

Potential role of *Nigella sativa* (NS) in abating oxidative stress-induced toxicity in rats: a possible protection mechanism

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Abstract : The seeds of *Nigella sativa* (NS), have been widely used in herbal medicines worldwide. It has been shown to possess prophylactic effects against oxidative stress. However, there is a paucity of information regarding the protective role of NS against oxidative stress, in the absence of toxic agents. The aim of the study was to elucidate the anti-oxidative stress pharmacodynamics of NS. Eighteen, 12-week-old Sprague-Dawley rats, weighing about 300 ± 25 gm were divided equally into six groups. Four of the groups were supplemented with NS at 100 mg/kg b.w/day orally (P.O.) and labeled as, 1st, 3rd, 5th and 6th day groups. The PCx (positive control) group was given distilled water orally, and the NCx (negative control) group rats were provided with food and water ad libitum. Blood samples were collected, and rats were sacrificed on days 1, 3, 5 and 6 (2h) post-treatment. The blood was used for oxidative stress enzymes analysis (SOD, GSH-Px and MDA), liver (ALT) and kidney (creatinine) function assay, and the liver, kidney and spleen were dissected for histology. The results revealed that NS exhibited an anti-oxidative stress effect in the liver and kidneys as indicated by the low levels of ALT and creatinine. In response to antioxidant enzymes, especially that of the 3rd-day treatment group, an increase in SOD and GSH-Px indirectly caused an alleviation of oxidative stress, leading to a much lower level of MDA. It was concluded that treatment with NS at 100 mg/kg b.w/per day for three consecutive days, demonstrated the highest efficacy in abating oxidative stress in rats.

Keywords: Antioxidant, GSH-Px, *Nigella sativa*, pharmacodynamic and prophylactic.

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I. Introduction

Over the past several decades, a growing interest in naturally occurring phytochemical compounds and herbal constituents with various pharmacological effects has led to a demand for using medicinal plants for the prevention and treatment of various diseases, particularly inflammatory diseases and cancer. The advancement in technology and science has led to the identification of various active components in herbal extracts that possess various beneficial effects such as, anti-cancer, anti-inflammatory, anti-diabetic, and antioxidant properties. Conventional drugs and treatment have been associated with adverse side effects and other complications, such as drug resistance. Moreover, some of the existing conventional drugs are not sufficient or effective in providing a complete treatment of certain diseases. Therefore, this warrants further investigation into the identification and discovery of new drugs for alternative therapy, either to complement or replace existing conventional drugs (Cragg, 1998; Woo *et al.*, 2012). Thus, traditional medicines of plant origin have recently garnered more attention, because of several factors such as easy availability, safety, affordability, and efficacy as well as cultural acceptability. As such, more than 25 % of drugs in current use are directly derived from plants, whereas, 25 % are chemically transformed natural products (Vuorela *et al.*, 2004; Alkharfy *et al.*, 2015). The medicinal properties, a potential mechanism of action, toxicological studies and safety evaluation, of a lot of the plants in use today, remain unclear (Ahmad *et al.*, 2013; Mollazadeh & Hosseinzadeh, 2014).

Among the different medicinal plants, *Nigella sativa* (NS), also known as black cumin, or habat-ul sauda in Arabic, is emerging as a miracle herb, due to its wide range of pharmacological potential. It has also been associated with a rich historical and religious background and has been used for centuries throughout the Middle East, India, and Northern Africa. It has not only been used as a medicine but also as a spice for culinary purposes. In the Muslim world, it has particular importance and is considered to possess various healing properties, as it has been mentioned by Prophet Muhammad, that, the black seed is the remedy for all diseases

except death. It has also been recommended to be used on a regular basis, in Tibb Al-nabwi (Prophetic Medicine) (Al-Bukhari & Sahi, 1976; Ahmad *et al.*, 2013).

The objectives of this study are, to determine the potential protective effects of NS seeds on anti-oxidative status, particularly, SOD and GSH-Px, and against lipid peroxidation (MDA) as well as cytosolic enzymes (liver, kidney enzymes). To evaluate the morphological and histopathological features in liver, kidney and spleen associated with oral administration of NS seed powder. The time required for the level of G-Px to reach its peak following administration with NS will be investigated and, the mechanisms and protective pathways of NS in abating free radical-induced hepatotoxicity in rats, will be elucidated.

II. Materials and Methods

Plant Materials

NS seeds (imported from India) were purchased from a local herb store in Serdang, Malaysia. Voucher specimens of seeds were kept at the Histopathology Laboratory of Faculty of Veterinary Medicine/UPM University, and the seeds were identified and authenticated by Dr Shamsul Khamis, Head of the Laboratory of Natural Products, Institute of Bioscience, University Putra Malaysia with reference number (UPM/IBS/UB/H 104-5) for notice of approval. The protocol used in this study was slightly modified from that of Dollah *et al.*, (2013). Initially, the seeds were washed under running tap water for 5 min, rinsed thrice with dH₂O and, subsequently dried in an oven at 38.5 °C overnight, until a constant weight was attained. Just prior to performing the gavage procedure, the seeds were crushed to a very fine powder, using an electric crusher (Hung Chuan Machinery, Model RT-08, 25000 rpm, Taiwan), for 8 min (2 min × 4 times). Then distilled water was added at room temperature via gentle shaking, for a few minutes to prepare a crude suspension at a dose of, 100 mg/kg body weight/day (Khan *et al.*, 2011; El-Far *et al.*, 2014).

Animals

The research was approved and conducted as per the guidelines of the Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, (UPM) with approval reference number, UPM/IACUC/AUP-R082/2015. Eighteen, 12-week-old Sprague-Dawley rats, weighing about 300 ± 25 gm (b.w), were supplied by the Laboratory Animal Resource Unit, UPM, and placed in the animal house.

They were housed in animal cages under standard laboratory conditions, with a period of 12 h light/dark at 21 ± 1 °C, and 60 to 65% relative humidity in the animal house, UPM. The animals were allowed to acclimatize for at least seven days before commencing the experiment. The rats were fed with a standard rat chow pellet and allowed to drink water *ad libitum* and animals handling was conducted between 08.00-10.00 am, to minimise the effects of environmental changes. The treatments were given to the rats for six consecutive days. The body weight, feed and water intake were measured once a day.

Experimental Design

Animals were equally divided into six groups, four of them called the treatment groups and two control groups. The treatment groups consisted of, (A) 1st, (B) 3rd, (C) 5th and (D) 6th day groups, which were supplemented with an NS aqueous suspension at a dose of 100 mg/kg/per day orally). Group E or the positive control groups were given distilled water orally, while Group F served as a negative control group. On days 1, 3, 5 and 6 post-treatment, animals were anaesthetized with 87 mg ketamine /kg mixed with 13 mg of xylazine /kg (b.w), to the collection of blood samples, for biochemical assay studies, and subsequently sacrificed 2h post each treatment.

Clinical signs, Body weight, Feed intake and water consumption

Rats were observed twice daily (morning and evening) for signs of aspiration pneumonia, oral gavage injuries, and NS toxicity. The body weight of all rats was recorded before commencing and daily thereafter until the end of the experiment, using an electronic balance. The feed and water that leftover was collected at the end of the day and weighed or measured.

