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Original Article

Nigella sativa Averts 5-Fluorouracil Induced Kidney Injury via Targeting Redox Imbalance and MAPK Pathway

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ABSTRACT

Introduction: Chemotherapy-induced organ toxicities are the most frequent toxic manifestation of 5-fluorouracil (5-Fu) action in cancer patients. Hence, new approaches are required to deter chemotherapy-induced kidney toxicity. *Nigella sativa* (NS) is recognized as black cumin and has been found to be antiapoptotic, antioxidant, antimicrobial, anti-inflammatory and mitigates renal damage. **Objectives:** Thus, designed this work to evaluate the effect of NS in averting Nephrotoxicity induced by 5-FU treatment. **Materials and Methods:** Male albino Wistar rats were grouped and administered with saline, 5-FU group (150 mg/kg), 5-FU+NS (200 mg/kg) and 5-FU+NS (400 mg/kg), respectively. Rats were sacrificed on the 21st day, and biochemical, histological, serological and molecular estimations were done with kidney tissues and blood. 5-FU caused kidney injury as demonstrated by variations in kidney function markers (BUN, Cr, Kim-1), lipid peroxidation, histology and diminution of antioxidant guard machinery (GSH, GR, GPx and CAT). Additionally, 5-FU action changed p38 MAPK pathway proteins (p-p38, pJNK, pERK1/2, pNFkB, TNF-α) significantly. **Conclusion:** NS may serve as a potential candidate against renal injury by mitigating redox signaling, inflammation and p38 MAPK pathway. Therefore, NS could be used in adjuvant therapy for the prevention of Nephrotoxicity in cancer patients caused by 5-FU.

Keywords: 5-FU, p38MAPK pathway, Inflammation, Oxidative stress, ROS, *Nigella sativa*.

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INTRODUCTION

Cancer is one of the top most reasons for mortality globally. The current therapeutic procedures/strategies are not suitable enough as prevalence and death rates are not falling huge. Chemotherapy is the most frequently used strategy to manage different types of malignancies. Nevertheless, it is allied with the utmost toxic outcomes.[1] 5-Fluorouracil (5-FU) is a chemotherapeutic drug used for treating neoplasia commonly, which include colon, breast, and lung. Most important impediment of 5-FU usage is its toxic manifestation in terms of damaging normal proliferating cells leading to organ damage and failure, which is well documented like diarrhoea, mucositis, myelosuppression, dermatitis, hepatic damage, renal toxicity, cardiotoxicity, and toxicity of genitals. 5-fluoro-2′-deoxyuridine-5′-triphosphate, 5-fluoro-uridine-5′-triphosphate and 5-fluoro-uridine-5′-monophosphate are the three major reactive metabolites which are formed on metabolic activation of 5-FU. These metabolites exert a cytotoxic action by an interruption in the synthesis of nucleic acid and thymidylate synthase in cancerous and normal proliferating cells also. 5-FU shows dose-dependent toxicity causing serious, painful side effects which differ from patient to patient leading to termination of the therapy.[2,3] Reports suggest that excessive generation of free radicals and inflammation-related intermediaries play a major part in 5-FU-influenced damage.[4] Therefore, compounds having antioxidant and anti-inflammatory efficacy may possibly alleviate the chemotherapy-induced toxicity. Combinational treatment therapies with 5-FU have been used to diminish the toxic effects and deprive of its anti-cancer action.

Plant foods like fruits, vegetables, grain, herbs, spices and others are natural compounds which have secondary plant compounds in them with health promotion and disease prevention attributes. The use of herbs is widespread and growing dramatically in medicine due to nourishing and synergistic effects, making them an exceptional treatment approach/strategy.[5,6] *Nigella sativa* (NS) belongs to the Ranunculaceae family, and can be an ideal compound having low toxicity and manifold mechanisms of action. NS is used in Iranian traditional medicine to treat numerous ailments like respiratory, gastrointestinal, hepatic, and renal diseases. Recent reports suggest that NS shows antimicrobial, antioxidant, anti-inflammatory, anti-cancer, hypoglycemic, spasmolytic and bronchodilator activities due to the diversity of mechanism of action of its active constituents present in it.[7-9]

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NS has deciphered robust tactics in the mitigation of various diseases because of its sturdy antioxidant and anti-inflammatory potential innately both at the preclinical and clinical levels.[10] Reports show that NS intervenes in a range of diverse signalling pathways.[11,12] Therefore, keeping all the above features of NS in mind, the current work was planned to discover the mitigation of NS against anti-cancer drug 5-FU encouraged kidney damage.

