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Original article

Ameliorative effect of *Nigella sativa* conjugated silver nanoparticles against chromium-induced hepatotoxicity and renal toxicity in mice

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ABSTRACT

Hexavalent chromium induces oxidative stress in the liver and kidney. Therefore an *in vivo* study was designed to investigate the modulatory effect of biosynthesized AgNP against Cr (VI) induced hepatotoxicity and nephrotoxicity. The organs index, serum level of ALT, AST, ALP, MDA, total protein and creatinine were measured. The histopathology and micrometry of the liver and kidney were examined. The liver index was significantly increased (0.098 ± 0.13 g) with slight increase in kidney index in Cr exposed group. The serum level of ALT (163.0 ± 5.5 U/L), AST (484.0 ± 10.7 U/L), ALP (337.6 ± 9.6 U/L), MDA (641.2 ± 29.2 U/L), and creatinine (2.9 ± 0.2 mg/dL) were significantly increased ($P \leq 0.05$) with significant decrease in total protein level (2.9 ± 0.2 g/dL) ($P \leq 0.05$) in chromium treated group. In histopathology, distorted hepatic cords, necrosis, damaged glomerulus and Bowman's capsule were observed. Micrometric studies of the liver and kidney showed significant increase in size of hepatocytes (1188.2 ± 467.7 μ^2) and their nuclei (456.4 ± 206.7 μ^2), ACSA of Bowman's capsule (11835.5 ± 336.7 μ^2) and glomerulus (9051.8 ± 249.8 $\mu^{2)}$ in Cr (VI) treated group. The size of brush border (10.1 ± 3.0 μ) was significantly reduced in Cr (VI) treated group however the ACSA of lumen was not significantly changed. With the administration of NSSE and *Nigella sativa* AgNPs, decreased the oxidative damage caused by Cr (V).

1. Introduction

During human evolution, advanced technologies may become the source of pollution in the air, soil, and water (Onita et al., 2021) including heavy metals (Suchana et al., 2021). The heavy metals (HMs), in the environment, remain stable and redistributed among different components of the biological systems (Suchana et al., 2021). As HMs persist for a long time, bio-accumulates and biomagnified in the food chain (Kumar et al., 2019), especially harmful for animals present at higher trophic levels of the food chain like humans (Ali et al., 2019a, 2019b). In the human body, heavy metals are distributed and compartmentalized in the tissues and cells, where bind to nucleic acid and proteins causing damage to these biomolecules, hence disrupting cellular functions and induce damage in the liver, kidney, lungs, central nervous system (Engwa et al., 2019) and reproductive system (He et al., 2020). Chromium (III) is an essential element required for the metabolism of proteins and lipids as well as a cofactor for insulin action (Balali-Mood et al., 2021) whereas Cr (VI) is expendable and destructive to life (Ukhurebor et al., 2021), linked with a chain of diseases. According to International Agency for Research on Cancer report (IARC 2018), Cr (VI) belongs to the group I category of an occupational cancer-causing agent (Balali-Mood et al., 2021). ROS, the most significant free radicals produced by (Selamoğlu et al., 2017) environmental exposure to Cr (VI), induces oxidative stress, multi-organ toxicity including anemia, respiratory tract dysfunction, asthma, liver, kidney, and reproductive system failure in males (Sankhla and Kumar, 2019). Oxidative stress may also result

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by weak antioxidant defense system, may lead to condition where antioxidant compounds from external sources must be delivered in order to maintain normal body functions (Selamoğlu et al., 2009, 2015; Selamoğlu & Yılmaz, 2014).

The liver is a vital organ that involves in the bioaccumulation, transformation, detoxification, and excretion of toxicants, more susceptible to damage (Suchana et al., 2021) resulting in hepatocyte toxicity and cirrhosis (Ye et al., 2018). Furthermore, the main site of serum protein especially albumin synthesis occurs in liver cells. Low level of protein content and low mobility present changes in the rate of production and degeneracy of proteins. Elevated activities of biochemical markers for the liver such as serum aspartate aminotransferase or glutamic-oxaloacetic transaminase, serum alanine aminotransferase or glutamic pyruvic transaminase, and alkaline phosphatase in blood or tissues reflect their outflow from the liver due to impairment in hepatocytes (Trivedi et al., 2021). Whereas the kidney is involved in osmoregulation as well as metabolic excretion of toxicants leading to histopathological alterations (Suchana et al., 2021). *Nigella sativa* (NS) belongs to the family Ranunculaceae (Bashir et al., 2021), a traditionally used medicinal plant (Hannan et al., 2021). *Nigella sativa* seed oil is a notable source of bioactive compounds, vital oils, fatty acids, polyphenols, tocopherols, and phytosterols. Thymoquinone (TQ) is the most important bioactive compound with lot of health-promoting properties (Mazaheri et al., 2019). Black seeds possess a broad spectrum of activities including antihypertensive (Maideen et al., 2021), antidiabetic (Vijayakumar et al., 2021), antimicrobial (Hossain et al., 2021), anticancer (Almatroudi et al., 2020), diuretic (Aghamirei et al., 2022), immunomodulatory, anti-inflammatory (Ikhsan et al., 2018), anti-schistosomiasis, analgesics, antioxidant, gastroprotective, hepatoprotective (Almatroudi et al., 2020) and renal protective activities (Hannan et al., 2021).

Various studies manifested the application of *Nigella sativa* in diverse fields, but meager reports exist to reveal the application of *Nigella sativa* against the Cr (VI) induced hepatotoxicity and renal toxicity. Recently, their scanty investigation found regarding the protective action of *Nigella sativa* against chromium caused hepatotoxicity and renal toxicity in mice. Hence the current study was designed to find out the protective potential of *Nigella sativa* against chromium (VI) induced damage to the liver and kidney in male albino mice.

2. Materials and methods

2.1 Paul###

The trial techniques for all animals were managed and regulated as per local and international controls. The protocol for the present study was allowed by the institutional bio-ethical committee of the Government College University, Lahore with reference No. GCU-IIB-364 dated 6th October 2020. All animal trials were executed according to local and worldwide procedures followed by the Wet op de dierproeven (article 9) of Dutch law (international) and an associated rule planned via the Bureau of Animal Research Licensing, Local University as detailed in our earlier papers (Hussain et al., 2020; Ara et al., 2020; Ali et al., 2019a, 2019b, 2020; Khan et al., 2019; Mumtaz et al., 2019; Mughal et al., 2019; Dar et al., 2019). The rearing and use of animal were carried out using NIH Publication "Guide for the Care and Use of Laboratory Animals" (NRC 2011) and by the local bioethical committee of the University on animal experimentation.

2.2. Chemicals used

The tested chemical $K_2Cr_2O_7$ with 99% purity was procured from Merck (Merck, Darmstad, Germany). Black seeds were used in the study and purchased from the local market in Lahore. All other chemicals of analytic grade were used in the experiment. Distilled water was used to prepare stock solutions. All working solutions were prepared fresh from stock solution with distilled water.

2.3. Preparation of Nigella sativa seed extract

The seed extract was prepared by using the method followed by (Kannayiram et al., 2019) with slight modification. *Nigella sativa* seeds were purchased from Akbari Store, Lahore, washed with water, and dried in air at room temperature. The seeds were ground into a fine powder via sieving mesh and soaked in ethanol (10 g powder into 100 mL ethanol). The mixture was allowed to shake well on an orbital shaker for 24 hrs. The NSSE was filtered by using Whatman filter paper No.1 and at 37 °C dried in the incubator. The resultant powder was kept at 4 °C for further use. The dose was prepared from NSSE by using body weight, 50 mg/ kg BW.

2.4. Preparation of Nigella sativa AgNPs

Nigella sativa seed powder (5 g) was mixed with 100 mL of distilled water, and boiled for 20 min at 100 °C. 10 mL seed extract was added with 0.1 mM solution of $AgNO_3$ with constant stirring for 10 min at 25 °C. The mixture was incubated for the next 2 h at 80 °C. The reaction mixture was placed at 25 °C for 24 h in a dark room. After 24 h, the mixture was turned from light brown to a dark brown color indicating the reduction of Ag+ ions. To remove impurities mixture was washed with ethanol (twice) and distilled water thrice at 12000 rpm, the pellet contained NS AgNPs. Prepared NS AgNPs were dried at 70 °C for 72 h. in over and stored at 4 °C for future use (Chand et al., 2021). The dose was prepared from *Nigella sativa* AgNPs by using body weight, 50 mg/ kg BW.

2.5. Experimental design

During the experiment, *Mus musculus* (male) of age 2 to 3 months, weight 30 to 40 g were kept as model animals. The albino male mice were purchased from the animal house of the Department of Zoology, Government College University, Lahore, Pakistan. Mice received feed no. 14 and drinking water ad-labium. The temperature in the animal house was maintained at 23 ± 2 °C, humidity at 45–50 %, and 12 hrs. dark-light cycle.

