proteins.

Histopathological examination of tissues

Histopathological slides of different tissue (liver and kidney) were prepared following the method of Bancroft and Gamble (2008). Briefly, already preserved kidney and liver samples were sliced into 5 mm thin pieces and placed in running tape water for 12 hours to ensure complete removal of fixative. After 24 hours the samples were dehydrated, cleared and infiltrated using different concentrations of ethanol, xylene and paraffin respectively. Embedding of samples into blocks was done using paraffin which were then utilized for sectioning through a standard microtome. The prepared sections were then mounted over clean glass slides which were then placed in oven for drying. After drying, standard protocol of hematoxylin and eosin (H&E) staining technique was adapted for the staining of the tissues.

Oxidative stress and antioxidant response

Oxidative stress and antioxidant response was measured by estimating the total antioxidant capacity (TAC) and total oxidant status/total oxidative stress (TOS) in all groups following below mentioned methods.

Total antioxidant capacity (TAC)

Serum samples already preserved at -80°C were processed for the estimation of total antioxidant capacity as per method described by Erel (2004) with few modifications. Briefly, blank solution was prepared by adding 5µl of each sample in 200µl acetate buffer (Reagent-I). Absorbance of blank solution was then estimated at 660 nm before adding reagent II (20µl, 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonate) into the blank solution. Then the prepared solution mixture was incubated for 5 minutes at a temperature of 37°C and the second absorbance reading was measured. Total antioxidant capacity (TAC) of all the groups was estimated through delta absorbance values of each sample, using plotted standard curve made against different standard concentrations.

Serum Total Oxidative Stress (TOS; µmol/L)

Total oxidant status or oxidative stress (TOS) was measured as per calorimetric method explained by Erel (2004) with few modifications. Briefly, 35 µl serum samples were added in Eppendorf tubes arranged in two rows and 225 µl of reagent 1 (R1) was disseminated in both rows. Absorption of the samples added in first row was evaluated at two wavelengths (560 & 800 nm) through standard spectrophotometer (Thermo scientific Multi-scan G0, Thermo Fisher®). Then reagent 2 (R2) was added in the second row of samples which were further placed for incubation at 25°C for 04 minutes. After incubation, absorption was again estimated at two wavelengths (560 & 800 nm) through spectrophotometer. The assay was standardized using H_2O_2 and all the values were calculated using the formula,

Total oxidative stress (TOS) µmol/L = Absorbance /0.85 -0.204

Statistical analysis

Obtained data was statistically evaluated using ANOVA through factorial statistical analysis and the mean values of different groups were compared through Duncan's multiple range test while final results of the experiment as per group level was estimated using statistical software (MSTATC) and the level of significance was $p \leq 0.05$.

RESULTS

Feed intake

Feed intake (g/mice/day) of the groups have been presented in Figure 1. Feed intake of group administered SP alone was non-significant while feed intake of groups fed malathion alone (M1 and M2) was significantly lower during all four weeks of experiment when compared with control. In combination groups (SPM1 and SPM2) feed intake of both groups were non-significant during all weeks of experiment except for SPM2 at week 2 where feed intake was significantly lower when compared with control.

Body weight

Weekly body weight of all groups has been presented in Figure 2. Body weight of groups administered malathion alone (M1 and M2) was significantly lower however, the values of groups SP and SPM1 were non-significantly different when compared with control during all four weeks of experiment. Unlike body weight of SPM2 during week 2 which was significantly lower, its body weight during all other weeks was non-significantly different with respect to control group.

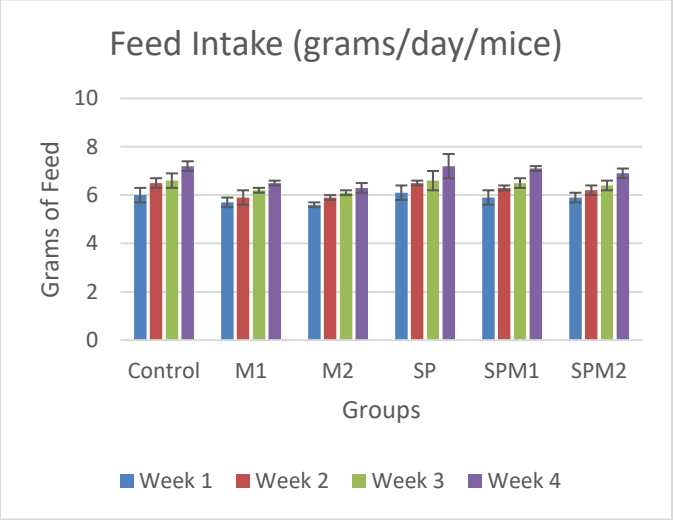

Figure 1: Feed intake (g/mice/day) of mice fed two levels of malathion (M1 and M2) and *Spirulina platensis* for 28 days of trial