MATERIALS AND METHODS

Plant material

Preparation of the plant extract

Soxhlet extractor having 70% of ethanol, 100 g powdered seeds of NS were extracted in it. The extract was concentrated under reduced pressure and stored at −20°C until it was used. The yield of the resulting extract was 32%, and it was finally dissolved in saline for final use in animals.[13]

Animal Study

Male albino Wistar rats having a weight of 180–200*g* were reared at the animal facility of Prince Sattam Bin Abdulaziz University, Al Kharj having ethical clearance no. BERC-015-05-21. Animals were kept in a controlled environment having a temperature as 21–25°C, relative humidity (50 ± 8%) and 12 hr of light and darkness to mimic normal conditions. Rats were having contact with water and food freely.

Animals and the experimental protocol

Twenty-four, 6 weeks old, male Wistar rats of weight 180 ± 200 g were separated into four groups randomly followed by the experimental protocol as given in the schematic treatment regimen in Table 1. Group I rats were administered with saline orally, also called as negative control rats. The animals in group II were administered with saline as a group I and was also injected once intraperitoneally 5-FU (150 mg/kg) on day 19th, also called 5-FU or positive control group. Group 3 (5-FU+NS1), and group 4 (5-FU+NS2) were treated orally daily for 20 days with 200 and 400 mg/kg of NS and an injection of 5-FU once i.p. was given on 19th day. Male albino Wistar rats were sacrificed on day 21^{st,} and the kidney tissue was perfused and harvested.[14-16] Blood was taken under mild anaesthesia before sacrifice to get the serum for checking serum toxicity markers. Biochemical parameters and protein estimations were executed with the kidney tissue homogenate. The homogenate was made in 0.1 M phosphate buffer 4°C at physiological pH using a homogenizer in KCl (1.17%) with the purged tissue which was washed with ice-cold saline. Some part of kidney tissue was kept in 10% buffered formalin for histology studies.[17]

Kidney tissue processing

Kidneys were cleaned and washed with chilled saline at physiological pH. 10% (w/v) tissue homogenate was produced in tris hydrochloride buffer (0.1 M) at physiological pH using a homogenizer. The homogenate was spined at 12000 rpm for twenty minutes at 4°C. The clear supernatant, after spinning, was operated to explore the incidence of numerous antioxidant marker enzymes like Catalase (CAT), Glutathione (GSH), Glutathione Reductase (GR), Glutathione Peroxidase (GPx), and Malondialdehyde (MDA). To quantify the absorbance, UV-1601 was used, and an Elisa Plate Reader to measure the biochemical parameters, serological markers and proteins of the p-p38 Mitogen-Activated Kinase Pathway (MAPK).[18]

Assessment of Lipid Peroxidation (LPO)

LPO assessed by published protocol.[19] 1 mL reaction was obtained by mixing 200 mL (100 mM) of ascorbic acid with the supernatant (200 mL), 20 mL of ferric chloride (100 mM) and phosphate buffer (580 mL, 0.1M) at physiological pH. This 1mL reaction mix was kept in a shaking water bath at 37°C for one hour. One mL of ten percent of trichloroacetic acid was used to stop the reaction subsequently, 10 mL of thiobarbituric acid (0.67%) was added, and all the reaction tubes were placed in a boiling water bath for 20 min. These reactions were then moved to an ice bath and then spined at 2500× *g* for ten minutes, and absorbances were taken.

Assessment of antioxidant enzyme machinery

Assessment of CAT activity

CAT was obtained as per the previous reported method.[20] The reaction began by mixing 50 mL of Post Mitochondrial Supernatant (10%), 1950 mL of 0.1 M phosphate buffer and 1000 mL of 0.10 mM H_2O_2 at physiological pH. The absorbance of the sample was taken finally.

Assessment of GSH

GSH assessed the following.[21] Equi-volume mixture of 1:1 (v/v) ratio of Post Mitochondrial Supernatant with 4.0% sulfosalicylic acid was done to initiate the mixture followed by incubation for 60 min at 4°C, and centrifugation at 4°C for 1200× *g* for 15 min. 10 mM DTNB (400 mL) was added to four hundred mL aliquots, phosphate buffer (0.1M, 2.2 mL, pH 7.4) followed by measuring of the absorbance.

Assessment of GR Activity

GR calculated by the method.[22] 50uL of 0.5 mM EDTA, 25uL of 1.0 mM oxidized glutathione, 0.1 M phosphate buffer (0.825 mL) at physiological pH, 50uL (0.1 mM NADPH) and 10% PMS (50 mL) to obtain 1 mL of the reaction mixture. Absorbance was measured.

Assessment of GPx

GPx measured.[23] The reaction started with a mixture of 1 mM sodium azide (100uL), 1 mM EDTA (100 uL), phosphate buffer

(1440 uL, 0.1 M) at 7.4 pH, 1 IU/ mL glutathione reductase (50uL), GSH (50 uL, 1mM), 0.2 mM NADPH (0.1 mL), 0.25 mM H_2O_2 (0.01 mL) and ten percent Post Mitochondrial Supernatant (100uL) to obtain volume in milli litre. The usage of NADPH was calculated at 340 nm absorbance at 25°C.