2.6. Administration of dose

After 15 days of acclimatization, forty mice were divided into 8 equal groups i.e., 5 mice each, including one control group and seven other treatment groups. The newly prepared solutions (0.2 mL for every one mouse of each representative group) of *Nigella sativa* seed extract and *Nigella sativa* mediated silver nanoparticles at the dose rate of 50 mg/kg BW were administered orally employing gavage once a day. Chromium solution 1.5 mg/kg BW, orally from $K_2Cr_2O_7$ was provided orally.

Control: Group I was continued to receive chromium-free drinking water (*ad-labitum*) (60 days).

Cr: Group II was continued to receive chromium 1.5 mg/kg BW, via gauge once a day (60 days).

NS: Group III was continued to receive *Nigella sativa* seed extract, 50 mg/kg BW, via gauge once a day (60 days).

NS + NP: Group IV was continued to receive *Nigella sativa* AgNPs, 50 mg/kg BW, via gauge once a day (60 days).

NS (P): Group V was continued to receive NS seed extract, 50 mg/kg BW + Cr, 1.5 mg/kg BW, via gauge once a day (60 days).

NS + NP (P): Group VI was continued to receive *Nigella sativa* AgNPs, 50 mg/kg BW + Cr, 1.5 mg/kg BW, via gauge once a day (60 days).

NS (T): Group VII was continued to receive Cr, 1.5 mg/kg BW (30 days) followed by *Nigella sativa* seed extract, 50 mg/kg BW, via gauge once a day (next 30 days).

NS + NP (T): Group VIII was continued to receive Cr, 1.5 mg/kg BW (30 days) followed by *Nigella sativa* AgNPs, 50 mg/kg BW, via gauge once a day (next 30 days) (Fig. 1).

2.7. Collection of Samples

The blood sample was collected by puncturing the vein from a cephalic vein or jugular vein of every mouse on the 61st day of the experiment. The blood collected from the mouse was shifted in EDTA coated 3 mL ImuMed vacutainer and stored at 4 °C for biochemical analysis. Blood was centrifuged to analyze biochemical parameters at 25 °C for 10 min at 2000 rpm to separate serum. Under deep anesthesia liver and both kidneys were removed via a long abdominal incision in the middle part. Samples were preserved in formalin for later use.

2.8. General observations

Behavioral modifications in mice for all groups were observed and recorded on regular basis. Each animal from all groups was weighed weekly, and the dose was administered accordingly. Similarly, the water consumption in terms of per gram body weight was obtained on daily basis. Liver and kidney weight were noted for each animal after collection.

2.9. Biochemical parameters

Blood was centrifuged to analyze biochemical parameters at 25 °C at 2000 rpm for 10 min to separate serum. The aliquots were stored at − 20 °C till further analysis. Alanine aminotransferase (ALT) (Helmy et al., 2019), aspartate aminotransferase (AST) (Helmy et al., 2019), alkaline phosphate (ALP) (Trapero et al., 2018, creatinine (Khamis et al., 2018) and total protein (Yılmaz and Ergün, 2018) were measured by commercially accessible diagnostic kits as described in Table 1.

2.10. Histopathology of liver and kidney

For histopathological analysis, the liver and kidney of each animal of each group, affixed with 10% formalin were processed by the traditional method using ascension grades of alcohol and xylene, paraffin wax embedding followed by microtomy at 5 µm. The tissue sections were stained by hematoxylin and eosin (H&E) staining for microscopic examination. The liver damage was standardized by steatosis, necrosis, hepatocyte degeneration, and micrometric analysis. Moreover, kidney criteria were steatosis, necrosis, regenerating nephron, fibrosis, nuclear fragmentation & condensation, and micrometric analysis (Khalaf et al., 2020).

2.11. Micrometric analysis

The micrometrical observations recorded include the size of hepatocytes and their nuclei and brush border. ACSA of Bowman's capsule, glomerulus, and lumen of proximal convoluted tubule were measured. All the data was put into a graph pad prism. The data was obtained from photographs at 40X from a microscope.

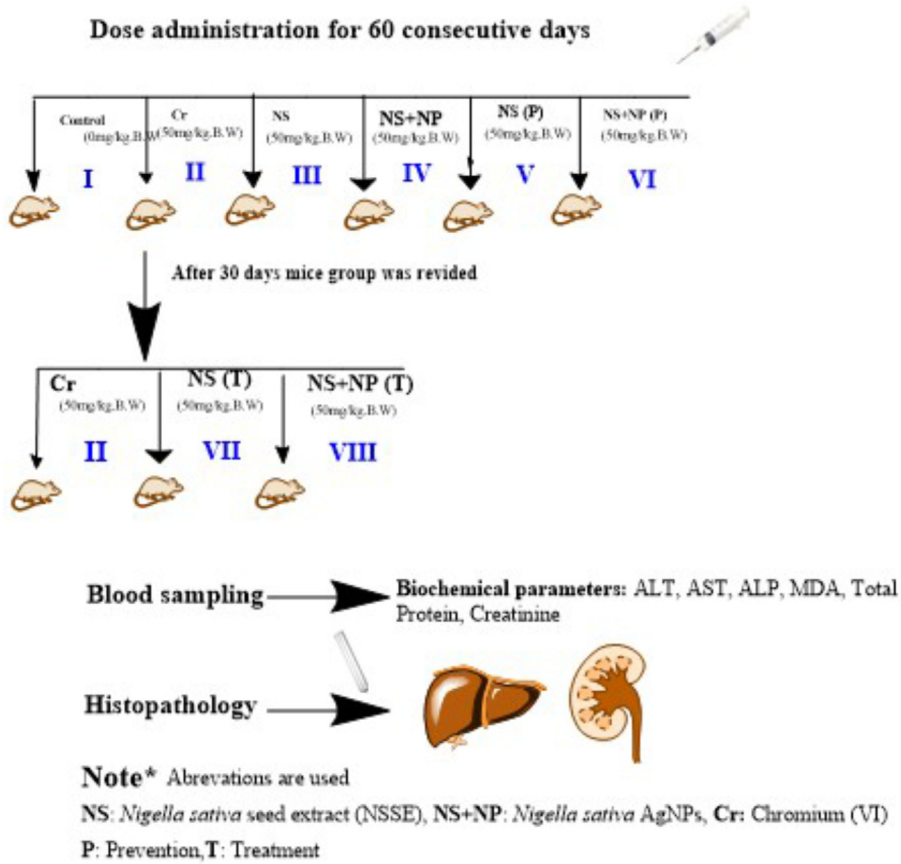

Fig. 1. Experimental scheme of study.

Table 1
Layout for kits used in current study.

Sr No	Biochemical parameter	Particulars of Kit	Reference
1	Alanine aminotransferase (ALT)	ALT diagnostic kit	(Helmy et al., 2019)
2	Aspartate aminotransferase (AST)	AST diagnostic kit	(Helmy et al., 2019)
3	Alkaline phosphate (ALP)	Commercial double-antibody sandwich ELISA kits	(Trapero et al., 2018)
4	Malondialdehyde (MDA)	Commercial Photometry diagnostic kits (Teb Pazhouhan Razi (TPR), Tehran, Iran)	(Amri et al., 2021)
5	Total protein	Commercial test kits (Bioanalytic Diagnostic Industry, Germany)	(Yılmaz and Ergün, 2018)
6	Creatinine	QuantiChrom™ CRNN assay kit (QC, CA)	(Khamis et al., 2018)

Following formula was used to calculate the CSA in each case (Ahmad et al., 2012):

$$CSA = \frac{(length \times width)}{4}\pi$$

2.12. Data analysis

Data obtained were analyzed and presented as mean ± SEM. Kolmogorov–Smirnov test was used to evaluate the normality of the data and then statistically analyzed by one-way ANOVA, with Dunnett's multiple comparison test, to find any significant difference among the means of treatment groups. GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was employed for data analysis. The $P \leq 0.05$ were observed as significant values.

3. Results

3.1. Water intake

Water intake was significantly different among different groups. In Cr treated group water intake was significantly increased (0.11782 ± 0.02222 mL/g/day) as compared to control (0.09024 ± 0.01412 mL/g/day) ($P \leq 0.05$). Administration of 50 mg/kg BW dose of NSSE and *Nigella sativa* AgNPs decreased the water intake as compared to Cr treated group. In treatment groups, water intake was reduced significantly in NS (P) (0.06935 ± 0.01357 mL/g/day) and NS (T) (0.06807 ± 0.01364 mL/g/day). However, in NS + NP (P) (0.09067 ± 0.01660 mL/g/day) and NS + NP (T) (0.11310 ± 0.02333 mL/g/day) reduction was not up to significant level (Fig. 2).

3.2. Physiological parameters

During the study, aggressive behavior was observed in chromium exposed mice that deceased over time. However, no signs of mortality were observed. An increase in hepatosomatic index (0.098 ± 0.13 g) up to a significant level ($p < 0.05$) was observed. However, in renal somatic index no significant difference was observed in chromium groups vs. treatment groups after 60 days (Table 2).