(Description of abbreviation: M1=40mg/kg BW malathion; M2=60mg/kg BW malathion; SP=1% *Spirulina platensis* in feed)

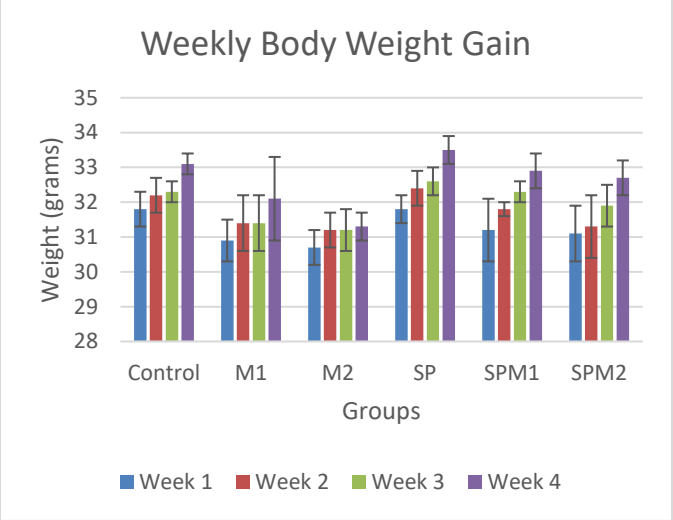

Figure 2: Weekly body weights of different groups fed malathion alone and/or in combination with *Spirulina platensis*

(Description of abbreviation: M1=40mg/kg BW malathion; M2=60mg/kg BW malathion; SP=1% *Spirulina platensis* in feed)

Relative organ weights

Relative weights of different organs in each group have been shown in Figure 3.

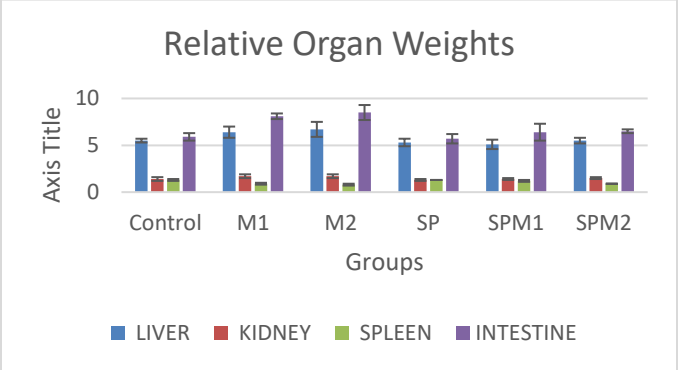

Figure 3: Relative organs weight of mice administered different levels of malathion and *Spirulina platensis* at day 28 of experiment.

(Description of abbreviation: M1=40mg/kg BW malathion; M2=60mg/kg BW malathion; SP=1% *Spirulina platensis* in feed)

Liver and Kidney:

Relative weights of liver and kidney of groups SP, SPM1 and SPM2 were non-significantly different while weights of groups M1 and M2 were significantly increased when compared with control.

Spleen:

Relative weight of spleen of groups fed malathion alone (M1 and M2) were significantly decreased while weights of groups SP and SPM1 were non-significantly different as compared to control.

Intestine

: Relative intestine weight of groups SP and SPM1 were non-significant while weights of groups M1, M2 and SPM2 were significantly increased when compared with control group.

Hematological parameters

The values of different hematological parameters of each group have been presented in Figure 4.

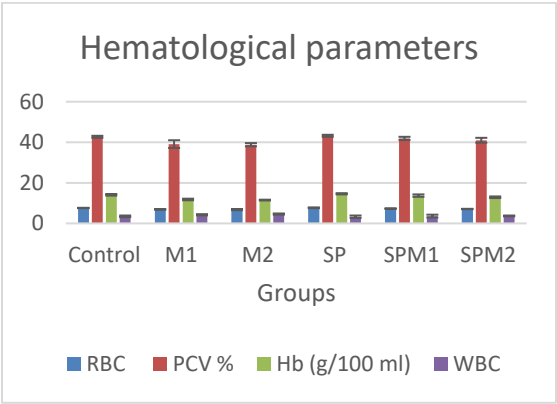

Figure 4: Hematological parameters of mice administered different levels of malathion and *Spirulina platensis* at day 28 of experiment

(Description of abbreviation: M1=40mg/kg BW malathion; M2=60mg/kg BW malathion; SP=1% *Spirulina platensis* in feed)

Erythrocytic count:

Erythrocytic count in groups M1, M2 and SPM2 were markedly lower while values of groups SP and SPM1 were non-significantly different when comparison was made with control group.