Biochemical analysis

The plasma, hemolysate RBCs and serum were extracted from blood samples, for spectrophotometric determination: Estimation of Plasma Malondialdehyde (MDA) Concentration as an oxidative stress marker (Pintea *et al.*, 2008). The thiobarbituric acid reactive substances (TBARS) assay, was carried out as described by (Lapenna *et al.*, 2001) with slight modifications. Estimation of Erythrocyte-Superoxide Dismutase (E-SOD) Activity as the first intracellular defense against free radicals. The Pyrogallol oxidation inhibition assay was carried out as described earlier by (Marklund, 1984) with minor modifications. Estimation of Erythrocyte-Glutathione Peroxidase (E-GSH-Px) Activity as a master antioxidant enzyme (Liu & Choi, 2000; Allen & Bradley, 2011). The DTNB direct method was conducted, as described by (Sazuka *et al.*, 1989) with slight

modifications (using the manual methods for all). Alanine Aminotransferase (ALT) test, which is usually used to detect hepatopathy (Limdi et al., 2003), and Creatinine (CREA), a test that measures kidney function, were conducted.

Histological Examination

Liver, kidney and spleen were resected and cleaned from surrounding adipose tissue. After washing gently with 0.9% physiological saline, and blotted dry, somatic index (SI) determination was carried out. During necropsy, the focus was on the liver, kidney and spleen. Pathological changes, if found, were scored from 0-3; {0=No lesion (no significant alterations) and 3=most severe}. The organs were checked to detect any gross lesions following visual examination (color and size) and palpation (consistency) before they were scored. The scoring was carried as follows; no significant lesions = 0, 5-10% organ affected considered mild and scored (1) point, 11-25% considered moderate and scored (2) points, and over 25% considered severe and scored (3) points. The samples were processed routinely for histopathology, using the paraffin embedded technique, sectioned at 4 microns and stained with hematoxylin and eosin (H&E) for histopathological evaluation.

Statistical analysis

Mean total clinical signs, biochemical tests, gross, histological lesions and scores were summarized and subjected to the Mann-Whitney test. All statistical procedures were estimated using the predictive analysis software 20 (PASW version 20; SPSS Inc., Chicago, IL, USA) and tested at 5% level of significance.

III. Results and Discussion

Clinical signs, Body weight, Feed intake and water consumption

All experimental animals were monitored twice daily, no clinical abnormalities were observed in all of the rat groups during the experimental period. The mean body weight, feed and water intake are represented graphically in (Fig. 1). Measurement of body weight, feed and water intake were used to evaluate the health status of the rats during the experimental period. There were no statistical differences in the treated groups compared to the negative control group, which was indicative of the healthy status of rats, following NS supplementation.

Biochemical test result

The results obtained in (Fig. 1) showed a gradual decrease in serum ALT and CREA levels, following supplementation with NS when compared with NCx. It was observed that levels of serum enzymes were affected by supplementation with 100 mg/kg/day (P.O.) NS. Furthermore, the results suggest that supplementation with NS does not induce hepato-nephrotoxicity or injury, especially in the cell membrane. In contrast, there was a slight (no significant) increase in the level of these enzymes, as detected in PCx as compared to NCx.

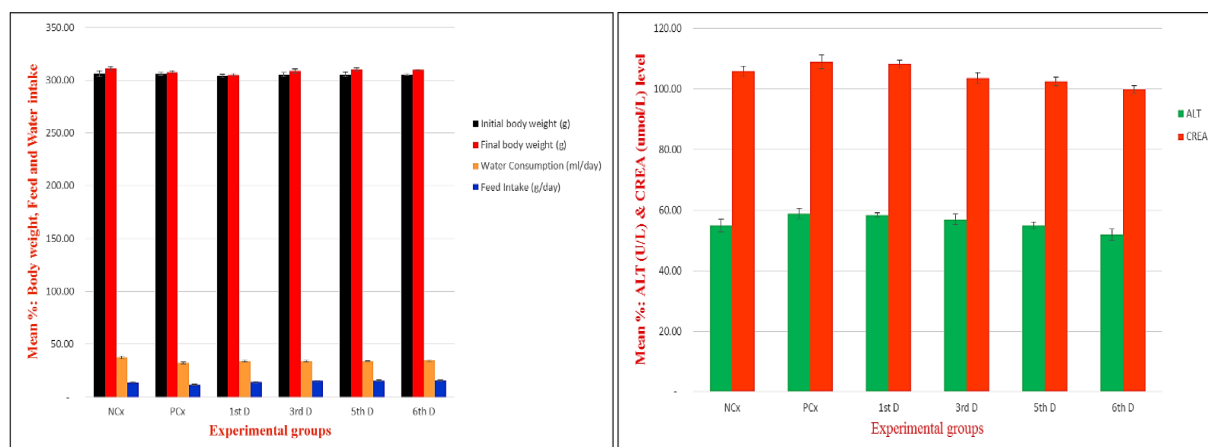


Fig. 1. Effects of (100 mg/kg/day (p.o.)) NS for six successive days (left bar graph): body weight, feed and water intake (right bar graph): serum ALT and CREA levels. No significant changes were observed, following supplementation. Each column represents the mean of three rats \pm SEM.

Oral treatment of healthy rats with 100 mg/kg/day (P.O.) NS for 6 successive days, induced a significant increase in erythrocytes (SOD and G-Px) and a significant decrease in plasma (MDA), during the third day only (group B) of administration when compared with the NCx group. While in the fifth and sixth days

of administration, these changes gradually started to return to the normal values. The inhibitory effect of NS on plasma MDA enzyme was associated with an increase of erythrocyte (SOD and G-Px) (Fig. 2 and 3).

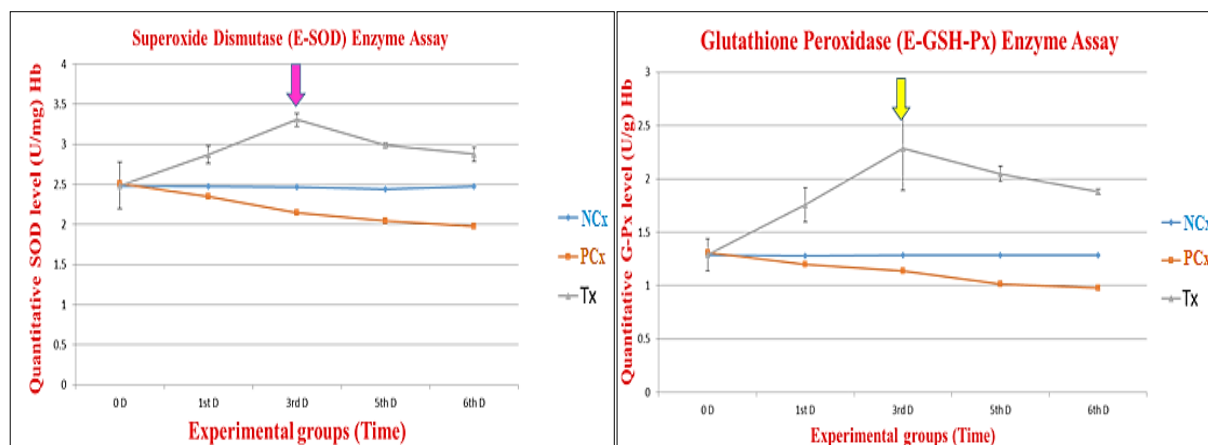


Fig. 2. Effects of 100 mg/kg/day (P.O.) of NS, for six successive days, (left line graph) E-SOD (U/mg Hb) level, (right line graph) E-G-Px (U/g Hb) level are plotted, the pink and yellow arrows indicate the point when the enzymes reached its peak (the 3rd day after administration).