3.3. Biochemical parameters

The values of biochemical parameters (ALT, AST, ALP, MDA, total protein, and creatinine) were measured after 60 days of exposure to 1.5 mg/kg BW Cr and other treatments including NSSE and *Nigella sativa* AgNPs. Continuous Cr intake exerted negative effects on redox balance and treatment of NS and NS + NP modulated the toxic effects significantly.

ALT level was significantly elevated in all Cr exposed animals (163 ± 4.1 U/L) as compared to control (42.4 ± 1.9 U/L) ($P \leq 0.05$). Administration of 50 mg/kg BW dose of NSSE and *Nigella sativa* AgNPs significantly inhibited the level of ALT as compared to Cr groups. In treatment groups, ALT level was reduced significantly in NS (P) (92 ± 4.4 U/L), NS + NP (P) (80.4 ± 3.6 U/L), NS (T) (121.8 ± 3.3 U/L), and NS + NP (T) (107.8 ± 3.5 U/L) (Fig. 3).

AST level was significantly elevated in all Cr exposed animals (484 ± 8.1 U/L) as compared to control (84.2 ± 1.5 U/L) ($P \leq 0.05$). Administration of 50 mg/kg BW dose of NSSE and *Nigella sativa* AgNPs significantly inhibited the level of AST as compared to Cr groups. In treatment groups, AST level was reduced significantly in NS (P) (188.4 ± 10.8 U/L), NS + NP (P) (145 ± 10.6 U/L), NS (T) (214.6 ± 13.9 U/L), and NS + NP (T) (200.2 ± 11.8 U/L) (Fig. 3).

ALP level was significantly elevated in all Cr exposed animals (337.6 ± 7.2 U/L) as compared to control (106.2 ± 4.9 U/L) ($P \leq 0.05$). Administration of 50 mg/kg BW dose of NSSE and *Nigella sativa* AgNPs significantly inhibited the level of ALP as compared to Cr groups. In treatment groups ALP level was reduced significantly in NS(P) (189.6 ± 15.6 U/L), NS + NP(P) (187.2 ± 8.8 U/L), NS (T) (245.8 ± 10.1 U/L) and NS + NP (T) (194.8 ± 7.1 U/L) (Fig. 3).

MDA level was significantly elevated in all Cr exposed animals (641.2 ± 22.1 U/L) as compared to control (158 ± 3.7 U/L) ($P \leq 0.05$). Administration of 50 mg/kg BW dose of NSSE and *Nigella sativa* AgNPs significantly decreased the level of MDA as compared to Cr groups. In treatment groups, MDA level was reduced significantly in NS (P) (288 ± 61.9 U/L), NS + NP (P) (253 ± 9.3 U/L), NS (T) (348 ± 41.2 U/L), and NS + NP (T) (349.6 ± 31.7 U/L) (Fig. 3).

Total protein level was significantly decreased in all Cr exposed animals (2.94 ± 0.2 g/dL) in comparison to control (8.42 ± 0.5 g/dL) ($P \leq 0.05$). Administration of 50 mg/kg BW dose of NSSE and *Nigella sativa* AgNPs significantly elevated the level of total proteins as compared to Cr groups. In treatment groups total proteins level was improved significantly in NS (P) (6.16 ± 0.5 g/dL), NS + NP (P) (7.96 ± 0.3 g/dL), NS (T) (4.36 ± 0.2 g/dL) and NS + NP (T) (5.28 ± 0.4 g/dL) (Fig. 3).

Creatinine level was significantly increased in all Cr exposed animals (4.0 ± 0.1 mg/dL) in comparison control (0.8 ± 0.0 mg/dL) ($P \leq 0.05$). Administration of 50 mg/kg BW dose of NSSE and *Nigella sativa* AgNPs significantly decreased the level of creatinine

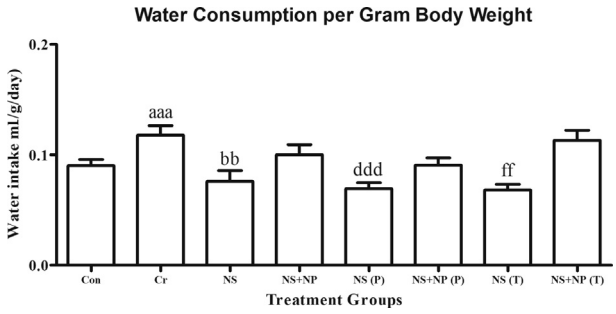

Fig. 2. Water intake measurement in experimental groups, Con; Control; Cr: chromium treated; NS: *NSSE* treated; NS + NP: *Nigella sativa* AgNPs treated; P: prevention; T: treatment. Different letters showing significant different of treatment groups with that of Cr treated group. Results represented in term of mean ± SEM. $P \leq 0.05$. Different letters showing significant different of treatment groups with that of Cr treated group. **Statistical icons:** a,b,c,d,e,f,g = $P \leq 0.05$. aa,bb, cc,dd,ee,ff,gg = $P \leq 0.01$. aaa,bbb,ccc,ddd,eee,fff,ggg = $P \leq 0.001$.

Table 2
Comparison in hepatosomatic index (HIS) and renal somatic index (RSI) of treatment groups.

	Treatment groups							
Organ	Con	Cr	NS	NS + NP	NS (P)	NS + NP (P)	NS (T)	NS + NP (T)
HIS (g)	0.05 ± 0.07	0.098 ± 0.13**aaa**	0.0577 ± 0.11**bbb**	0.0493 ± 0.041**ccc**	0.0474 ± 0.031**ddd**	0.0548 ± 0.0307**eee**	0.0545±0.0793**fff**	0.047 ± 0.03**ggg**
RSI (g)	0.01 ± 0.02	0.02 ± 0.06	0.02 ± 0.004	0.015 ± 0.007	0.015 ± 0.01	0.01616 ± 0.0246	0.01551 ± 0.0244	0.015 ± 0.0184

Con; Control; Cr: chromium treated; NS: *NSSE* treated; NS + NP: *Nigella sativa* AgNPs treated; P: prevention; T: treatment. Different letters showing significant different of treatment groups with that of Cr treated group. Results represented in term of mean ± SEM. $P \leq 0.05$. Different letters showing significant different of treatment groups with that of Cr treated group. **Statistical icons:** a,b,c,d,e,f,g = $P \leq 0.05$. aa,bb,cc,dd,ee,ff,gg = $P \leq 0.01$. aaa,bbb,ccc,ddd,eee,fff,ggg = $P \leq 0.001$.