Leukocytic count:

Leukocytic count of groups fed malathion alone (M1 and M2) were significantly increased while leukocytic counts of groups SP, SPM1 and SPM2 were non-significantly different compared to control group.

Hemoglobin:

Hemoglobin concentration of groups M1, M2 and SPM2 were significantly lower while values of groups SP and SPM1 were non-significant compared with control group.

Hematocrit:

Hematocrit percentage of groups M1, M2 and SPM2 were significantly lower while values of groups SP and SPM1 were non-significantly different compared to control.

Serum biochemical parameters

The values of different serum biochemical parameters of all groups have been presented in Table 2.

Total proteins, albumin and globulin:

The values of serum albumin, total protein and globulin of groups M1, M2 and SPM2 were significantly lower while these values in groups SP and SPM1 were non-significantly different compared to control:

Alanine aminotransferase (ALT):

Serum ALT values of groups M1, M2 and SPM2 were significantly raised while these values of groups SP and SPM1 were non-significant when comparison was made with control group.

Urea and creatinine:

Serum urea and creatinine values of groups SP and SPM1 were non-significant while these values of groups M1, M2 and SPM2 were significantly increased compared to control group.

Gross lesions

Mice in control group did not exhibit any type of gross pathology on any of the visceral organ. Liver and kidney were normal in size, color and consistency (Figure 5A and 5C). Spleen and intestine were also normal and no hemorrhages were noticed. Similar pattern was also noticed in SP group where *Spirulina platensis* was given alone. Group M1 receiving 40 mg/kg malathion showed enlarged liver and kidney with mild to moderate hemorrhages upon their surfaces. Swelling was noticed on intestine and there was regression of the size of spleen when compared to control group. In group M2 (60 mg/kg malathion), color of liver was darker with friable consistency while size of the kidneys were also increased with several hemorrhages on the surface (Figure 5E). Pin-point hemorrhages were noticed upon the surfaces of both liver and kidney. In group SPM1, no anatomical as well as gross pathological deviation was noticed. Liver and kidney were normal in size and color. Similarly spleen and intestine were more or less similar to control. In group SPM2, liver (Figure 5B), kidney (Figure 5D) and intestine were normal except the presence of few hemorrhages on their surfaces while spleen was congested as compared to control.

Table 2: Serum biochemical parameters of mice administered different levels of malathion and *Spirulina platensis* at day 28 of experiment[1, 2]

Group	T. Proteins	Albumin	Globulin	Urea	Creatinine	ALT
Control	6.1±0.1	3.1±0.1	3.0±0.1	23.6±1.2	0.3±0.1	51.1±3.1
M1	4.9±0.2*	2.2±0.1*	2.7±0.1*	38.5±2.1*	0.5±0.3*	101.1±2.9*
M2	4.5±0.1*	2.1±0.0*	2.5±0.1*	43.0±1.4*	0.5±0.2*	109.5±4.8*
SP	6.2±0.1	3.1±0.0	3.1±0.0	24.1±0.7	0.3±0.0	52.4±2.9
SPM1	5.6±0.1	3.0±0.1	3.0±0.0	25.0±1.5	0.3±0.0	54.6±3.3
SPM2	5.8±0.1*	2.8±0.1*	2.9±0.0*	28.5±1.3*	0.4±0.1*	68.1±3.6*

[1]Values with (*) are significantly different from control (P>0.05)

[2]Description of abbreviation: M1=40mg/kg BW malathion; M2=60mg/kg BW malathion; SP=1% Spirulina platensis in feed