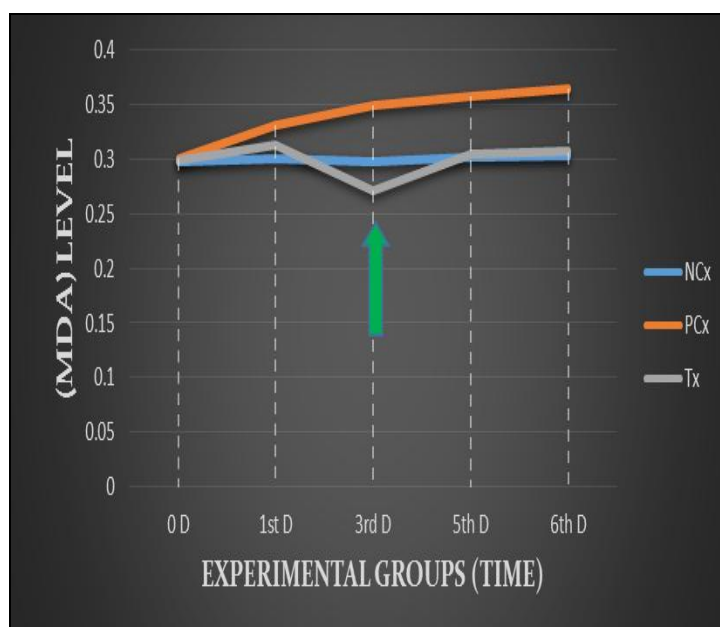


Fig 3. Effects of 100 mg/kg/day (P.O.) of NS, for six successive days on the oxidative stress marker enzyme level (MDA). The mean \pm SE of Plasma-MDA (nmol/mL) levels are plotted, the green arrow indicates the point when enzyme reached the lowest level (the 3rd day after administration).

Histopathological findings

Gross evaluations (Organs weight, size, color and consistency)

According to the somatic index determinations (SI) for rats, as illustrated graphically in Fig. 4, there were no significant changes in liver, spleen and kidney weight observed in all experimental animals as compared to NCx. Also, following palpation and visual examination of these organs, they exhibited normal consistency, color and size (No gross abnormalities related to 100 mg/kg NS freshly suspension administration was detected in the 6 days) (as in photographs) Fig. 5, 6 and 7.

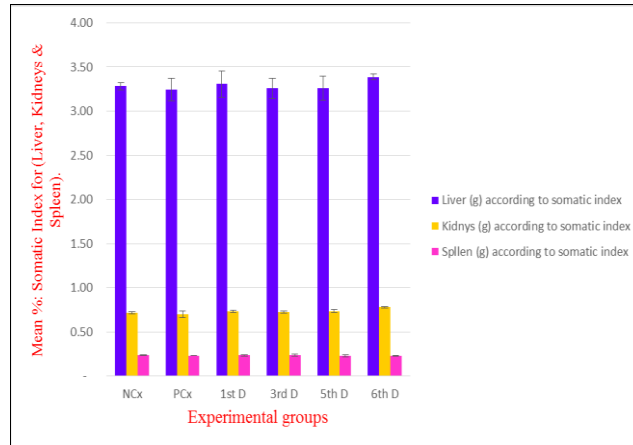


Fig. 4. Effects of (100 mg/kg/day (P.O.)) of NS, for six successive days on the mean \pm SE of (liver, kidney and spleen) somatic index (SI). No significant changes from NCx were observed following supplementation. Each column represents the mean of three rats \pm SEM, [SI = Organ W./body W.].

Microscopic analysis

The results of the histopathologic examination are shown in Table (1). The histological architecture of all rats' groups revealed:

Liver: normal hepatic cells with well-preserved cytoplasm, prominent nucleoli and central veins.

Kidney: normal cellular architecture and tubules with intact glomeruli.

Spleen: normal structure of red and white pulp, as in photomicrographs.

Table 1. Lesion scoring for tissues after NS supplementation for six consecutive days.

Organ	Lesion	Control		1 st D	3 rd D	5 th D	6 th D
		NCx	PCx				
Liver	Congestion	0.00	0.00	0.00	0.03 \pm 0.01	0.04 \pm 0.01	0.05 \pm 0.006
	Dilation of sinusoid	0.00	0.00	0.00	0.01 \pm 0.006	0.01 \pm 0.006	0.02 \pm 0.01
	Inflammatory cells infiltration	0.00	0.00	0.00	0.00	0.01 \pm 0.006	0.01 \pm 0.013
	Hepatocellular degeneration	0.00	0.00	0.00	0.00	0.00	0.00
	Hepatocellular Necrosis	0.00	0.00	0.00	0.00	0.00	0.00
Kidney	Congestion	0.00	0.00	0.00	0.00	0.01 \pm 0.006	0.02 \pm 0.011
	Interstitial leukocytic infiltration	0.00	0.00	0.00	0.00	0.00	0.00
Spleen	Congestion	0.00	0.00	0.00	0.00	0.01 \pm 0.006	0.01 \pm 0.006
	Lymphoid depletion SWP	0.00	0.00	0.00	0.00	0.00	0.00
	Mononuclear infiltration SRP	0.00	0.00	0.00	0.00	0.00	0.00
Total lesions score		0.00	0.00	0.00	0.04	0.08	0.11

NCx: negative control, PCx: positive control, SWP: splenic white pulp, SRP: splenic red pulp, *: Significantly different at $P < 0.05$.