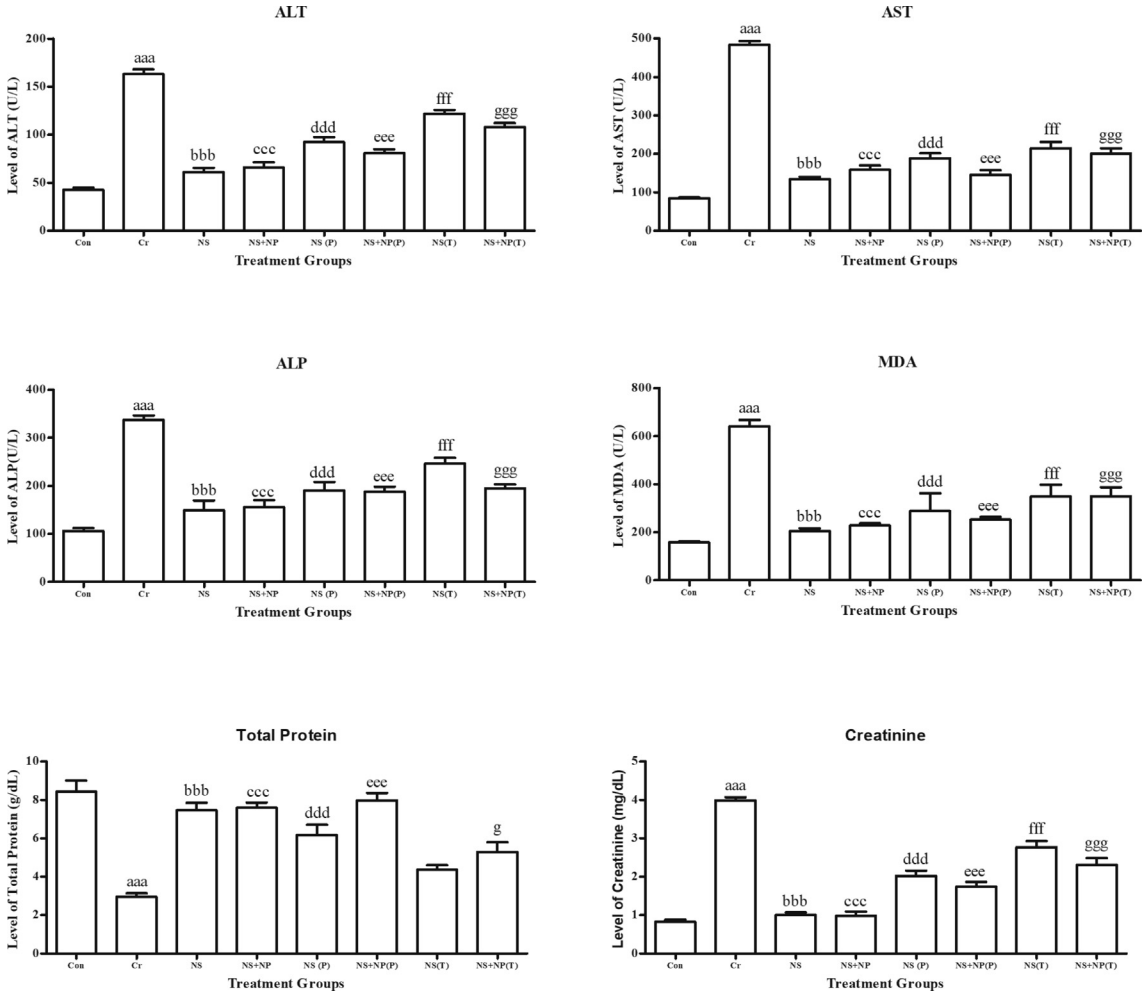

Fig. 3. Analysis of ALT, AST, ALP, MDA, Total Protein and Creatinine measurement in experimental groups. Con; Control; Cr: chromium treated; NS: *NSSE* treated; NS + NP: *Nigella sativa* AgNPs treated; P: prevention; T: treatment. Results represented in term of mean ± SEM. $P \leq 0.05$. Different letters showing significant different of treatment groups with that of Cr treated group. **Statistical icons:** a,b,c,d,e,f,g = $P \leq 0.05$. aa,bb,cc,dd,ee,ff,gg = $P \leq 0.01$. aaa, bbb, ccc, ddd,eee,fff,ggg = $P \leq 0.001$.

as compared to Cr groups. In treatment groups creatinine level was reduced significantly in NS (P) (2.0 ± 0.1 mg/dL), NS + NP (P) (1.7 ± 0.1 mg/dL), NS (T) (2.8 ± 0.1 mg/dL) and NS + NP (T) (2.3 ± 0.2 mg/dL) (Fig. 3).

3.4. Histopathology of liver and kidney

Histological slides of liver and kidney tissue of control groups showed well-organized histological structures including hepatocytes, hepatic cords, central vein, glomerulus was encircled by bowman's capsule, proximal convoluted tubules were with brush border, however distal convoluted tubule with intact endothelium. In Cr treated group abnormalities in liver and kidney sections were observed. During the microscopic examination, different alterations were found including nuclear fragmentation, atrophid nuclei of hepatocytes, derangement in hepatic cords and abnormal sinusoids, microvesicles, and microvesicles in hepatocytes indicating steatosis, massive vacuolar degeneration of hepatocytes with necrosis, a large number of Kupfer cells (Fig. 4) and bile duct metaplasia were the notable features (Supplementary Fig. 1). The different tissue section of kidney in the Cr group represented numerous histological alterations including degeneration of renal tubules, glomerular degeneration and disorganized bowman's capsule, necrosis (Fig. 5), fatty cell infiltration and microvesicles indicating steatosis, fibrocytes infiltration and fibrosis in interstitial tissues, nuclear fragmentation, and condensation, eccentric nuclei, and vacuolation (Fig. 6) were major features observed. However, in the treatment groups, liver and kidney structures recovered significantly.

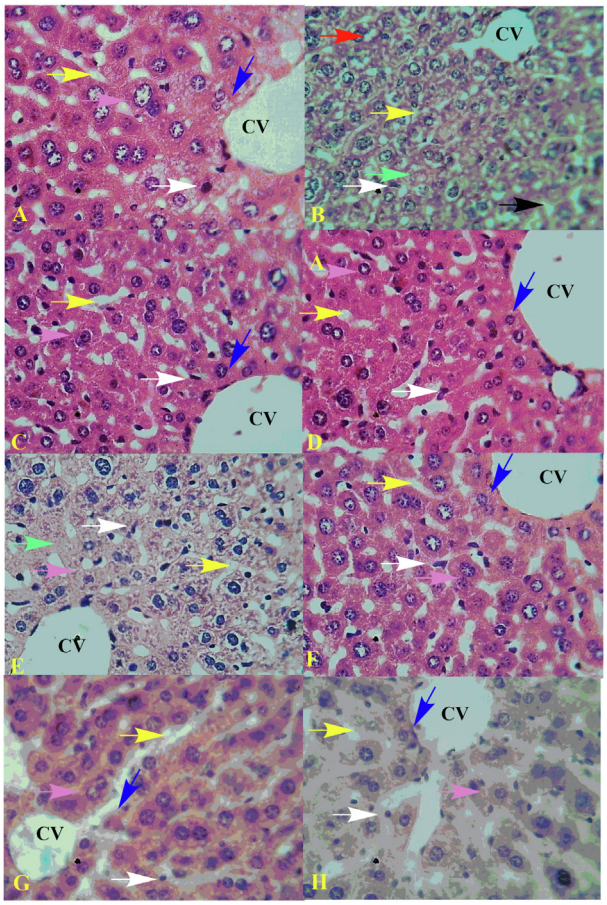

Fig. 4. Histopathology of Liver (40X) A: control; B: Cr; C: NS; D: NS + NP; E: NS (P); F: N + NP (P); G: NS (T); H: N + NP (T) treated groups. CV: central vein, Yellow arrows: sinusoids, purple arrow: normal hepatocytes, white arrow: kupfer cells: black arrow: necrosis, red arrow: micro vesicles in hepatocytes, green arrow: degenerating hepatocytes, blue arrows: hepatic cords.

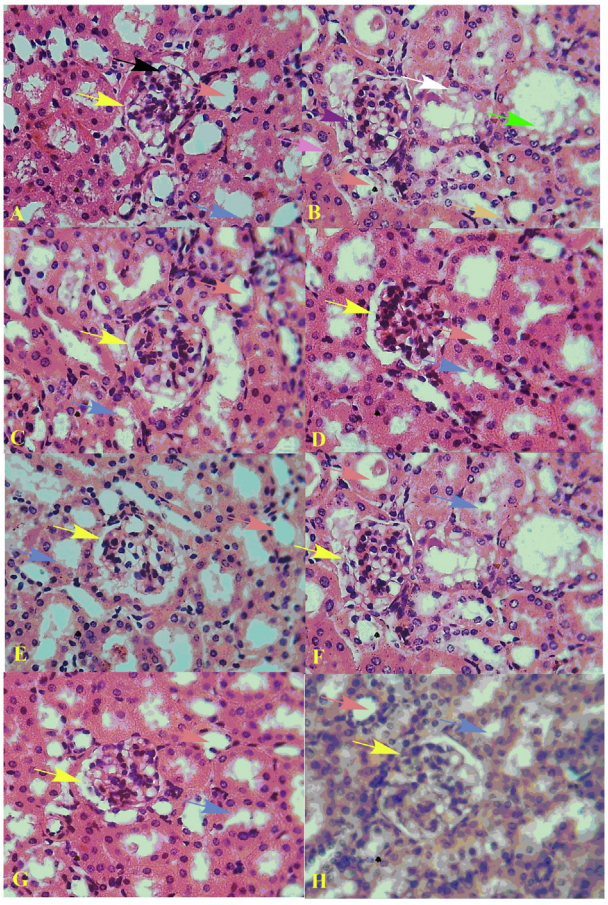

Fig. 5. Histopathology of Kidney (40X). Yellow arrows: Bowman's capsule: black arrow: glomerulus: orange arrow: distal convoluted tubule, blue arrow: proximal convoluted tubule, white arrow: micro vesicles and eccentric nucleus, black arrow: distorted glomerulus, green arrow: necrosis.

3.5. Micrometry of liver and kidney

The micrometric analysis of the liver includes the size of hepatocytes and size of hepatocytes nucleus in Fig. 7. Whereas kidney micrometric analysis includes measurement of ACSA of Bowman's capsule, ACSA of the glomerulus, ACSA of the lumen of proximal convoluted tubule, size of brush border in Fig. 7. A significant increase in the size of hepatocytes and their nucleus were observed in Cr treated groups 1188.191 ± 467.698 μ^2 and 456.419 ± 206.739 μ^2 ($P \leq 0.05$) as compared to control (836.778 ± 223.984 μ^2, 254. 269 ± 105.453 μ^2) respectively. With the administration of 50 mg/kg BW dose of *Nigella sativa* seed extract and *Nigella sativa* AgNPs, the size of hepatocytes was reduced up to a significant level in treatment groups including NS (P) (631.595 ± 155.019 μ^2), NS + NP (P) (669.454 ± 165.959 μ^2), NS (T) (707.928 ± 233.903 μ^2) and NS + NP (T) (707.928 ± 707.928 μ^2) up to significant level (Fig. 7).