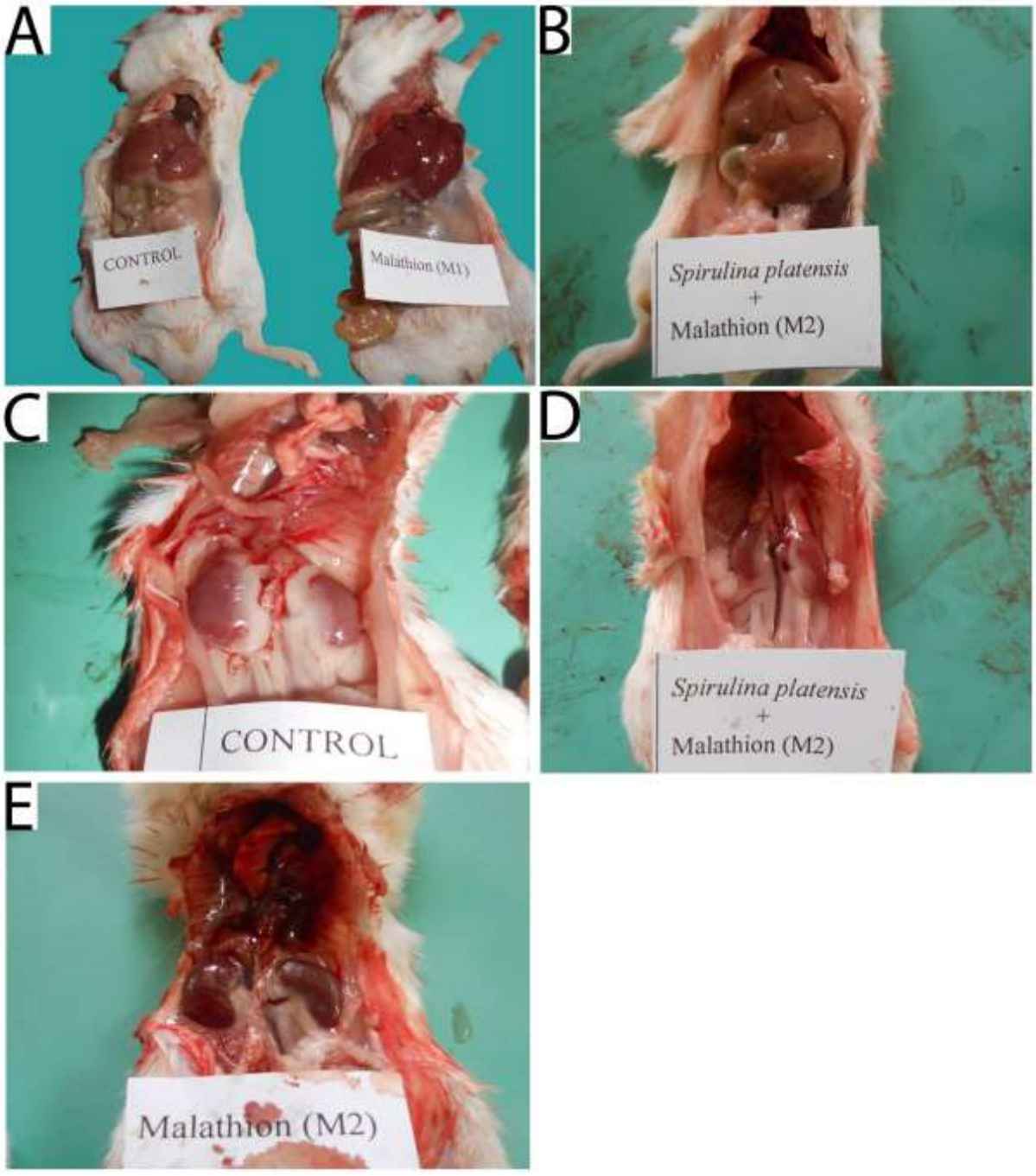

Figure 5: Gross pathology of liver and kidney; 5A: Normal colored liver in control group as compared to larger and dark colored liver in malathion treated mice; 5B: Mice of group SPM2 presented liver with normal size and color; 5C: Mice of control group presenting normal kidneys embedded within fat tissues; 5D: Mice of group SPM2 showed normal sized kidneys; 5E: Mice of group M2 showing enlarged and dark colored kidneys.

Histopathological examinations

Liver

A normal picture of hepatic parenchyma was observed in control group with well-arranged hepatic cords, normal sinusoidal spaces and regular hepatocytes. The nuclei within hepatocytes were normal and chromatin material was well enclosed within nucleolus (Figure 6A). Similar pattern of hepatic parenchyma was observed in group given *Spirulina platensis* alone.

In M1 group, vacuolar degeneration (mild degree) was observed in cytoplasm. Nuclei were pyknotic nuclei within hepatocytes at some places along with moderate degree of congestion and cellular infiltration throughout the parenchyma of lover (Figure 6B). In M2 group much severe alterations were observed as compared to M1 group. Cell necrosis was prominent at some places which was well corroborated by the pyknosis of hepatocytes. Cellular infiltration was also prominent and vacuolar degeneration (moderate degree) was also noticed while cell swelling caused reduction in sinusoidal spaces (Figure 6C).

In group E (SP+M1), normal parenchyma of the hepatocytes was noticed and hepatocytes were present in a normal state within distinct hepatic cords. Apart from cellular infiltration at few places, hepatic nuclei were normal in appearance containing fine chromatin material suggesting the mitigating effects of *Spirulina platensis* against malathion (Figure 6D). In group F (SP+M2) liver parenchyma was normal however nuclei were pyknotic nuclei at some places suggesting necrosis of cells. Cellular infiltration was also present at some places and in short the intensity of lesions was comparatively lesser when compared to individual malathion groups.