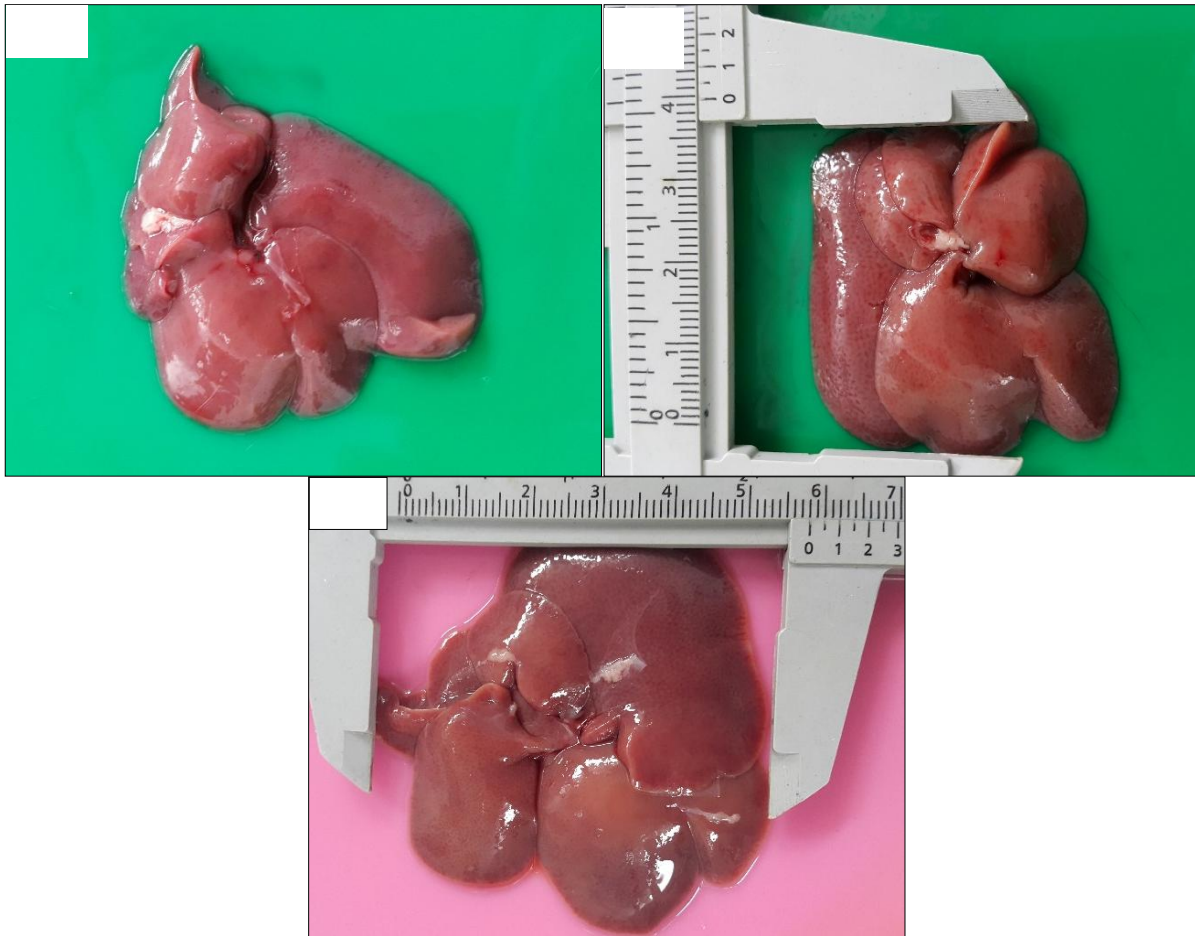


Fig. 5. Photograph illustrating the gross morphological changes of liver. At necropsy, (control and NS groups) showing a normal structure, color and size for all rats.



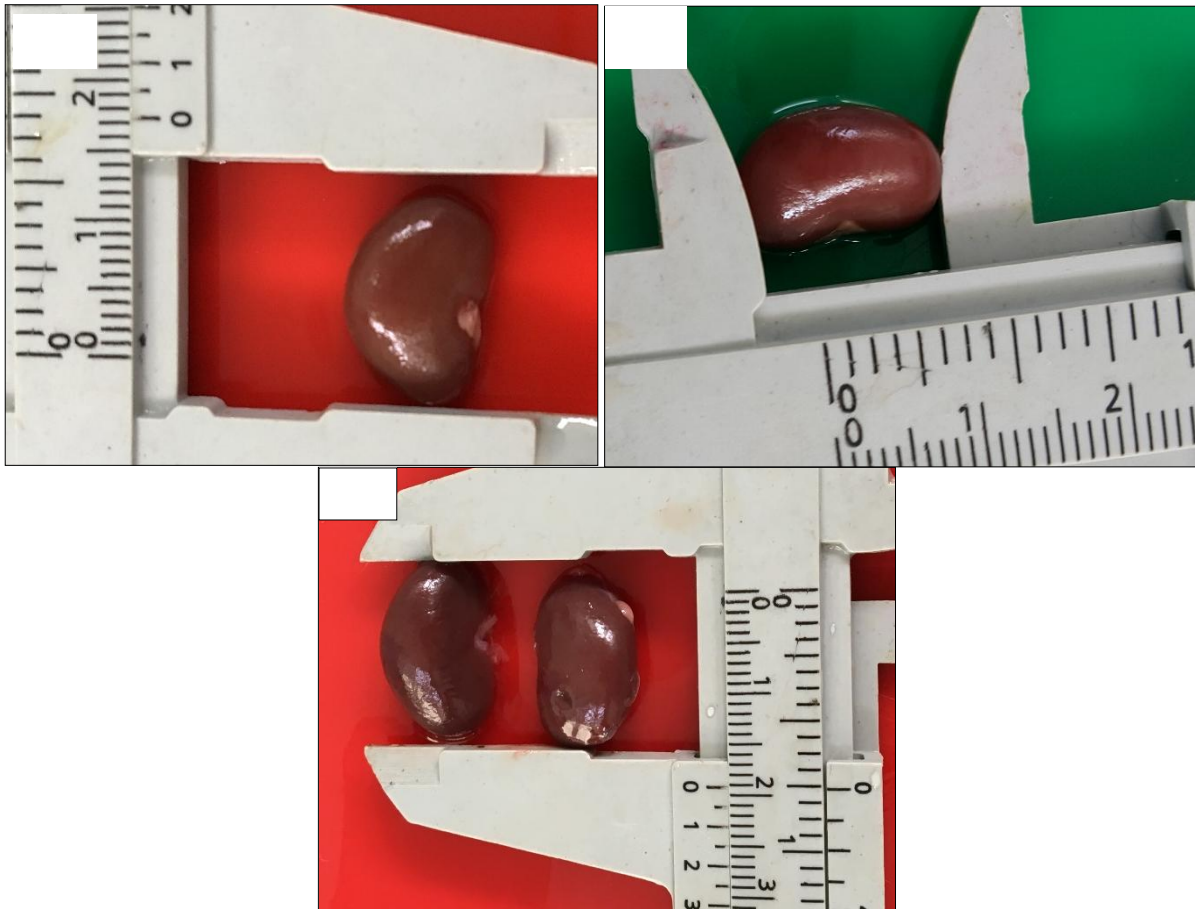


Fig. 6 Photograph illustrating the gross morphological changes of kidney. At necropsy, (control and NS groups) showing a normal structure, color, size and weight for all rats.