Assessment of p38 MAPK pathway proteins viz phospho-p38 Mitogen Activated Protein Kinases (MAPK), phospho-Extracellular Signal-Regulated Kinases (p-ERK1/2), phospho c-Jun N Terminal Kinase (p-JNK), Nuclear Factor-kappa B (pNF-kB) and Tumour Necrosis Factor-alpha (TNF-α).

MAPK signalling pathway-related factors via ELISA in strict accordance with the instructions. NF-kB, TNF-α, p-ERK1/2, p-p38MAPK, and p-JNK, were measured by eBioscience assay kit and Invitrogen, CA, USA, respectively.

Protein estimation

The protein was estimated.[24]

Serum renal toxicity diagnostic markers

Male albino Wistar rats were anaesthetized following the experiment protocol. Blood was withdrawn by cardiac puncture, followed by sacrificing the animals. Serum was obtained by spinning for ten minutes at 10000 × g to evaluate the Kidney Injury Molecule (KIM-1), Creatinine (Cr), and Blood Urea Nitrogen (BUN) in serum samples from all the groups of animals.

Assay for BUN

BUN assessed[25]

An equivalent amount of 10% TCA and serum was centrifuged at 2000 rpm to obtain supernatant without protein. To this supernatant (500 uL), 3.5 mL of distilled H_2O and 3.2 mL of H_2SO_4–H_3PO_4 reagents, 0.8 mL of 2% diacetylmonoxime were added. The H_2SO_4–H_3PO_4 reagent is made of 85% phosphoric acid (150 mL) and concentrated H_2SO_4 (50 mL), and milliq (140 mL). Then placed on a boiling water bath for 30 min, this mixture was cooled, and then absorbance was taken.

Assay for Cr

Cr calculated by alkaline picrate protocol.[26] Initially, 1mL of the serum was added to 5% sodium tungstate (1.0 mL), 0.6 M H_2SO_4 (1.0 mL) and milliq (1.0 mL) mixed and spined at 800 g for 5 min. The improved upper layer/supernatant was joined with 1mL of 0.75 M NaOH and 1mL of 1.05% picric acid. Absorbance was taken precisely at 520 nm after 20 min.

KIM-1 measurement

The levels of KIM-1 were calculated using by ELISA-based kit (Adipo Bioscience®, Inc., USA) as per the protocol mentioned with the kit.

Histology

After sacrifice, kidneys were rapidly detached and conserved in 10% neutral buffered formalin for histology. The kidney was fixed in paraffin wax and lengthwise sectioned with a microtome, and then hematoxylin and eosin staining was done. Lastly, the stained paraffin-stained kidney sections were observed under a microscope.

Statistical investigation

The data is accessible as the mean ± Standard Error of the Mean (SEM) for each group. An Analysis of Variance (ANOVA) was used to determine the variances among groups. Subsequently, a Tukey-Kramer multiple comparisons test was done. The statistical significance is set at $p < 0.05$ for all contrasts as a criterion unless otherwise noted.

RESULTS

Effect of 5-FU and NS on MDA Levels

Increase in MDA level in group II given 5-FU in contrast to the group I (p<0·001) significantly. We detected treatment at both doses of NS led to the significant renewal (p<0·01, p<0·001) of membrane structure in renal tissue in contrast to the 5-FU group Table 3.

NS restores antioxidant machinery

Administration of 5-FU evidently exhausted kidney GSH reserves and repressed GPx, GR and CAT activities in contrast to the group I (p<0.001, p<0.01) (Tables 2, 3). Nonetheless, NS dosing at both doses resulted in dose-dependent (ns-non significant, p<0.05: p<0.01: p<0.001) retrieval in GSH reserves and activities of the above-mentioned antioxidant enzymes significantly in contrast to 5-FU group Tables 2 and 3.

NS protects kidney function markers

Administration of 5-FU demonstrated an elevation in kidney damage/diagnostic biomarkers (BUN, Cr and Kim-1) in 5-FU treated rats in contrast to the group I (Figures 1 and 2). Group II exhibited raised BUN, Cr and Kim-1 (p<0·001) in contrast to the group I significantly. There was a noticeable inhibition observed in BUN, Cr and Kim-1 (p<0·05, p<0·01, p<0·001) at both doses, respectively (Figures 1, 2) of NS treatment (Figures 1, 2).

NS downregulates p38 MAPK pathway proteins

P38 MAPK pathway plays an imperative part in the progress of 5-FU-initiated kidney toxicity. 5-FU administration caused a significant upsurge in p-p38 MAPK, pERK1/2, pJNK, pNFkB, and TNF-α in 5-FU in contrast to the control group (Figures 3, 4, 5, 6). Treatment with NS at both doses markedly (p<0.05, p<0.01, p<0.001) downregulated the levels of p38 MAPK pathway proteins in contrast to the 5-FU group (Figures 3, 4, 5, 6).