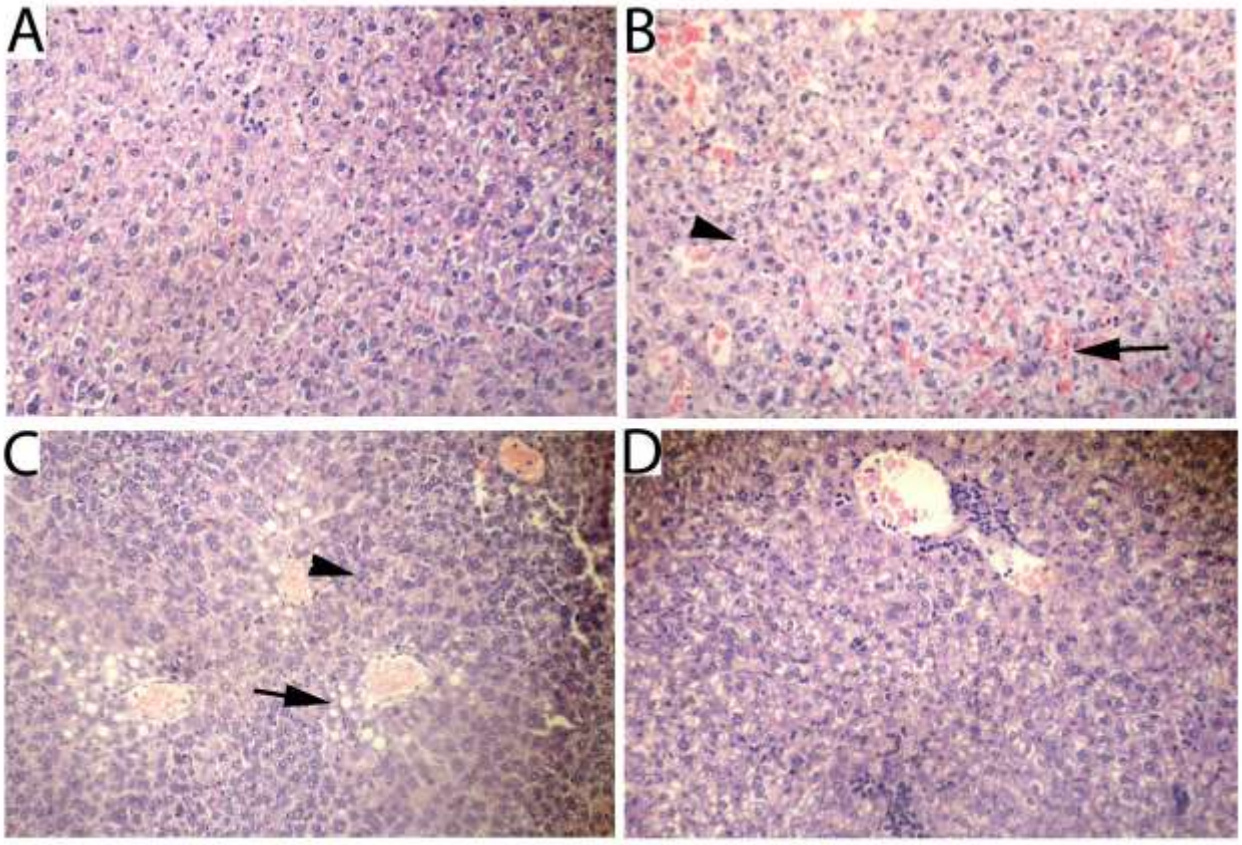

Figure 6: Photomicrographs of liver of different groups (H & E Staining 200X); 6A: Group A (control) showing normal hepatic parenchyma; 6B: Group B (M1) showing congestion (arrow) and pyknotic nuclei (arrow head); 6C: Group C (M2) showing fatty change (arrow) and pyknotic nuclei (arrow head); 6D: Group E (SPM1) showing normal hepatic parenchyma with only few pyknotic nuclei at scattered places.

Kidney

The parenchyma of kidney in control group was regular with normal nuclei within the epithelial cells of the tubules. The control group also presented clear urinary spaces and glomeruli were also normal in appearance (Figure 7A). Renal parenchyma of group fed SP alone was normal in appearance just like control group. Renal

cells contained normal nuclei with nucleolus presenting fine and well-arranged chromatin material and the urinary spaces were also normal.

In M1 group renal parenchyma showed cellular infiltration. The pyknotic nuclei were observed in the epithelial cells of tubules at few places while at few places fluid was present in lumen of renal tubules along with mild to moderate degree congestion in the renal parenchyma. The pathological alterations became severe in M2 group as compared to M1 group. Moderate to severe degree of congestion was noticed throughout the renal parenchyma along with moderate degree of pyknotic nuclei in the medullary region. Occlusion of fluid in urinary spaces was also noticed along with tubular degeneration throughout renal parenchyma (Figure 7B).