A significant increase in bowman's capsule and glomerulus was observed in Cr treated groups 11835.47 ± 336.66 μ^2 and 9051.82 ± 249.81 μ^2 ($P \leq 0.05$) as compared to control (9106.81 ± 522.18 μ^2,7136.26 ± 261.61 μ^2) respectively. Administration of 50 mg/kg BW dose of *Nigella sativa* seed extract and *Nigella sativa* AgNPs, the size of Bowman's capsule was reduced up to the significant level in treatment groups including NS (P) (7481.91 ± 455.10 μ^2), NS + NP (P) (9304.56 ± 658.89 μ^2) and NS (T) (7128.01 ± 320.03 μ^2), however in NS + NP (T) (10586.26 ± 871.53 μ^2) reduction was not up to significant level (Fig. 8). In glomerulus size was also reduced in treatment groups NS (P) (6291.00 ± 631.46 μ^2), NS + NP (P) (5676.29 ± 326.25 μ^2) and NS (T) (6070.99 ± 213.25 μ^2), how-

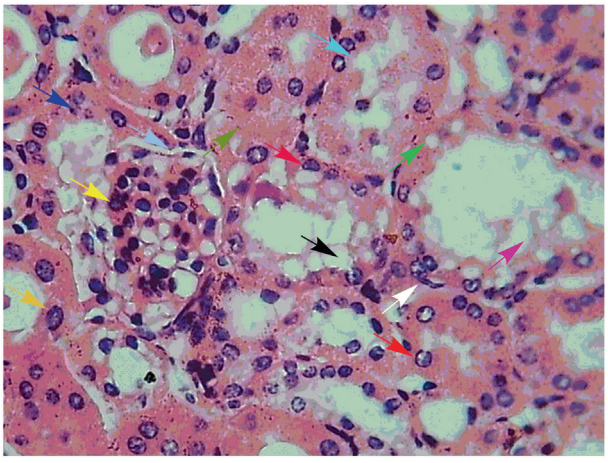

Fig. 6. Selected regions of the histological section of Cr (VI) treated kidney showing histopathology (40X). Blue arrows: bowman's capsule: yellow arrow: atrophy of glomerulus: white arrow: fibrocytes, dark green arrow: fibrosis in interstitial tissues, light green arrow: micro vesicles and nucleus disappeared, black arrow: macrovesivles, green arrow: necrosis, light blue arrow: brush border, dark blue arrow: necrosis, red arrow: nuclear fragmentation and condensation, dark pink arrow: eccentric nucleus, light pink arrow: vacuolation, orange arrow: mage nucleus.

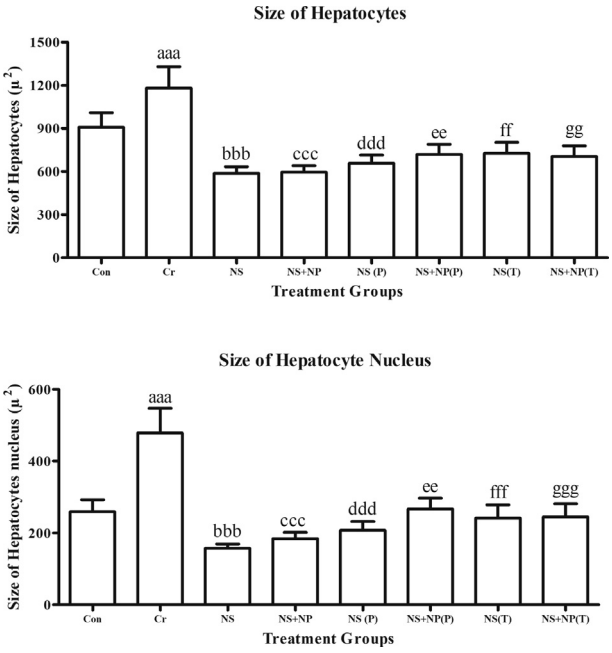

Fig. 7. Analysis of Hepatocyte and their nucleus size measurement in experimental groups. Con; Control; Cr: chromium treated; NS: *NSSE* treated; NS + NP: *Nigella sativa* AgNPs treated; P: prevention; T: treatment. Results represented in term of mean ± SEM. $P \leq 0.05$. Different letters showing significant different of treatment groups with that of Cr treated group. **Statistical icons:** a,b,c,d,e,f,g = $P \leq 0.05$. aa,bb, cc,dd,ee,ff,gg = $P \leq 0.01$. aaa, bbb, ccc, ddd,eee,fff,ggg = $P \leq 0.001$.

ever in NS + NP (T) (7951.64 ± 1134.21 μ^2) reduction was not up to significant level.

In the glomerulus, size was also reduced in treatment groups NS (P) (20.249 ± 3.130 μ^2), NS + NP (P) (19.324 ± 4.897 μ^2), NS (T) (19.070 ± 8.116 μ^2), and NS + NP (T) (17.819 ± 3.766 μ^2) up to significant level (Fig. 8).

The lumen of the proximal convoluted tubule was also not changed up to the significant level in chromium treated group (1049.63 ± 168.57 μ^2) as compared to the control group (841.85 ± 114.96 μ^2) ($p > 0.05$) (Fig. 8). In the proximal convoluted tubule, the brush border decreased in Cr group and changed up to a significant level in chromium treated group (10.070 ± 3.031 μ) as compared to the control group (20.086 ± 2.593 μ) (Fig. 8).

4. Discussion

Chromium compounds switch their oxidative state and do not withdraw into the ecosystem. Cr (VI) in comparison to Cr (III) is more toxic and easily passes through cell membranes with the aid of anion carriers (Xueting et al., 2018). The current study on albino mice aimed to evaluate whether *Nigella sativa* seed extract and *Nigella sativa* AgNPs supplementation can turn down the effects of Cr (VI) toxicity (by examining the level of ALT, AST, ALP, MDA, total protein, creatinine, and histopathology of liver and kidney). We observed that *Nigella sativa* seed extract and *Nigella sativa* AgNPs supplementation played a protecting role by turning down the Cr (VI) induced toxicity in the liver and kidney. Although *Nigella sativa* is a top-ranked herbal medicine with miraculous healing power (Kazmi et al., 2019) effective in various chronic diseases (Rashidmayvan et al., 2019). This is the foremost study on the ameliorative potential of *Nigella sativa* seed extract and *Nigella sativa* AgNPs on water intake HIS, RSI, ALT, AST, ALP, MDA, creatinine, and histopathology of liver and kidney against Cr (VI). That's why data is not sufficiently available for comparison.

There was an inexplicable change in the body weight with Cr (VI) administration (Shil and Pal, 2019) whereas the weight of the liver was increased (El-Demerdash et al., 2021). According to already published data Cr (VI) caused lesions, chronic inflammation, an increase in Kupfer cells, and fatty degeneration resulting change in the weight of the liver (Suljević et al., 2021), resulting in high HIS whereas RSI was not changed up to the detectable level when compared to control. However, mice treated with NSSE showed a detectable recovery in liver weight. It is established that Cr (VI) toxicity causes oxidative stress and cellular damage (Awasthi et al., 2018). The liver is involved in detoxification, Cr (VI) exposure induces ROS synthesis to result in oxidative damage that is visible in liver tissue sections (Yang et al., 2022). Liver dystrophy and inflammation were observed, and hepatocytes with fat vesicles were related to poor regulation of lipid reserves due to toxicity. As similar to previous studies hepatocytes atrophy, nuclear condensation and fragmentation, derangement of hepatic cords, and lateral side arrangement of the nucleus were seen in tissue section that may be due to deposition of lipids and glycogen precipitation. Necrosis in hepatocytes was because of the increased number of neutrophils and lymphocytes (Buchko and Havryliak, 2021). With the administration of NSSE and biosynthesized AgNP in the present study, the liver structure was improved.

Evaluation of the kidney functions had been made in the current study by estimating ALT, AST, ALP, and total protein. In the current study the elevated level of ALT, AST, and ALP can be attributed to a high level of ROS and pointed out liver damage that modified the membrane permeability and transport function along with the outflow of enzymes from hepatocytes into the bloodstream a clear indication of hepatotoxicity (El-Demerdash et al., 2021). With the administration of NSSE levels of these enzymes were reduced (Mazhar, 2022) due to the action of TQ (Fadishei et al., 2021). Cr (VI) decreases the protein level due to impaired protein synthesis machinery, most probably due to bioaccumulation of Cr (VI) (Trivedi et al., 2021), NSSE and biosynthesized AgNP treatment improved the level of total proteins.