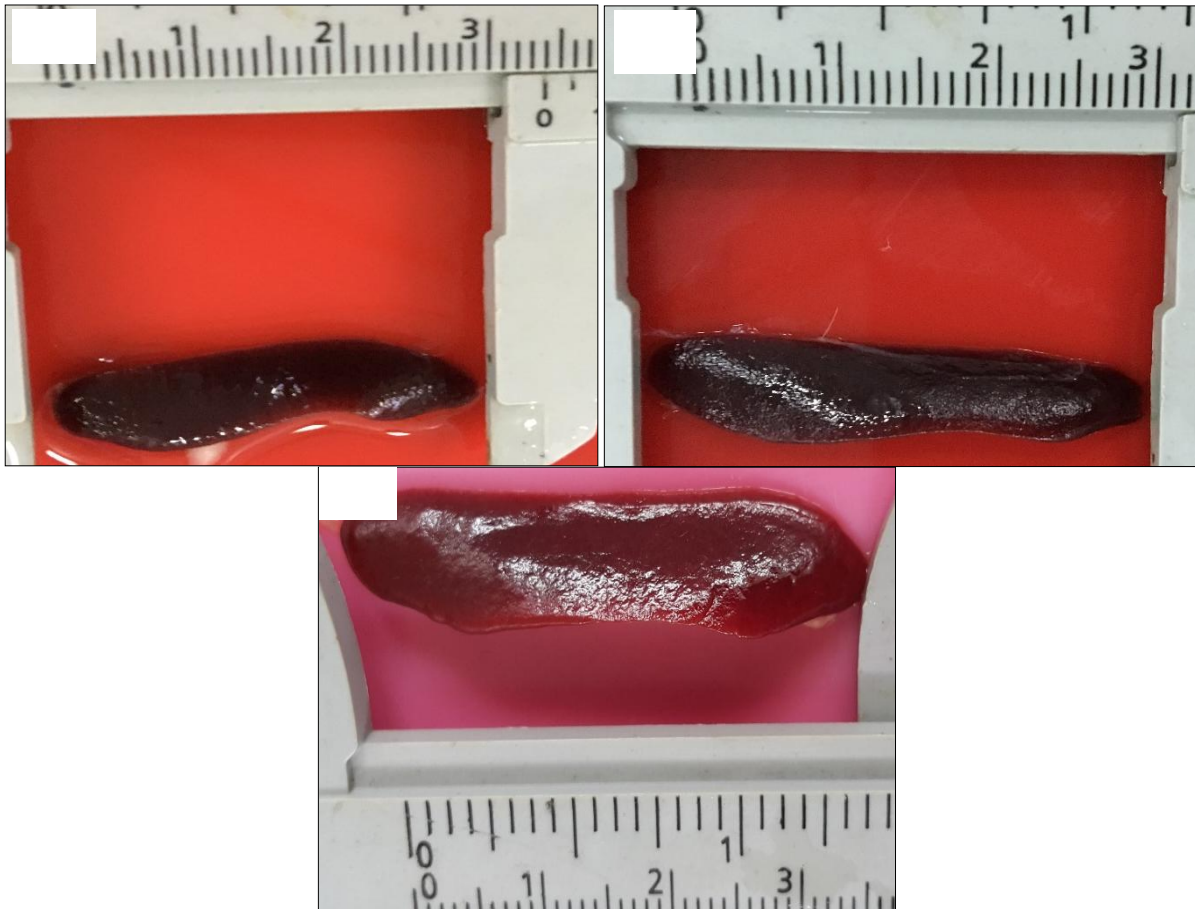
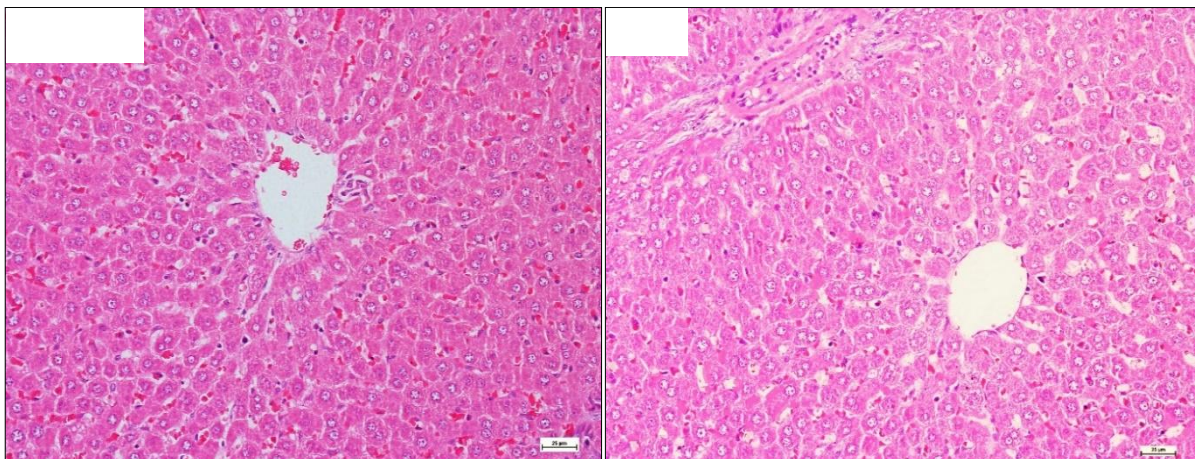


Fig. 7. Photograph showing the gross morphological changes of spleen. At necropsy, (control and NS groups) displaying a normal structure, color and size for all rats.



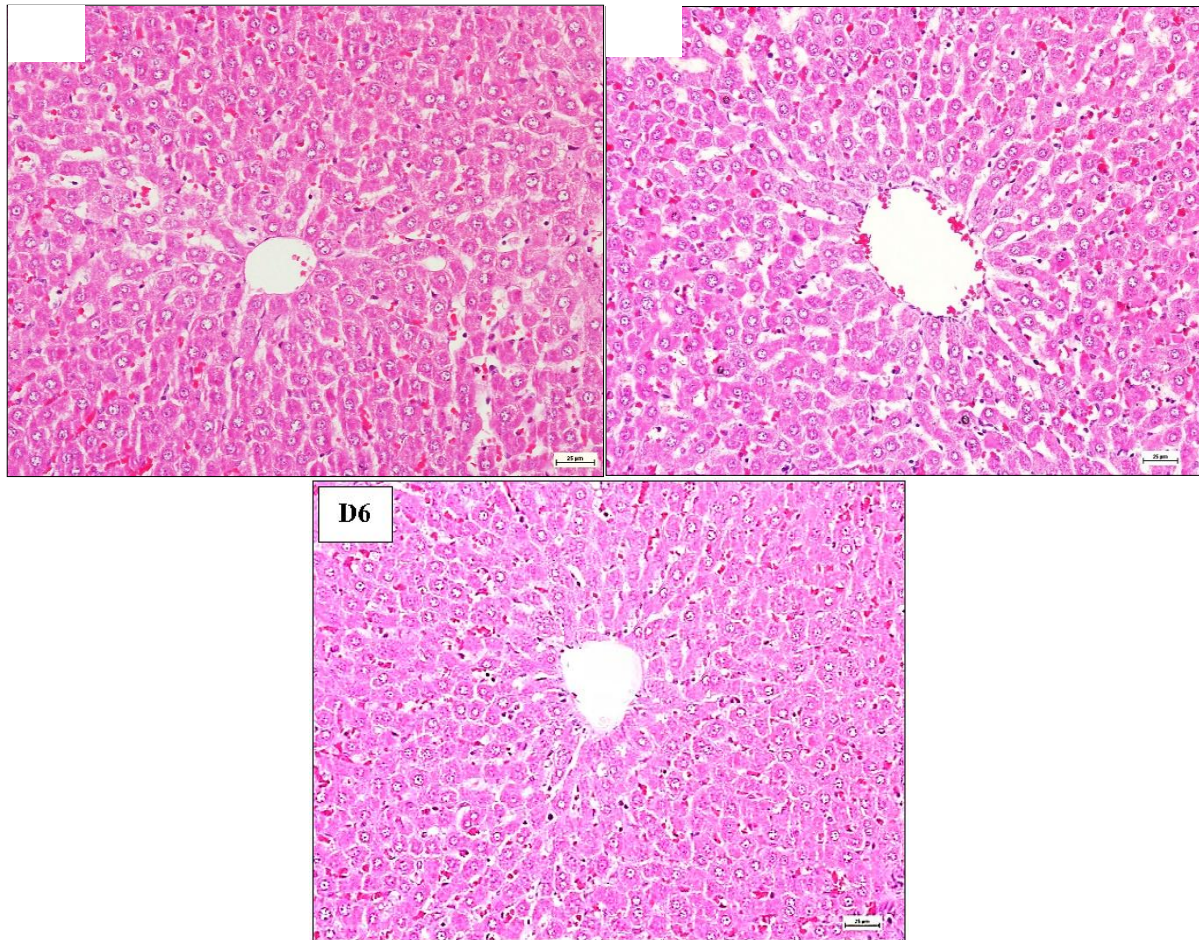
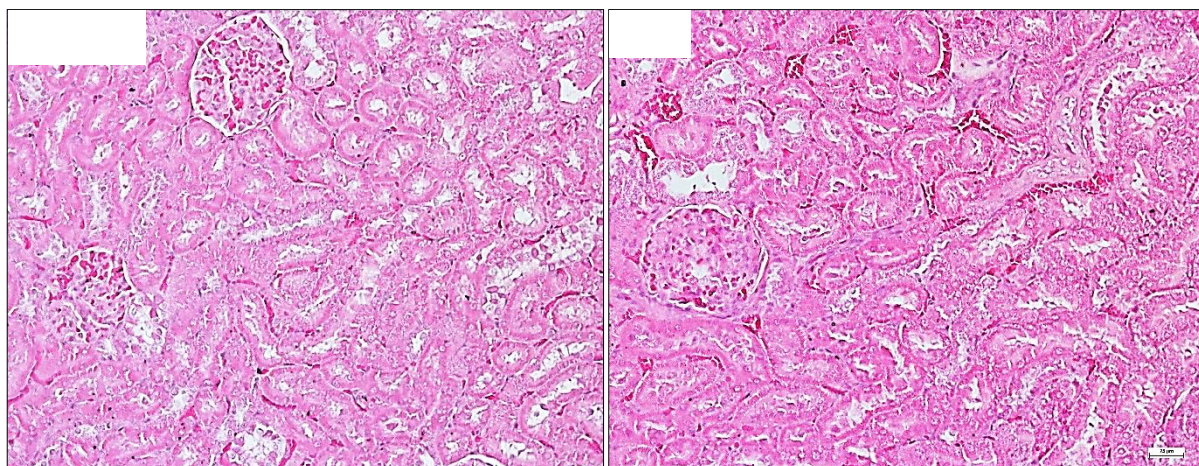