In group E (SP+M1) nuclei of cells were normal in appearance with fine nucleolus containing clear chromatin material. Only mild degree congestion was observed at few places. The renal parenchyma was almost normal suggesting the mitigative effects of *Spirulina platensis* against malathion associated alterations as observed in the group fed M1 alone. In group F (SP+M2) renal parenchyma was nearly normal with normal nuclei of epithelial cells in the tubules having fine nucleolus and chromatin material (Figure 7C). However urinary spaces at some places were obstructed with fluid and mild degree congestion was also observed at some places suggesting partial amelioration caused by *Spirulina platensis* when coupled with M2 level of malathion.

Total antioxidant capacity (TAC) and oxidative stress (TOS)

The values of total antioxidant capacity (TAC) and total oxidative stress (TOS) have been presented in Figure 8. TAC values of groups fed malathion alone (M1 and M2) along with group SPM2 were significantly lower while that of groups SP and SPM1 were non-significant when compared with control. However the values of TOS were significantly raised in groups M1, M2 and SPM2 while that of groups SP and SPM1 were non-significantly different when compared to control group.

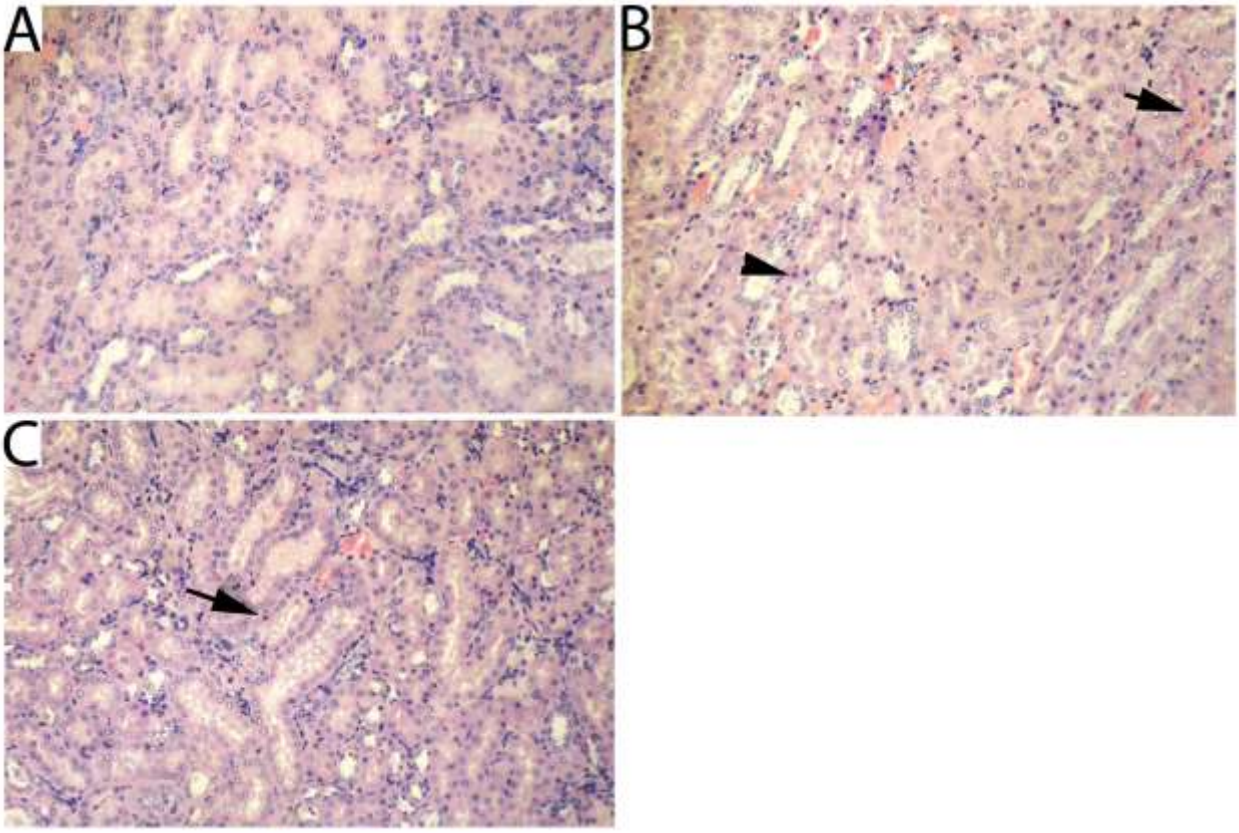

Figure 7: Photomicrograph of kidneys of different groups (H & E Staining 200X); 7A: Control showing normal renal parenchyma with nuclei having nucleolus in tubular epithelial cells; 7B: Group C (M2) showing congestion (arrow) and pyknotic nuclei (arrow head) in tubular epithelial cells; 7C: Group F (SPM2) showing normal renal parenchyma with mild degree of pyknotic nuclei (arrow head) indicating amelioration with *Spirulina platensis.*