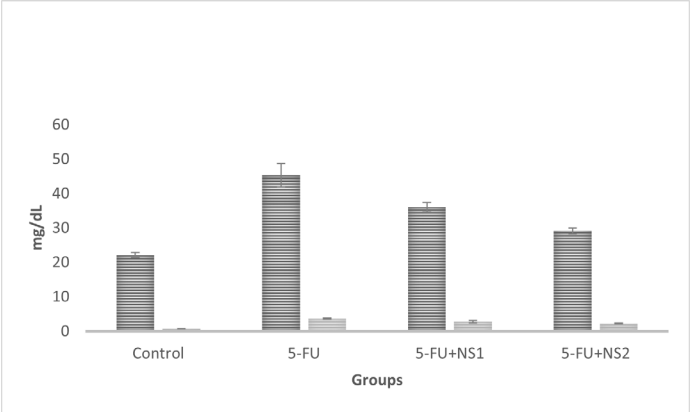

Figure 1: Effect of low and high prophylactic dose of NS on diagnostic serum toxicity markers (BUN-series 1 and Cr-series 2) in 5-FU induced kidney damage.

*Results are representative of mean ± SE of six rats per group. Values are expressed as the means ± standard error. Results that we got are significantly different from 5-FU treated group as the main comparison is with 5-FU treated group (***) $p < 0.001$ versus Control; (#) $p < 0.05$ versus 5-FU; (##) $p < 0.01$ versus 5-FU; (###) $p < 0.001$versus 5-FU; (**) $p < 0.01$ versus Control and (*) $p < 0.05$ versus Control. Also series 1 represents BUN and series 2 Cr.
Group I: Normal control, Group II: 5-Flurouracil (5-FU) treated (150mg/kg b.w), Group III: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Lower dose) (200 mg/kg b.w), Group IV: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Higher dose) (400 mg/kg b.w).

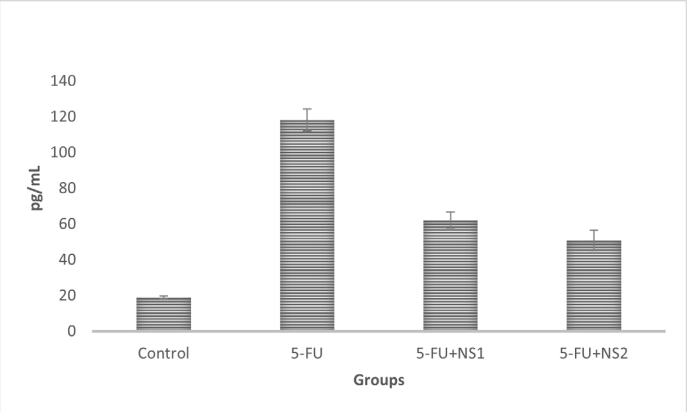

Figure 2: Effect of low and high prophylactic dose of NS on Kim-1 in 5-FU induced kidney damage.

*Results are representative of mean ± SE of six rats per group. Values are expressed as the means ± standard error. Results that we got are significantly different from 5-FU treated group as the main comparison is with 5-FU treated group (***) $p < 0.001$ versus Control; (#) $p < 0.05$ versus 5-FU; (##) $p < 0.01$ versus 5-FU; (###) $p < 0.001$versus 5-FU; (**) $p < 0.01$ versus Control and (*) $p < 0.05$ versus Control.
Group I: Normal control, Group II: 5-Flurouracil (5-FU) treated (150mg/kg b.w), Group III: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Lower dose) (200 mg/kg b.w), Group IV: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Higher dose) (400 mg/kg b.w).

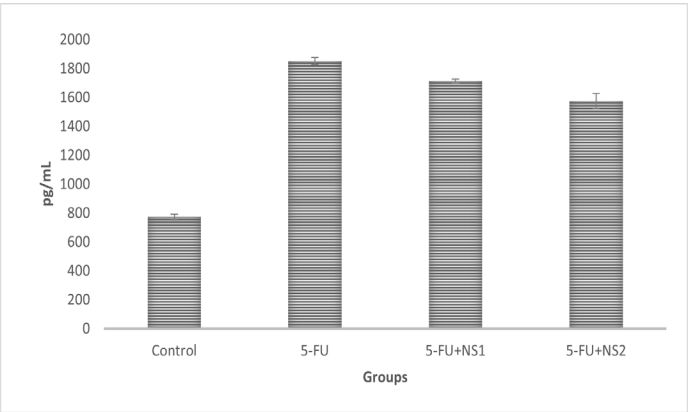

Figure 3: Effect of low and high prophylactic dose of NS on pNFkB in 5-FU induced kidney damage.