The kidney is the vital target organ for the accumulation of Cr (VI), and the elimination of toxicant (Kakade et al., 2020) reactive species synthesized by Cr (VI) effect cell membrane lipids and induce peroxidation of unsaturated fatty acids (Suljević et al., 2021) and necrosis in kidney tissue (Yin et al., 2019). Upon exposure to Cr (VI), massive destruction was found in the renal cortex including fatty cell infiltration representing lipid accumulation due to ROS species (Hanan et al., 2019). Regarding renal filtration, in our study, there were inconsistent forms of histopathological changes were observed such as glomerulus degeneration, necrosis, fibrosis, sloughing of tubular epithelial cells, and cytoplasmic vacuolation. According to previous studies, cytoplasmic vacuolation is the job of reactive species that aid the discharge of lysosomal enzymes and upcoming protein oxidation of the cell, convert them into fragments, and set apart them into vacuoles as part of the cellular defense mechanism, protecting the interference with cell metabolism (Sorour and Abd-Elgalil, 2019). As nephritis in the tubule and glomerulus damage are linked with irregular flow of the glomerular filtrate. The slow or blocked condition of glomerular filtrate results in shrinkage of the glomerulus (Hanan et al., 2019). In Cr treated animals water consumption was maximum, as ammonia execration requires more water and animals drink more water compared to other groups. This indicates renohepatic toxicity (Abbas et al., 2016). With the administration of NSSE in the present study, the architecture of the renal cortex was improved (Baloch et al., 2020) and water consumption was reduced as observed in our study.

Evaluation of the kidney functions had been made in the current study by estimating MDA and creatinine. In our study Cr (VI) exposure elevated the level of MDA and creatinine in serum.

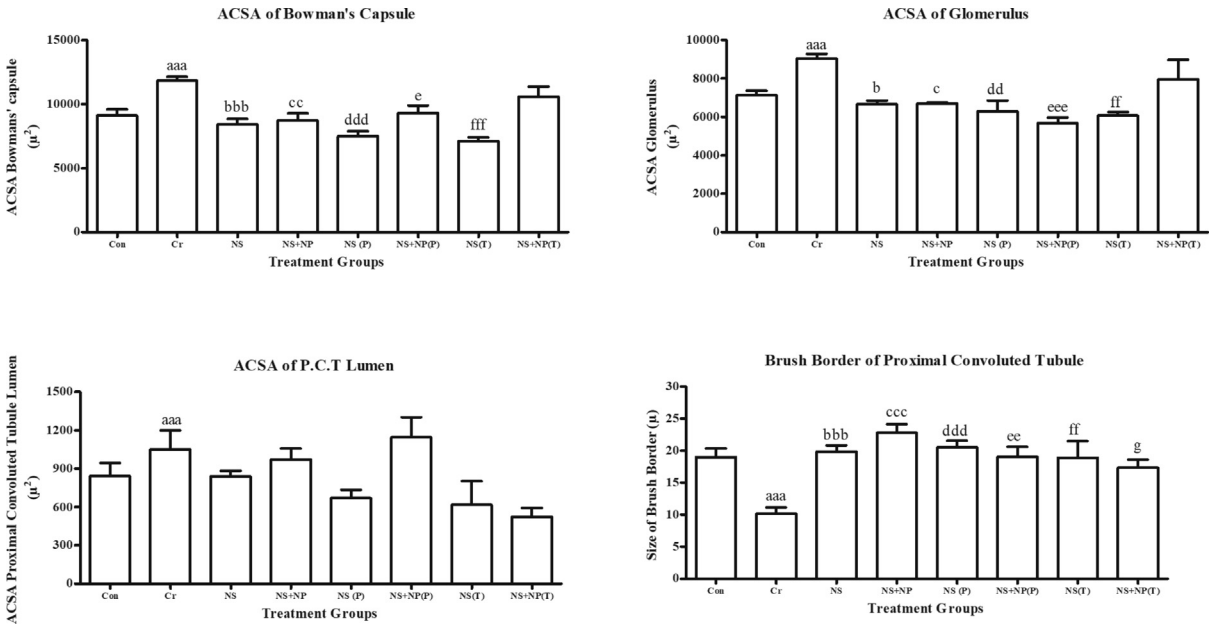

Fig. 8. Analysis of ACSA of Bowman's capsule, glomerulus, lumen of proximal convoluted tubule and brush border size measurement in experimental groups. Con; Control; Cr: chromium treated; NS: NSSE treated; NS + NP: *Nigella sativa* AgNPs treated; P: prevention; T: treatment. Results represented in term of mean ± SEM. $P \leq 0.05$. Different letters showing significant different of treatment groups with that of Cr treated group. **Statistical icons:** a,b,c,d,e,f,g = $P \leq 0.05$. aa,bb,cc,dd,ee,ff,gg = $P \leq 0.01$. aaa, bbb, ccc, ddd,eee,fff,ggg = $P \leq 0.001$.

MDA is the biomarker for lipid peroxidation and protein damage results in an elevated level of MDA in serum (Muller et al., 2022). MDA, the final product of lipid peroxidation, used as indicator for liver injury (Selamoğlu et al., 2014). Serum creatinine level indicates acute tubular necrosis caused by renal toxicity (Alsuhaibani, 2018) and also provides a rough estimate of the rate of glomerular filtration (Franca and Prince, 2019), the glomerulus injury leads to a reduction in the diffusion process by thickening of capillary basement membrane resulting slower rate of filtration (Deshmukh and Manjalkar, 2021) and NSSE and biosynthesized AgNP administration reduced level of creatinine.

As per the micrometric analysis of the liver showed in Cr group the size of hepatocytes and their nucleus were increased may be due to disruption of nuclear function, which might be due to protein DNA interaction and transcriptional activity of hepatocytes (Fedchenko et al., 2022). In the kidney, an increase in glomerular size increase may be due to glomerulus damage, and crescent formation (Bouteldja et al., 2021) may increase in the size of the Bowman's capsule. In NSSE and biosynthesized AgNP treated groups size were significantly reduced.

Our results indicated that the administration of *Nigella sativa* and *Nigella sativa* mediated AgNPs in mice produced marked modulatory effects against chromium (VI) and caused hepatic, renal, and biochemical alterations. Based on the results, *Nigella sativa* and *Nigella sativa* mediated AgNPs seems to have an ameliorative mechanism in hepatotoxicity and renal toxicity. We selected equal quantity of dose for *Nigella sativa* seed extract and *Nigella sativa* mediated AgNPs in order to compare the ameliorative potential against particular quantity of chromium. If we use small quantity of dose of nanoparticles then it may be more beneficial. Further we will investigate this dose dependent response". Also, further studies are required to explicitly interpret the definite mechanism of mitigation by *Nigella sativa* and *Nigella sativa* mediated AgNPs.

5. Conclusion

In conclusion, chromium (VI) treatment resulted in lipid peroxidation, interference in the natural antioxidant defense system, biochemical parameters, and histological alterations in the liver and kidney. Furthermore, *Nigella sativa* supplementations to chromium Cr (VI) treated mice modulated oxidative damage and rehabilitate the disturbance in the majority of the measured parameters. Its effect is noticeable in the prevention group. So, *Nigella sativa* had strong antioxidant potential in amelioration of Cr (VI) toxicity by reduction of free radicals synthesis and enhancing the antioxidant defense system; additionally, our data reinforce the use of *Nigella sativa* as a hepatic protective and renal protective natural-born product.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103571.

References

Abbas, T., Ahmad, K.R., Asmatullah, K.P.L., Kanwal, M.A., Suleman, S., 2016. Reno-hepatic protective effects of jambul against chromium induced anomalies in mice. Punjab Univ. J. Zool. 31 (1), 059–067.

Aghamirei, Z., Hajian, S., Mohamadi-Zeidi, E., Ferasat, A., Karami, A., Javadi, M., 2022. The effect of the alcoholic extract of black cumin (*Nigella sativa*) on stone size in patients with oxalate calcium kidney stones. J. Isfahan Med. School 40 (661), 103–108.

Ahmad, K.R., Nauroze, T., Raees, K., Abbas, T., Kanwal, M.A., Noor, S., Jabeen, S., 2012. Protective role of jambul (*Syzygium cumini*) fruit-pulp extract against fluoride-induced toxicity in mice testis: a histopathological study. Fluoride 45 (3 Pt 2), 281–289.

Ali, S., Hussain, S., Khan, R., Mumtaz, S., Ashraf, N., Andleeb, S., Shakir, H.A., Tahir, H. M., Khan, M.K.A., Ulhaq, M., 2019b. Renal toxicity of heavy metals (cadmium and mercury) and their amelioration with ascorbic acid in rabbits. Environ. Sci. Pollut. Res. Int. 26 (4), 3909–3920.