Fig. 8. Photomicrograph of liver from, Control and NS (100 mg/kg) groups show a normal limit, represented by normally arranged hepatocytes around the central vein together with normal sinusoidal capillaries (H&E 20_x).



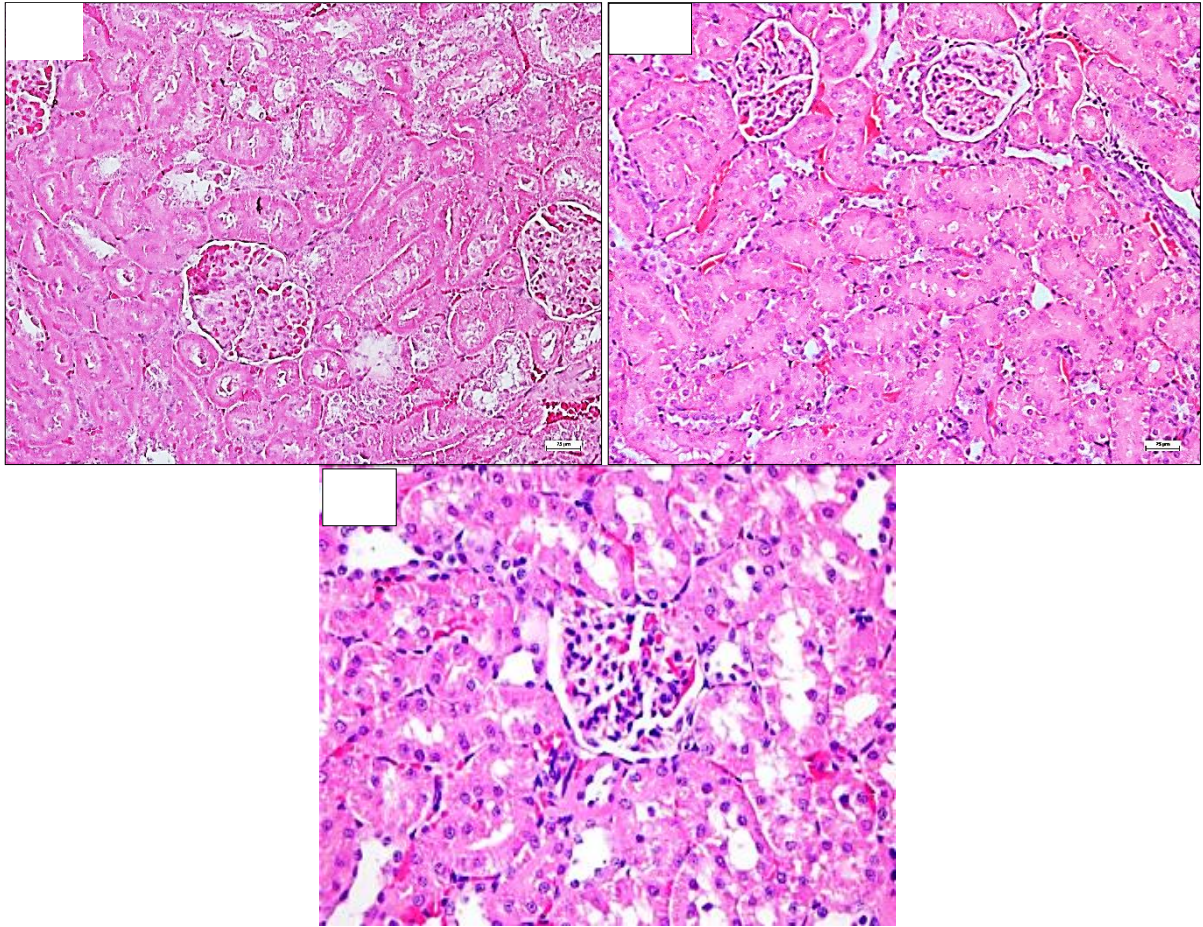
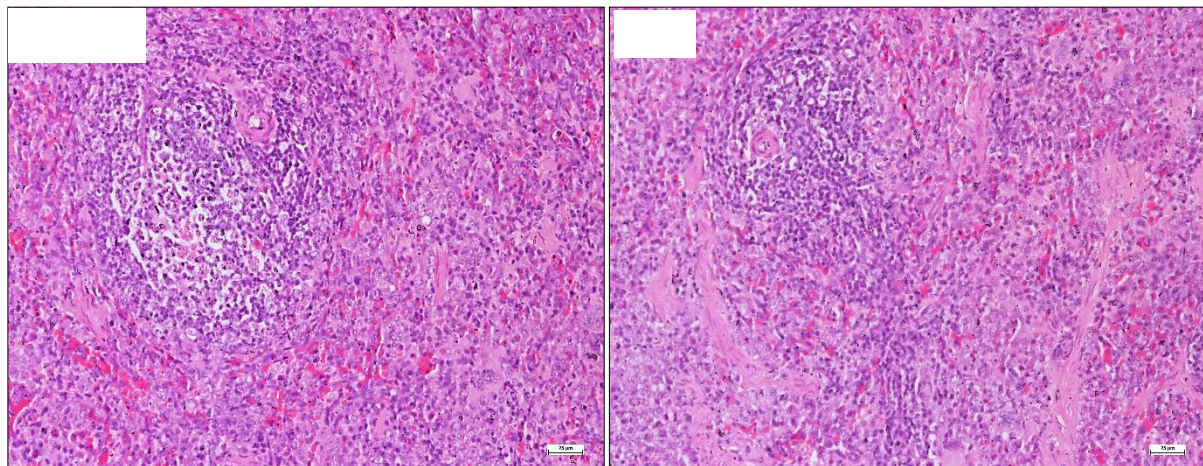


Fig. 9. Photomicrograph of Kidney from, Control and NS (100 mg/kg) groups show a normal limit, represented by normal glomeruli and tubules (H&E 20&40_x).