*Results are representative of mean ± SE of six rats per group. Values are expressed as the means ± standard error. Results that we got are significantly different from 5-FU treated group as the main comparison is with 5-FU treated group (***) $p < 0.001$ versus Control; (#) $p < 0.05$ versus 5-FU; (##) $p < 0.01$ versus 5-FU; (###) $p < 0.001$versus 5-FU; (**) $p < 0.01$ versus Control and (*) $p < 0.05$ versus Control.
Group I: Normal control, Group II: 5-Flurouracil (5-FU) treated (150mg/kg b.w), Group III: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Lower dose) (200 mg/kg b.w), Group IV: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Higher dose) (400 mg/kg b.w).

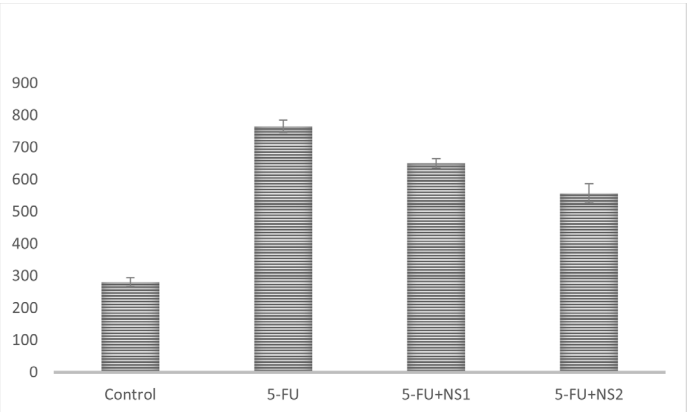

Figure 4: Effect of low and high prophylactic dose of NS on TNF-alpha in 5-FU induced kidney damage.

*Results are representative of mean ± SE of six rats per group. Values are expressed as the means ± standard error. Results that we got are significantly different from 5-FU treated group as the main comparison is with 5-FU treated group (***) $p < 0.001$ versus Control; (#) $p < 0.05$ versus 5-FU; (##) $p < 0.01$ versus 5-FU; (###) $p < 0.001$versus 5-FU; (**) $p < 0.01$ versus Control and (*) $p < 0.05$ versus Control.
Group I: Normal control, Group II: 5-Flurouracil (5-FU) treated (150mg/kg b.w), Group III: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Lower dose) (200 mg/kg b.w), Group IV: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Higher dose) (400 mg/kg b.w).

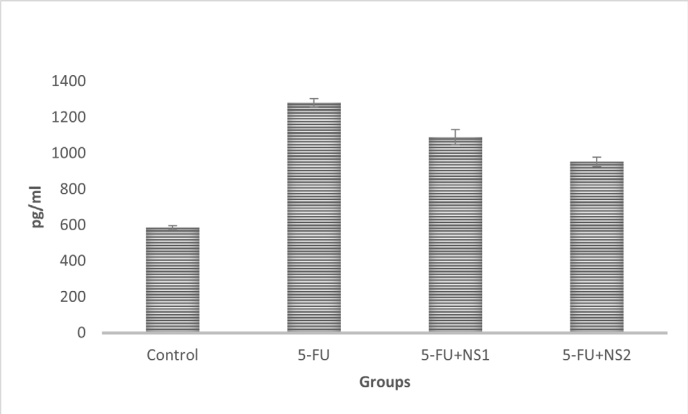

Figure 5: Effect of low and high prophylactic dose of NS on p38 MAPK in 5-FU induced kidney damage.

*Results are representative of mean ± SE of six rats per group. Values are expressed as the means ± standard error. Results that we got are significantly different from 5-FU treated group as the main comparison is with 5-FU treated group (***) $p < 0.001$ versus Control; (#) $p < 0.05$ versus 5-FU; (##) $p < 0.01$ versus 5-FU; (###) $p < 0.001$versus 5-FU; (**) $p < 0.01$ versus Control and (*) $p < 0.05$ versus Control.
Group I: Normal control, Group II: 5-Flurouracil (5-FU) treated (150mg/kg b.w), Group III: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Lower dose) (200 mg/kg b.w), Group IV: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Higher dose) (400 mg/kg b.w).

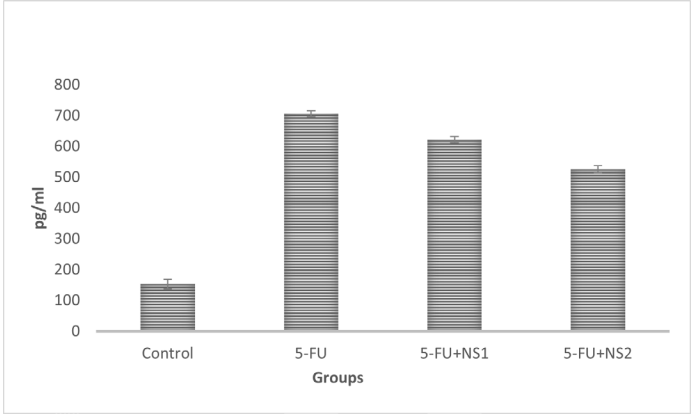

Figure 6: Effect of low and high prophylactic dose of NS on pERK1/2 in 5-FU induced kidney damage.