Ali, S., Awan, Z., Mumtaz, S., Shakir, H.A., Ahmad, F., Ulhaq, M., Tahir, H.M., Awan, M. S., Sharif, S., Irfan, M., Khan, M.A., 2020. Cardiac toxicity of heavy metals

(cadmium and mercury) and pharmacological intervention by vitamin C in rabbits. Environ. Sci. Pollut. Res. 27 (23), 29266–29279.
Ali, H., Khan, E., Ilahi, I., 2019a. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. J. Chem. 2019, 14.
Almatroudi, A., Khadri, H., Azam, M., Rahmani, A.H., Al-Khaleefah, F.K., Khateef, R., Ansari, M.A., Allemailem, K.S., 2020. Antibacterial, antibiofilm and anticancer activity of biologically synthesized silver nanoparticles using seed extract of *Nigella sativa*. Processes 8 (4), 388.
Alsuhaibani, A.M., 2018. Effect of *Nigella sativa* against cisplatin induced nephrotoxicity in rats. Italian J. Food Saf. 7 (2).
Amri, J., Alaee, M., Babaei, R., Salemi, Z., Meshkani, R., Ghazavi, A., Akbari, A., Salehi, M., 2021. Biochanin-a has antidiabetic, antihyperlipidemic, antioxidant, and protective effects on diabetic nephropathy via suppression of tgf-β1 and par-2 genes expression in kidney tissues of stz-induced diabetic rats. Biotechnol. Appl. Biochem., 1–10
Ara, C., Asmatullah, N.B., Ali, S., Batool, F., Shakir, H.A., Arshad, A., 2020. Abnormal, steroidogenesis, oxidative stress, and reprotoxicity following prepubertal exposure to, butylparaben in mice and protective effect of *Curcuma longa*. Environ. Sci. Pollut. Res. 28 (9), 1, 11.
Awasthi, Y., Ratn, A., Prasad, R., Kumar, M., Trivedi, S.P., 2018. An *in vivo* analysis of cr6+ induced biochemical, genotoxicological and transcriptional profiling of genes related to oxidative stress, DNA damage and apoptosis in liver of fish, *Channa punctatus* (bloch, 1793). Aquat. Toxicol. 200, 158–167.
Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M.R., Sadeghi, M., 2021. Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. Front. Pharmacol. 12, 643972.
Baloch, W.A., Zafar, S., Ullah, I., Khan, M.I., Yaseen, M.S., Ameer, M.K., 2020. Doxorubicin induced histomorphometric changes in the kidney of albino rats and protective role of *Nigella sativa*. Int. J. Front. Sci. 4 (2), 88–91.
Bashir, O., Jan, N., Gani, G., Naik, H., Hussain, S.Z., Reshi, M., Amin, T., 2021. Food applications of *Nigella sativa* seeds. In: Black cumin (*Nigella sativa*) seeds: Chemistry, Technology, Functionality, and Applications. Springer, pp. 191–207.
Bouteldja, N., Klinkhammer, B.M., Bülow, R.D., Droste, P., Otten, S.W., Von-Stillfried, S.F., Moellmann, J., Sheehan, S.M., Korstanje, R., Menzel, S., 2021. Deep learning–based segmentation and quantification in experimental kidney histopathology. J. Am. Soc. Nephrol. 32 (1), 52–68.
Buchko, O., Havryliak, V., 2021. Effect of the supplement of humic origin on the free radical processes and histological changes in the tissues of rats affected by chromium (vi). Biointerf. Res. Appl. Chem. 11 (3), 10996–11008.
Chand, K., Jiao, C., Lakhan, M.N., Shah, A.H., Kumar, V., Fouad, D.E., Chandio, M.B., Maitlo, A.A., Ahmed, M., Cao, D., 2021. Green synthesis, characterization and photocatalytic activity of silver nanoparticles synthesized with *Nigella sativa* seed extract. Chem. Phys. Lett. 763, 13821.
Dar, K.K., Ali, S., Ejaz, M., Nasreen, S., Ashraf, N., Gillani, S.F., Shafi, N., Safeer, S., Khan, M.A., Andleeb, S., Mughal, T.A., 2019. In vivo induction of hepatocellular carcinoma by diethylnitrosoamine and pharmacological intervention in balb c mice using Bergenia ciliate extracts. Braz. J. Biol. 79 (4), 629–638.
Deshmukh, A., Manjalkar, P., 2021. Synergistic effect of micronutrients and metformin in alleviating diabetic nephropathy and cardiovascular dysfunctioning in diabetic rat. J. Diabetes Metab. Disord. 20 (1), 533–541.
El-Demerdash, F.M., El-Sayed, R.A., Abdel-Daim, M.M., 2021. Hepatoprotective potential of rosmarinus officinalis essential oil against hexavalent chromium-induced hematotoxicity, biochemical, histological, and immunohistochemical changes in male rats. Environ. Sci. Pollut. Res. 28 (14), 17445–17456.
Engwa, G.A., Ferdinand, P.U., Nwalo, F.N., Unachukwu, M.N., 2019. Mechanism and health effects of heavy metal toxicity in humans. Poisoning in the modern world-new tricks for an old dog 10.
Fadishei, M., Ghasemzadeh, R.M., Imenshahidi, M., Mohajeri, A., Razavi, B.M., Hosseinzadeh, H., 2021. Effects of *Nigella sativa* oil and thymoquinone against bisphenol a-induced metabolic disorder in rats. Phytother. Res. 35 (4), 2005–2024.
Fedchenko, T., Izmailova, O., Shynkevych, V., Shlykova, O., Kaidashev, I., 2022. Ppar-γ agonist pioglitazone restored mouse liver mrna expression of clock genes and inflammation-related genes disrupted by reversed feeding. PPAR research, 2022.
Franca, O. N., Prince, B., 2019. Effects of aqueous extracts of *Nigella sativa* and *Ocimum gratissimum* on electrolyte, urea, creatinine of wistar rat.
Hanan, E.M., Alshmyaa, O.A., Samaa, S., Helmy, H.O., 2019. Structural changes induced by potassium dichromate in renal cortex of adult male albino rats and the possible protective role of selenium. Med. J. Cairo Univ. 87, 661–675.
Hannan, M.A., Zahan, M.S., Sarker, P.P., Moni, A., Ha, H., Uddin, M.J., 2021. Protective effects of black cumin (*Nigella sativa*) and its bioactive constituent, thymoquinone against kidney injury: an aspect on pharmacological insights. Int. J. Mol. Sci. 22 (16), 9078.
He, Y., Zou, L., Luo, W., Yi, Z., Yang, P., Yu, S., Liu, N., Ji, J., Guo, Y., Liu, P., 2020. Heavy metal exposure, oxidative stress and semen quality: exploring associations and mediation effects in reproductive-aged men. Chemosphere 244, 125498.
Helmy, M.S., Masoud, H.M., Darwish, D.A., Abdel-Monsef, M.M., Ibrahim, M.A., 2019. Isolation and properties of lactate dehydrogenase isoenzyme from buffalo liver: application in ast and alt assay diagnostic kits. J. Appl. Pharmaceut. Sci. 9 (10), 054–060.
Hossain, M.S., Sharfaraz, A., Dutta, A., Ahsan, A., Masud, M.A., Ahmed, I.A., Goh, B.H., Urbi, Z., Sarker, M.M.R., Ming, L.C., 2021. A review of ethnobotany, phytochemistry, antimicrobial pharmacology and toxicology of *Nigella sativa* l. Biomed. Pharmacother. 143, 112182.
Hussain, S., Ali, S., Mumtaz, S., Shakir, H.A., Ahmad, F., Tahir, H.M., Ulhaq, M., Khan, M.A., Zahid, M.T., 2020. Dose and duration-dependent toxicological evaluation of lead acetate in chicks. Environ. Sci. Pollut. Res. 18, 1–6.
Ikhsan, M., Hiedayati, N., Maeyama, K., Nurwidya, F., 2018. *Nigella sativa* as an anti-inflammatory agent in asthma. BMC Res. Notes 11 (1), 1–5.
Kakade, A., Salama, E.-S., Pengya, F., Liu, P., Li, X., 2020. Long-term exposure of high concentration heavy metals induced toxicity, fatality, and gut microbial dysbiosis in common carp, *Cyprinus carpio*. Environ. Pollut. 266, 115293.
Kannayiram, G., Sandhya, A., Sowmiya, S., Valarmathi, S., Joseph, D., 2019. Anti-inflammatory activity of *Nigella sativa* silver nanoparticles: biochemical study. Asian J. Pharmaceut. Clin. Res., 346–349
Kazmi, A., Khan, M.A., Huma, A., 2019. Biotechnological approaches for production of bioactive secondary metabolites in *Nigella sativa*: An up-to-date review. Int. J. Sec. Metab. 6 (2), 172–195.
Khalaf, A.A., Hassanen, E.I., Ibrahim, M.A., Tohamy, A.F., Aboseada, M.A., Hassan, H. M., Zaki, A.R., 2020. Rosmarinic acid attenuates chromium-induced hepatic and renal oxidative damage and DNA damage in rats. J. Biochem. Mol. Toxicol. 34 (11), e22579.
Khamis, M.M., Holt, T., Awad, H., El-Aneed, A., Adamko, D.J., 2018. Comparative analysis of creatinine and osmolality as urine normalization strategies in targeted metabolomics for the differential diagnosis of asthma and copd. Metabolomics 14 (9), 1–8.
Khan, R., Ali, S., Mumtaz, S., Andleeb, S., Ulhaq, M., Tahir, H.M., Khan, M.K.A., Khan, M.A., Shakir, H.A., 2019. Toxicological efects of toxic metals (cadmium and mercury) on blood and the thyroid gland and pharmacological intervention by vitamin C in rabbits. Environ. Sci. Poll. Res. 26 (16), 16727–16741.
Kumar, V., Parihar, R.D., Sharma, A., Bakshi, P., Sidhu, G.P.S., Bali, A.S., Karaouzas, I., Bhardwaj, R., Thukral, A.K., Gyasi-Agyei, Y., 2019. Global evaluation of heavy metal content in surface water bodies: a meta-analysis using heavy metal pollution indices and multivariate statistical analyses. Chemosphere 236, 124364.
Maideen, N.M., Balasubramanian, R., Ramanathan, S., 2021. Nigella sativa (black seeds), a potential herb for the pharmacotherapeutic management of hypertension – a review. Curr. Cardiol. Rev. 17 (4), 7–13.
Mazaheri, Y., Torbati, M., Azadmard-Damirchi, S., Savage, G.P., 2019. A comprehensive review of the physicochemical, quality and nutritional properties of *Nigella sativa* oil. Food Rev. Intl. 35 (4), 342–362.
Mazhar, M.W., 2022. Antioxidant and protective effect of andldquo; artemesia absinthiumandrdquo; and andldquo; *Nigella sativa* andrdquo; on albino mice mode of hepatic injury. J. Basic Clin. Pharm. 13 (2).
Mughal, T.A., Saleem, M.Z., Ali, S., Anwar, K.K., Bashir, M.M., Babar, M., Khan, M.A., 2019. Evaluation of hepatotoxicity of carbon tetrachloride and pharmacological intervention by vitamin E in Balb c mice. Pak. J. Zool. 51 (2), 755–761.
Muller, C.D., Garcia, S.C., Brucker, N., Goethel, G., Sauer, E., Lacerda, L.M., Oliveira, E., Trombini, T.L., Machado, A.B., Pressotto, A., 2022. Occupational risk assessment of exposure to metals in chrome plating workers. Drug Chem. Toxicol. 45 (2), 560–567.
Mumtaz, S., Ali, S., Khan, R., Andleeb, S., Ulhaq, M., Khan, M.A., Shakir, H.A., 2019. The protective role of ascorbic acid in the hepatotoxicity of cadmium and mercury in rabbits. Environ. Sci. Pollut. Res. Int. 26 (14), 14087–14096.
Onita, B., Albu, P., Herman, H., Balta, C., Lazar, V., Fulop, A., Baranyai, E., Harangi, S., Keki, S., Nagy, L., 2021. Correlation between heavy metal-induced histopathological changes and trophic interactions between different fish species. Appl. Sci. 11 (9), 3760.
Rashidmayvan, M., Mohammadshahi, M., Seyedian, S.S., Haghighizadeh, M.H., 2019. The effect of *Nigella sativa* oil on serum levels of inflammatory markers, liver enzymes, lipid profile, insulin and fasting blood sugar in patients with non-alcoholic fatty liver. J. Diabetes Metab. Disord. 18 (2), 453–459.
Sankhla, M.S., Kumar, R., 2019. Contaminant of heavy metals in groundwater & its toxic effects on human health & environment. Available at SSRN 3490718.
Selamoğlu, Z., Özdemir, İ., Yılmaz, İ., Gök, Y., 2009. Antioxidative effects of novel synthetic organoselenium compound in rat lung and kidney. Ecotoxicol. Environ. Saf. 72 (3), 916–921.
Selamoğlu, Z., Özdemir, İ., Çiftçi, O., Çakır, O., Gülhan, M., F., Oğuz, M. P., 2014. Role of propolis on biochemical parameters in kidney and heart tissues against L NAME induced oxidative injury in rats. Clin. Exp. Hypertens. 7 (36), 492–496.
Selamoğlu, Z., Özdemir, İ., Ciftci, O., Gülhan, M.F., Savcı, A., 2015. Antioxidant effect of ethanolic extract of propolis in liver of L-NAME treated rats. 24 (2), 227–232.
Selamoğlu, Z., Gülhan, M., Fuat, S., Ramin, E., Şahna, E., Durdagi, S., Abdullah, I., 2017. Effects of propolis, caffeic acid phenethyl ester, and pollen on renal injury in hypertensive rat: an experimental and theoreticalapproach. Cell Biochem. Funct. 35, 304–314.
Selamoğlu, Z., Yılmaz, İ., 2014. The investigation of the antioxidative properties of the synthetic organoselenium compounds in liver tissue of rat with histological and biochemical analyses. J. Pharmaceut. Care 4 (2), 162–169.
Shil, K., Pal, S., 2019. Metabolic and morphological disorientations in the liver and skeletal muscle of mice exposed to hexavalent chromium. Comp. Clin. Pathol. 28 (6), 1729–1741.
Sorour, H., Abd-Elgalil, M., 2019. Mesenchymal stem cells ameliorate diabetic renocortical changes in a rat model: histological, morphometrical and biochemical study. J. Med. Histol. 3 (2), 132–161.
Suchana, S.A., Ahmed, M.S., Islam, S., Rahman, M.L., Rohani, M.F., Ferdusi, T., Ahmmad, A., Fatema, M.K., Badruzzaman, M., Shahjahan, M., 2021. Chromium exposure causes structural aberrations of erythrocytes, gills, liver, kidney, and genetic damage in striped catfish *Pangasianodon hypophthalmus*. Biol. Trace Elem. Res. 199 (10), 3869–3885.