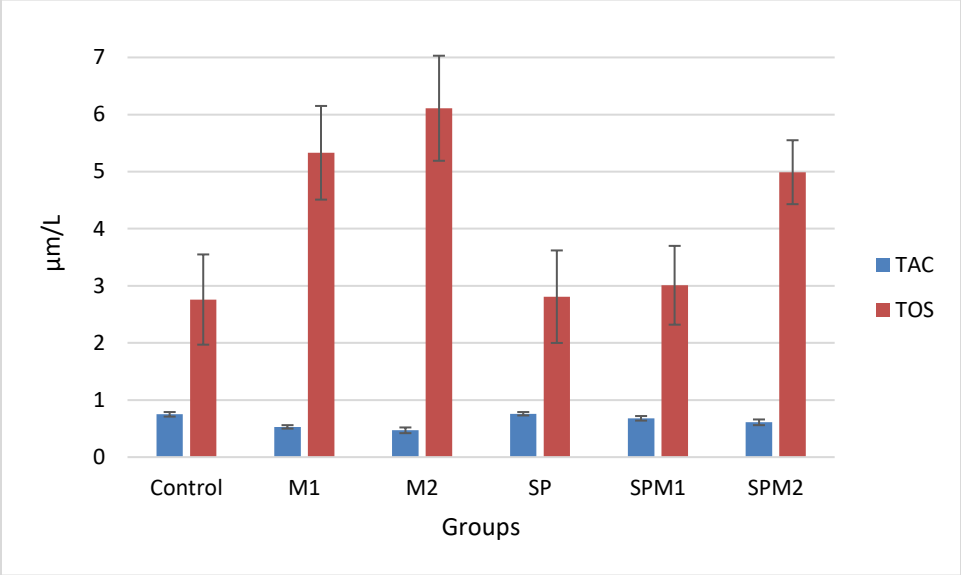

Figure 8: Total antioxidant capacity (TAC) and total oxidative stress (TOS) of groups fed different combinations of malathion and *Spirulina platensis* (Mean±SD)

(Description of abbreviation: M1=40mg/kg BW malathion; M2=60mg/kg BW malathion; SP=1% *Spirulina platensis* in feed)

DISCUSSION

Organophosphates (OP) are the chemical compounds which are often referred to as "anticholinesterases" due to their ability of inhibiting cholinesterase activity. After Second World War, thousands of different chemicals have been synthesized in order to achieve species specificity i.e. less toxic to mammals and more toxic to insects and malathion is one of the example of those chemicals. This compound is efficiently being used since half century as a potent insecticide to crops. Their extensive use in the crops have severely contaminated the environment of some non-targeted species like humans, animals and aquatic ecosystems. The potential routes of OP contamination are oral, dermal and inhalation (Ahmad et al. 2021).

The present experiment aimed to describe the mitigation potential of *Spirulina platensis* upon malathion induced toxicopathological, haematological and serum biochemical alterations in white Swiss albino mice. Use of 1% SP in feed was found sufficient to ameliorate malathion induced alterations at 40mg/kg level while such mitigation became absent or partially present when malathion levels were increased to 60mg/kg ultimately leading to the fact that 1% SP level became saturated at higher levels of malathion.

Feed intake of the mice supplemented with malathion alone was significantly lower when compared with control confirming the adverse effects of malathion upon the feed utilization of individual mice. When SP was given with malathion, adverse effects upon feed intake at lower levels of malathion got ameliorated while only a partial amelioration was observed in case of 60mg/kg malathion. Body weight gain was adversely affected with supplementation of malathion in feed and such suppression in growth parameter was observed throughout the experiment. Body weight was found ameliorated when SP was given with lower malathion level but when level of malathion was increased scenario of amelioration got partial. Decrease in the body weight gain may be associated with a reduced pattern of feed intake in malathion treated individuals as observed in this study. Relative liver, kidney and intestine weights were significantly higher in malathion treated groups while SP addition significantly ameliorated these alterations at lower malathion levels while amelioration became partial when higher level (60mg/kg) was used. However malathion treated groups represented reduced spleen weight which was ameliorated in lower malathion treatment group. For a normal body liver is meant to detoxify and remove the hazardous/toxic materials from the body and hence these metabolized/detoxified materials are being removed from the body through kidneys. An increase in the relative kidney and liver weights may occur possibly in an attempt to eradicate malathion from the body. The accessible literature does not present any study which confirm the mitigation of malathion associated alterations by *Spirulina platensis*.