*Results are representative of mean ± SE of six rats per group. Values are expressed as the means ± standard error. Results that we got are significantly different from 5-FU treated group as the main comparison is with 5-FU treated group (***) $p < 0.001$ versus Control; (#) $p < 0.05$ versus 5-FU; (##) $p < 0.01$ versus 5-FU; (###) $p < 0.001$versus 5-FU; (**) $p < 0.01$ versus Control and (*) $p < 0.05$ versus Control.
Group I: Normal control, Group II: 5-Flurouracil (5-FU) treated (150mg/kg b.w), Group III: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Lower dose) (200 mg/kg b.w), Group IV: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Higher dose) (400 mg/kg b.w).

Effect of 5-FU and NS on histology of kidneys

Light microscopic examination revealed a standard histological assembly of the renal tissue of the negative control group (Figure 7A). However, 5-FU treated renal tissue showed severe histopathological modifications. Renal corpuscles reveal expanded Bowman's capsules, congested glomerular capillaries, haemorrhage in the interstitial tissue, and infiltration of inflammatory cells in addition to enlarged and congested blood vessels (Figure 7B). (Figures 7C, D) demonstrates that pre-, co-, and post-treatment of NS for 21 days through 5-FU administration intensely upgraded the pathological deviations induced by 5-FU in the tissue of the treated rats manifested evident from vibrant symbols of retrieval as their Bowman's capsules and glomeruli appeared nearly usual and standard.

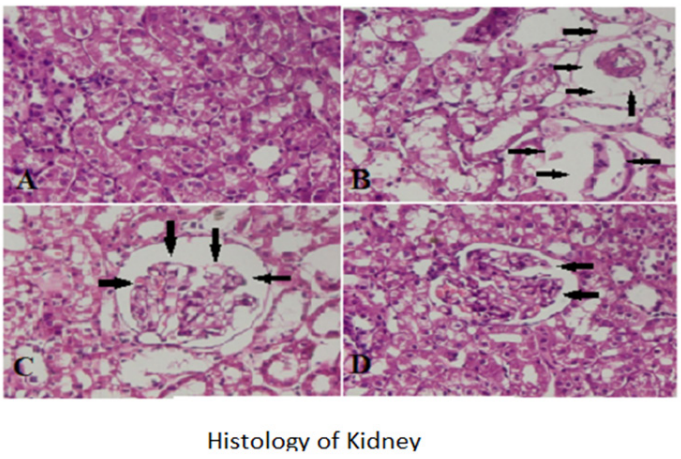

Figure 7: Effect of low and high prophylactic dose of NS on histological changes in 5-FU induced kidney damage.

Group I: Normal control (slide A), Group II: 5-Flurouracil (5-FU) treated (150mg/kg b.w) (slide B), Group III: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Lower dose) (200 mg/kg b.w) (slide C), Group IV: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Higher dose) (400 mg/kg b.w) (slide D).Magnification (40X).

DISCUSSION

Presently, chemotherapy is the pivotal management choice for cancer patients with serious drawbacks clinically, which restricts its use.[27] In the present communication, we explored the prophylactic result of NS on 5-FU–induced renal oxidative damage and inflammation leading to dysfunction. Though, the mechanistic pathway to kidney damage instigated by 5-FU is entirely unclear. But free radical production followed by peroxidation of lipids, impairment of cell membrane, dearth of antioxidant system, inflammation, and apoptosis could be the mechanism postulated by numerous investigators.[27,28] The defensive role of NS observed in the current work is associated with the lessening of oxidative stress and inflammation and henceforth, cell death in the kidneys of 5-FU administered Wistar rats.[29]

The most projecting determining factors of kidney injury/dysfunction are BUN, Creatinine (Cr) and Kim-1. 5-FU executed kidney injury is apparent from previous reports by elevated BUN, Cr and Kim-1. Our current results are continuous and well aligned

Table 1: Schematic treatment regimen.

Group 1 (control)	Drinking water	Normal saline only
Group 2 (only 5-FU)	Drinking water	5-FU 150mg/kg b.wt.i.p (19th day)
Group 3 (5-FU+NS1) (200mg/kg b.wt.)	NS1 200 mg/kg b.wt.	5-FU 150mg/kg b.wt.i.p (19th day)
Group 4 (5-FU+NS2) (400mg/kg b.wt.)	NS2 400mg/kg b.wt.	5-FU 150mg/kg b.wt.i.p (19th day)

Table 2: Effect of low and high prophylactic dose of NS on antioxidant reservoirs in 5-FU induced kidney damage.