Suljević, D., Sulejmanović, J., Fočak, M., Halilović, E., Pupalović, D., Hasić, A., Alijagic, A., 2021. Assessing hexavalent chromium tissue-specific accumulation patterns and induced physiological responses to probe chromium toxicity in coturnix japonica quail. Chemosphere 266, 129005.

Trapero, C., Jover, L., Fernández-Montolí, M.E., García-Tejedor, A., Vidal, A., Gomez, de Aranda, I., Ponce, J., Matias-Guiu, X., Martín-Satué, M., 2018. Analysis of the ectoenzymes ada, alp, enpp1, and enpp3, in the contents of ovarian endometriomas as candidate biomarkers of endometriosis. Am. J. Reprod. Immunol. 79 (2), e12794.

Trivedi, S., Kumar, V., Singh, S., Kumar, M., 2021. Efficacy evaluation of *Rauwolfia serpentina* against chromium (vi) toxicity in fish, channa punctatus. J. Environ. Biol. 42 (3), 659–667.

Ukhurebor, K.E., Aigbe, U.O., Onyancha, R.B., Nwankwo, W., Osibote, O.A., Paumo, H. K., Ama, O.M., Adetunji, C.O., Siloko, I.U., 2021. Effect of hexavalent chromium on the environment and removal techniques: a review. J. Environ. Manage. 280, 111809.

Vijayakumar, S., Divya, M., Vaseeharan, B., Chen, J., Biruntha, M., Silva, L.P., Durán-Lara, E.F., Shreema, K., Ranjan, S., Dasgupta, N., 2021. Biological compound capping of silver nanoparticle with the seed extracts of blackcumin (*Nigella sativa*): a potential antibacterial, antidiabetic, anti-inflammatory, and antioxidant. J. Inorg. Organomet. Polym Mater. 31 (2), 624–635.

Xueting, L., Rehman, M.U., Mehmood, K., Huang, S., Tian, X., Wu, X., Zhou, D., 2018. Ameliorative effects of nano-elemental selenium against hexavalent chromium-induced apoptosis in broiler liver. Environ. Sci. Pollut. Res. 25 (16), 15609–15615.

Yang, Q., Han, B., Li, S., Wang, X., Wu, P., Liu, Y., Li, J., Han, B., Deng, N., Zhang, Z., 2022. The link between deacetylation and hepatotoxicity induced by exposure to hexavalent chromium. J. Adv. Res. 35, 129–140.

Ye, H., Nelson, L.J., Del, M., M. G., Martínez-Naves, E., Cubero, F. J., 2018. Dissecting the molecular pathophysiology of drug-induced liver injury. World J. Gastroenterol. 24 (13), 1373.

Yılmaz, S., Ergün, S., 2018. Trans-cinnamic acid application for rainbow trout (oncorhynchus mykiss): I. Effects on haematological, serum biochemical, non-specific immune and head kidney gene expression responses. Fish Shellfish Immunol. 78, 140–157.

Yin, F., Yan, J., Zhao, Y., Guo, K.-J., Zhang, Z.-L., Li, A.-P., Meng, C.-Y., Guo, L., 2019. Bone marrow mesenchymal stem cells repair cr (vi)-injured kidney by regulating mitochondria-mediated apoptosis and mitophagy mediated via the mapk signaling pathway. Ecotoxicol. Environ. Saf. 176, 234–241.