Regarding hematological parameters, malathion treated groups presented a reduction in erythrocytic count, hematocrit percentage and hemoglobin concentration while an increase in leukocytic count of mice. Anaemia is a condition characterized by decreased red blood cells in the peripheral blood and is

identified by a reduced erythrocytic count, haemoglobin concentration and hematocrit percentage below the reference values for a given specie (Abidin et al. 2013). The haematological alterations observed in this study are generally associated with suppression of hematopoietic system and sometimes due to iron deficiency anaemia preferably due to improper iron absorption (Abidin et al. 2013). Results of different experiments have suggested the altered hematological parameters due to malathion (Kalender et al. 2006; Yehia et al. 2007) while Celik et al. (2009) has reported that few OPs can increase WBC levels which has also been noticed in this study. Leukocytosis can occur due to increased mobilization of leukocytes causing a state of stress in the animals. Increase in WBC observed in this study may occur due to response of body's immune system upon the stress conferred by malathion. Certain other alterations associated with malathion (e.g. Inflammatory cell infiltration, tissue damage and necrosis) may also be a factor for increased leukocytic count in mice (Kalender et al. 2006).

Serum total protein, albumin and globulin concentrations were significantly reduced while significant increase in ALT, urea and creatinine concentrations were observed in malathion treated groups. Albumen is a protein which is being produced by liver and its concentration gets altered by OPs administration (Yousef et al. 2006; Ogutcu et al. 2008; El Okle et al. 2022). As reduction of albumen levels are normally indicative of liver damage so it can be well attributed that malathion, an organophosphate, may alter metabolism and synthesis of protein and free amino acids within liver. When cell membrane of hepatocytes is damaged certain enzymes including ALT are being secreted into blood stream causing an increase in ALT levels within serum and are considered as markers of liver damage (Ncibi et al. 2008; Makki et al. 2022). Increase in ALT has been reported by many authors in different animals administered with certain organophosphates (Khan et al. 2005; Celik et al. 2009; Ncibi et al. 2008; El Okle et al. 2022). Similarly higher urea and creatinine levels are considered to be the markers for kidney damages (Abidin et al. 2013).

Malathion treated groups exhibited reduced antioxidant capacity when compared with control. Similarly significant increase in total oxidant status was also noticed in these groups confirming the oxidative stress associated with dietary supplementation of malathion in Swiss albino mice. Our results are in agreement with Selmi et al. (2015); Saulsbury et al. (2009) and Shafiee et al. (2010) who also reported malathion induced oxidative stress in mice. Malathion causes oxidative stress through the generation of certain reactive oxygen species (ROS) leading to reduced cellular antioxidant defense activity within the body. Addition of SP in diet efficiently ameliorated these alterations in mice at lower levels while amelioration became partial when SP was used with higher Malathion levels. Such findings have not been reported previously by any researcher. However, few reports are available in which different organic extracts have been shown to ameliorate chemical-specific oxidative stress in animals (Liu et al. 2021; Widowati et al. 2022).

Using certain herbal and natural products is a latest and most appealing trend nowadays to combat the adverse effects of different hazardous materials and chemicals. One such compound is Spirulina platensis (SP) which is officially being cultured for food purposes due to its high nutritious and therapeutic values. SP can successfully ameliorate the liver damages induced by carbon tetrachloride, mercury and cadmium in different laboratory animals (Sharma et al. 2007; Karadeniz et al. 2009). The effectiveness of SP against toxic effects of deltamethrin (a pesticide) have been reported in rats (Abdel-Daim et al. 2013) however; no study is available in the accessible literature which declares the mitigation potential of SP against malathion. Results of this study showed a good ameliorative of malathion (40 mg/kg BW) induced oxidative stress and toxicopathological alterations by SP (1% in feed) however, such mitigation became limited when 60mg/kg malathion was given to the mice.

CONCLUSIONS

From these findings, it can be concluded that 1% *Spirulina Platensis* efficiently ameliorated the adverse effects of 40mg/kg malathion, however; this amelioration became partial when 60 mg/kg malathion was used. However, the precise percentage levels of malathion and SP to cause such mitigation is yet to be determined.

Supplementary materials

Not applicable

Author contributions

AK and ZA conceptualized the study, wrote and proof read the manuscript; IM, MAQ, ARK, AR, ZF and MAI performed the experimentation and RK proof read the manuscript.

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Institutional Review Board Statement

The study was approved by University of Agriculture Faisalabad Pakistan Synopsis Approval Committee and an ethics committee for use of animals in research prior to commencing the study and performed in accordance with relevant institutional and national guidelines and regulations.

Informed Consent Statement

Not applicable.

Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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