Groups	GSH (nmol GSH/g tissue)	GPx (nmol NADPH Oxidized/min/mg protein)	GR (nmol NADPH Oxidized/min/mg protein)
Control	0.91±0.02	224.19±13.3	202.32±16.5
5-FU	0.38±0.03***	121.63±11.6**	11.23±17.5**
5-FU+NS1	0.57±0.06$^{\#}$	186.37±16.9$^{\#}$	152.32±11.4^{ns}
5-FU+NS2	0.72±0.05$^{\#\#}$	199.21±20.4$^{\#}$	175.8±15.8$^{\#}$

*Results are illustrative of mean ± SE of 6 rats per group. Values are articulated as the means ± standard error. Results infer that there is a comparison between 5-FU treated group (***) $p < 0.001$ versus Control, (**) $p < 0.01$ versus Control and (*) $p < 0.05$ versus Control. Then the treatment groups are compared with 5-FU treated group ($^{\#}$) $p < 0.05$ versus 5-FU; ($^{\#\#}$) $p < 0.01$ versus 5-FU; ($^{\#\#\#}$) $p < 0.001$versus 5-FU;.Group I: Normal control, Group II: 5-Flurouracil (5-FU) treated (150mg/kg b.w), Group III: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Lower dose) (200 mg/kg b.w), Group IV: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Higher dose) (400 mg/kg b.w).

Table 3: Effect of low and high prophylactic dose of NS on antioxidant and oxidative stress marker in 5-FU induced kidney damage.

Groups	MDA (nmol MDA formed/g tissue)*	CAT (nmol H_2O_2 consumed/min/mg protein)*
Control	12.2±0.7	42.49±3.02
5-FU	37.5±2.9***	21.34±2.18***
5-FU+NS1	27.6±2.2$^{\#}$	31.01±1.84^{ns}
5-FU+NS2	23.1±1.0$^{\#\#}$	36.18±2.66$^{\#\#}$

* Results are illustrative of mean ± SE of six rats per group. Values are articulated as the means ± standard error. Results infer that there is a comparison between 5-FU treated group (***) $p < 0.001$ versus Control, (**) $p < 0.01$ versus Control and (*) $p < 0.05$ versus Control. Then the treatment groups are compared with 5-FU treated group ($^{\#}$) $p < 0.05$ versus 5-FU; ($^{\#\#}$) $p < 0.01$ versus 5-FU; ($^{\#\#\#}$) $p < 0.001$versus 5-FU;.Group I: Normal control, Group II: 5-Flurouracil (5-FU) treated (150mg/kg b.w), Group III: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Lower dose) (200 mg/kg b.w), Group IV: 5-Flurouracil (5-FU) treated (150mg/kg b.w) + *Nigella sativa* (NS) (Higher dose) (400 mg/kg b.w).

with the previous results where the serum BUN, Cr and Kim-1of 5-FU administered animals are raised.[29-31] Nevertheless, in the present communication, NS treatment deciphers ameliorated serum BUN, Cr and Kim-1 levels, respectively, which deciphers the nephroprotective potential of NS.[32]

Lipid peroxidation is measured by MDA, which depicts the mechanisms of ROS production in tissue damage. In the present work, there was a steep upsurge in MDA in renal tissue administered with 5-FU in rats. The results of the present work align with the previous data.[18,28,33] NS treatment was seen to alleviate MDA levels at both doses. However, the higher dose had a sharp, significant effect, as reported.[34]

In biological systems, there are innumerable antioxidants, both enzymatic and non-enzymatic which are effective guards against ROS by removing free radicals. GSH is an indispensable antioxidative representative in the biological system, which is formed of three peptides, a non-enzyme that networks straight with its thiol group with free radicals. We found in the present work there was deprivation of GSH reservoirs in the 5-FU administered group due to exhaustion of thiol in cleaning ROS, as reported earlier.[35] Yet, NS supplementation refilled GSH in prophylactic and post-phylactic treatment groups 3 and 4, as mentioned in the previous reports.[34,36]

The depletion of antioxidative enzymes like GR, GPx, and CAT in the cell is correlated with ROS generation. These antioxidative enzymes avoid toxic damage and protect cells against ROS by eliminating of free radicals. The reserves of antioxidants like GPx, GR, and CAT decrease substantially in the present work rendering the role of ROS and free radical production in the pathology of 5-FU-induced renal damage. CAT works with H_2O_2 eradicating

enzymes establishing the primary antioxidant in the body which resulted in production of H_2O_2 and O_2. H_2O_2 and other free radicals are additionally converted to H_2O and O_2 by CAT, GR.[27,21] Superoxide anion radicals are converted to H_2O_2 by SOD and H_2O_2 is then further eradicated by catalase or with the help of GPx. We found in the present study that the antioxidative enzymes and non-enzymes, which include GSH, CAT, GPx, and GR, were diminished in group 2 significantly signifying the production of ROS because of damage to both antioxidative enzyme and non-enzymes reservoirs.[18,34,35] However, NS supplementation reloaded GSH, GPx, GR, and CAT, demonstrating its antioxidant potential conceivably by attacking singlet oxygen, superoxide radicals, peroxide, and peroxy radicals, as reported earlier. It was confirmed that the data obtained were in line with previously reported data.[32,33,37] Histological slides showed standard glomeruli and renal tubules in the control group. Group 2 kidneys showed distension in the kidney tubules, deterioration of tubular epithelium, hyperplasia and necrotic tissue. We confirm that deviations instigated by 5-FU in renal tissue are in constant with the preceding findings.[18,35,38] We detected in the treatment groups (group III and IV) there was a reversal of the pathologies that were found in 5-FU administered group.[39]