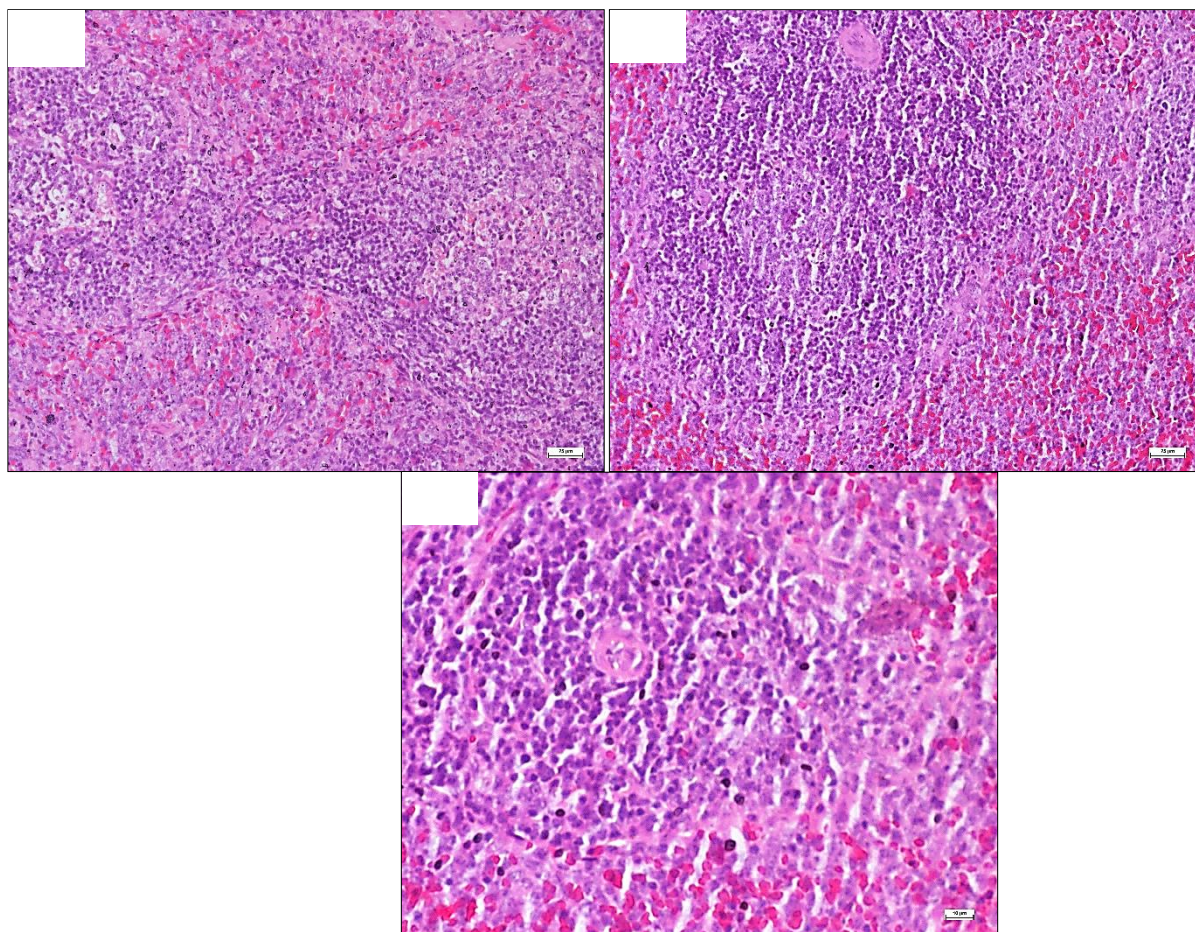


Fig. 10. Photomicrograph of spleen from, Control and NS (100 mg/kg) groups show a normal limit, represented by normal splenic white and red pulp (H&E 20, 40_x).

There is a paucity of information or studies regarding the relationship between the health status of an animal and the route of administration. To the best of our knowledge, this study appears to be the first to report the influence of oral administration on oxidative stress. This study aimed to determine the adverse effects associated with the dosing route, particularly oral gavage (P.O.). Up until now, it was believed that, since oral gavage causes passive reflux, resulting in overfilling of the stomach, it may lead to esophageal, and gastric irritation (Turner *et al.*, 2011). An increase in MDA levels and a decrease in SOD and G-Px concentrations, through the investigated routes in the PCx group as compared to the NCx group in our study, was suggestive of the role of oral gavage-induced oxidative stress, even in the absence of toxic agents. The decrease in antioxidant defense activity of SOD, G-Px could be attributed to the oxidative inactivation of enzymes, due to excess ROS generation, which could also lead to an increase in MDA levels. The generation of hydroxyl radicals may lead to inactivation of these enzymes (Krishnakantha & Lokesh, 1993; Nehar, 2014). Also, in our study the decreased activity of G-Px, could be attributed to a reduction in reduced glutathione GSH (by oral gavage induced-oxidative stress), which could result in an increase in peroxides. A reduction in glutathione GSH leads to a proportional decrease in H₂O₂ detoxification, by glutathione peroxidase (Sodhi *et al.*, 2008). Therefore, the balance of this enzyme system is essential, in removing superoxide anion and peroxides generated in the liver. When animals are exposed to stress, they are more vulnerable and susceptible to infectious pathogens, and subsequent shedding (Fitzgerald *et al.*, 2003).

To the best of our knowledge, this study was the first to demonstrate the effects of oral administration of 100 mg/kg (b.w)/per day of NS freshly suspension for six consecutive days, on Sprague-Dawley rats. The results revealed that treatment up to day three was highly efficacious in increasing antioxidant enzymes and abating oxidative stress and lipid peroxidation in NS-treated groups (Tx). These findings are of utmost importance, as NS is currently being subjected to several pharmacological screening studies, as a potential candidate for the prevention of carcinogenesis (Mansour *et al.*, 2002). The treatment groups (Tx) of rats with (100 mg/kg) NS induced an increase in antioxidant enzymes (E-SOD & E-G-Px) activity, which peaked on the 3rd day of administration. In the PCx group, which were not treated with NS, the antioxidant enzyme levels declined and remained normal in the NCx group. The prophylactic role and antioxidant effects of NS were

further supported by the significant reduction of lipid peroxidation and oxidative stress during the same period in (Tx) groups, indicated by the levels of plasma malondialdehyde (MDA). The inhibition of lipid peroxides (MDA) as well as an increase in SOD and G-Px activity, among the treatment groups (Tx) immediately after oral administration of NS (which peaked after three days) in the current investigation, could presumably be explained as protective effects of NS against oxidative damage. It was reported that the fixed oil of NS has both antioxidant and anti-eicosanoid effects greater than thymoquinone (the active constituent) alone (**Kanter et al., 2005**). Furthermore, a decrease observed in MDA levels in the Tx groups following NS supplementation, is suggestive that NS has appreciable free radical scavenger properties and possesses the ability to suppress lipid peroxidation as similarly reported in previous studies (**Yaman & Balikci, 2010; Al-okaily et al., 2012**). Regulation of lipid peroxidation, radical scavenging and antioxidant status may be one of the important mechanisms by which NS exerts its toxic inhibitory effect. The results of this study corroborate with the results of previous studies in which the effects of black cumin seeds was investigated (**Nagi et al., 1999; Burits & Bucar, 2000; Meral et al., 2001; Tuluce et al., 2009**). The previous studies reported that, the positive effects of black cumin seeds such as, inhibiting lipid peroxidation of biological membranes, activities of liver enzymes and contributing to the antioxidant defence system, could be attributed to the main active constituent, thymoquinone as well as other components namely, carvacrol, anethole, and 4-terpinol of black cumin essential oil (**Tuluce et al., 2009**). However, from the fourth day onwards following NS administration for the Tx groups, a decline in SOD and G-Px levels to the normal value, was observed. Although no studies have evaluated the effects of NS feeding following stress induced by oral gavage even in the absence of toxic agents, the decline observed could be associated with the consequence of less availability of free radicals or superoxide radicals (substrate for these antioxidant enzymes), which decreased gradually after administration of NS. It could be due to the reported potent superoxide radical scavenging property of NS, which has been shown to be as effective as superoxide dismutase.

The findings of the present trial are consistent with a previous study that, demonstrated how prior treatment with NS resulted in a reduction in the levels of lipid peroxidation and oxidative stress (**Mansour et al., 2002**). Another study reported similar results, which oral administration of TQ effectively induced an increase in quinone reductase and glutathione transferase activity, even in the absence of toxic agents. They also reported that administration with NSO or TQ inhibited ROS generation and caused an increase in the levels of antioxidant enzymes such as SOD and GSH.

The present study investigated the effect of NS on (ALT) and creatinine (CREA) as well as the pathological changes of liver and kidneys (macro and microscopic pathological changes). ALT is an enzyme normally present in high levels in the liver and less in the other cells. When the liver is damaged, the levels of ALT in the blood increases, thus indicative of liver injury. Creatinine is also considered a good indicator for kidney health or integrity. During hepatocellular injury, and kidney disorder, enzymes that are usually located in the cytosol are subsequently released into the bloodstream. Therefore, the quantification in plasma is a useful biomarker, to determine the extent and type of damage (**Pari & Murugan, 2004**). Although both ALT and AST are used to evaluate the hepatocellular integrity of the liver tissue, ALT is found more in the liver, while AST is usually found in equal amounts in the liver, heart, muscle, kidney and brain (**Dollah et al., 2013**). Therefore, ALT is more liver-specific than AST, and for this reason, ALT was chosen for this study. The results of the present study showed that supplementation of (100 mg/kg) NS for six days did not induce significant changes in the biochemical parameters of liver and kidney function, and gross, histopathological examinations revealed the normal architecture of liver, kidney and spleen. It was further supported by a decrease (no significant reduction) of serum ALT and creatinine level in Tx compared to NCx. While in the PCx group there were slight gradual increases in the level of these enzymes from the first day of the experiment. These observed results could be due to the oral method of administration, which inherently could be the cause of adverse effects, such as increased oxidative stress in PCx. This induced toxicity even in the absence of toxic compounds, and due to the short duration of the experiment, histological changes were not observed. While a decrease in the levels of these enzymes observed in the treatment groups, following administration of NS, was due to the anti-oxidative stress effect and free radical scavenger role of NS. The lesions score technique was employed, to confirm the findings, and the absence of pathological changes in the tissues, especially in the histological evaluation and lesion scores, confirmed the results. Previous studies also reported similar findings that, NS seeds extract increased the activity of SOD, GPx and CAT enzymes (**Kaleem et al., 2006**). Also, oral administration of aqueous extract of NS seeds did not cause any significant changes in liver function, demonstrated by hepatic enzymes levels and histopathological changes of liver tissue (**Mohammed, 2010**). Furthermore, **Al-Ghamdi, (2003)** reported no toxic effects of NS on hepatic enzymes among asthmatic patients. Another study also reported the absence of toxicity in mice treated with NS fixed oil (**Zaoui et al., 2000; Al-Ghamdi, 2003**).

The current study showed that six days of (100 mg/kg) NS (P.O.) administration, did not cause/give rise to any toxic effects on vital organs, specifically the liver, kidneys and spleen. However, prior feeding of NS, induced-protective effects for these organs against oxidative effects caused by oral gavage. Most previous

studies have suggested a hepatoprotective effect of NS, due to some components such as thymoquinone, monoterpenes, (El Tahir *et al.*, 1993) tocopherols, phytosterols, or phenols (Ramadan *et al.*, 2003).

However, Türkdougan *et al.* (2001) found that NS had the ability to prevent liver fibrosis and cirrhosis, indicating that NS protects the liver against fibrosis possibly through immunomodulator and antioxidant activities. Similar to our results, Meral *et al.* (2001) also suggested that NS treatment increased the antioxidant defence system activity in experimentally induced diabetic rabbits. While (Kanter *et al.*, 2003) concluded that NS caused a decrease in lipid peroxidation and liver enzymes and an increase in antioxidant defense system activity in the CCl₄-treated rats. They also reported that it prevented weight loss induced by the CCl₄ treatment. Our findings are in accordance with previous reports of other related studies, (Gul *et al.*, 2000; Kaleem *et al.*, 2006) that have suggested the protective role of NS seeds extract, possibly due to the antioxidative effect of flavonoids present in the seeds, that act as strong superoxide radicals and singlet oxygen quenchers. Also, we agree with (Inoue *et al.*, 1987) who concluded, treatment with NS seeds extracts caused an elevation of GSH levels, which protects the membranes against oxidative damage through regulation of the redox status of protein in the membrane.

Treatment with NS even in the absence of toxic agents may (strengthen, enhance, reinforce) antioxidant defence status of the body such as intracellular glutathione to induce anti-oxidative stress and free radical scavenger activity, following NS administration. This could explain the reason why an increase and decrease in SOD and G-Px levels were observed in a very short duration (peaked at the 3rd day of administration) and decreased to normal levels immediately after this period. This observation is in accordance with previous related studies by (Khalife *et al.*, 2007), where antioxidant and free radicals scavenging activities of glutathionyl–dihydrothymoquinone and DHTQ were observed, which resulted from the reaction that occurred between TQ and glutathione (GSH), NADH and NADPH, following NS administration. The possibility of an intracellular non-enzymatic metabolic activation of TQ dependent on GSH, NADH or NADPH, has also been suggested that could act as a “cellular switch”, which could regulate cellular antioxidant defences. The results of this study are in agreement with the observations of other related studies (Darakhshan *et al.*, 2015), which reported TQ induction of the expression and/or activity of GST, GSH-Px, SOD, and glutathione reductase. Consequently, TQ improved plasma and liver anti-oxidant ability and increased expression of liver anti-oxidant genes.

IV. Conclusion

The results of this study provided evidence that NS is a promising medicinal plant with many therapeutic properties, and it appeared to be safe with possible protective properties against free radicals induced hepato-nephropathy. When administered orally, NS lead to reduced impairment of liver functions by the preservation of intracellular GSH-Px, which could explain the hepatoprotective effect, decreased MDA concentration and increased SOD, GSH-Px concentrations, as a result of its antioxidant nature. It also prevented lipid peroxidation induced liver damage, by its radical scavenging nature. With the evidence of normal histological findings for all treated groups, it suggests that NS does not have any toxic effects on liver and kidneys at a dose (100 mg/kg/day/orally) for six consecutive days.

Treatment with NS at 100 mg/kg/per day for three consecutive days is highly efficacious in abating oxidative stress in rats.

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