There is a cross-talk in anti-cancer drug-induced organ toxicities, in particular with 5-FU between oxidative stress and inflammation. The immune responses are triggered by an increase in oxidative stress and depletion in antioxidant. ROS production is an imperative progression that occurs in antineoplastic drug-interceded injury/damage. Activation of NF-kB pathways could be one possible mechanism that initiates cell death and inflammation.[21] Available reports advocate that instigation of the inflammatory pathway is one of the grounds of 5-FU mediated kidney toxicity. Administration of 5-FU may generate a microenvironment that provisions the stimulation of NF-kB and downstream pro-inflammatory molecules like TNF-a and others.[36] However, treatment with NS at lower and higher doses moderated the transformation of NfkB, TNF-a, which proposes an anti-inflammatory efficacy of NS. Reports suggest that 5-FU management results in oxidation and activation of TNF-α following the production of ROS and RNS, resulting in organ damage, inflammation and non-targeted non-cancerous cell death.[41] MAPKs superfamily is an upstream element in the inflammatory pathway which comprises p38 MAP kinases, ERK, JNK. Pro-inflammatory cytokines like TNF-α and various cellular stimuli like ROS cause the instigation of p38 MAPK and ERK, JNK.[36] The participation of MAPKs instigation in facilitating the distressing measures of 5-FU chemotherapy in animal kidneys have been reported. JNK and p38 MAPK activation results in pro-inflammatory cytokine formation and, finally kidney cell death which could be a possible mechanism for kidney dysfunction in human and investigational/preclinical models.[42,43] Additionally, ERK stimulus activates downstream transcription factors like NF-κB and TNF-α has been reported previously.[40-43] The current work results demonstrated that NS treatment at both doses efficiently blocked MAPK and NF-κB trails as well as downstream cytokines, portentous the multipronged potential of NS. Similar results with NS were obtained in Adjuvant arthritis and LPS-induced respiratory distress in harmony with the current reports of NS suppression of MAPKs pathway and NF-κB in wounds of diabetic animals.[44,45] Remarkably, obstruction of MAPKs and its signalling pathways particularly p38 and JNK, have been associated with protection against multiple kidney injury models deciphering acute kidney catastrophe, tubular cell death and pathological tissue.[46] Outcomes of current work reveal the treatment with NS impedes MAPKs pathway along with its downstream proteins like NF-κB and downstream molecules are viewed as an operative approach for managing varied kidney damage. When the histopathological data were assessed, the control group showed normal glomerular and normal renal tubular structures like healthy kidneys. Nonetheless, 5-FU group kidney tissue showed dilatation in tubules of the kidney, degeneration of tubular epithelium, hyperplasia, necrosis, and inflammatory cell production. These deviations produced by 5-FU in renal tissue are constant with the preceding reported results.[28] We found treatment with NS at both doses decreased disintegration and necrosis in renal tissue in contrast to 5-FU group kidneys.

CONCLUSION

In conclusion, NS can be used as an adjuvant therapy with 5-FU to protect against kidney injury associated with 5-FU through further animal experimental and clinical studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

5-FU: 5-Fluorouracil; **NS:** *Nigella sativa*; **CAT:** Catalase; **GSH:** Glutathione; **GR:** Glutathione reductase; **GPx:** Glutathione peroxidase; **MDA:** Malondialdehyde; **MAPK:** p38 Mitogen activated kinase pathway; **LPO:** Lipid Peroxidation; **p-ERK1/2:** phospho-Extracellular signal regulated kinases; **p-JNK:** phospho c-Jun N terminal kinase; **pNF-kB:** Nuclear factor-kappa B; **TNF-α:** Tumor Necrosis Factor-alpha; **KIM-1:** Kidney injury molecule; **Cr:** Creatinine; **BUN:** Blood urea nitrogen; **ANOVA:** Analysis of variance; **ROS:** Reactive oxygen species; **SOD:** Superoxide dismutase.

SUMMARY

NS mitigates 5-FU-induced kidney injury by restoring antioxidant status, scavenging ROS, diminution of damage to serum by decreasing serum diagnostic toxicity markers. Moreover, it also downregulates MAPK pathway proteins, downstream inflammatory mediators and histological damage, which helps in the regulation of healthy kidney functioning.

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