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Umbelliferone β-D-galactopyranoside inhibits chemically induced renal carcinogenesis via alteration of oxidative stress, hyperproliferation and inflammation: possible role of NF-kB

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Abstract

Umbelliferone (7hydroxycoumarin) compound possesses strong anti-inflammatory and free radical scavenging activity. The intend of the current study was to conclude the renalprotective efficacy of Umbelliferone β-D-galactopyranoside (UFG) over diethylinitrosamine (DEN) initiated and ferric nitrilotriacetate (Fe-NTA) promoted the oxidative stress, inflammation and renal injury in Wistar rats. The capacity of UFG to scavenge the reactive nitrogen species (RNS) and reactive oxygen species (ROS) was evaluated and its also scrutinized scavenging against the hydroxyl (OH), superoxide (O_2), nitric acid (NO) and hydrogen peroxide (H_2O_2) radicals. Renal carcinoma was induced by single intraperitoneal injection of DEN (200 mg/kg, b.w.) and promoted by twice weekly treatment of Fe-NTA (9 mg/kg, b.w.) for 22 weeks. To estimated the molecular mechanism implicated in the antitumor potential of UFG, its consequence was appraised on renal tumor inflammation; proinflammatory cytokines including interleukin-1β (IL-1β), interlukin-6 (IL-6) and tumor necrosis factor (TNF-α); inflammatory mediator including prostaglandin E_2 (PGE_2), ornithine decarboxylase (ODC) and nuclear factor kappa B cell (NFκB). It's also induced the serum abnormability including creatinine, lactate dehydrogensae (LDH) blood urea nitrogen (BUN) and [^{3}H] thymidine incorporation. Further, augments the renal lipid peroxidation (LPO), endogenous antioxidant enzymes, phase II metabolizing enzymes and concomitant reduction in glutathione (GSH). UFG showed the 95% and 99% antioxidant activity in 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) model. UFG significantly inhibited the RNS and ROS radical and

indicated the antioxidant activity (in vitro). The results showed momentous renal markers and oxidative stress protection impaired by UFG. UFG also restored the altered inflammatory and proinflammatory cytokines, which further strengthens the renal protection of UFG in DEN+Fe-NTA induced renal carcinogenesis. These results recommend that UFG as an efficient chemoprotective agent having the ability to thwart the DEN induced and Fe-NTA promoted renal carcinoma in experimental rats.

Keywords: Renal, Hyper proliferative, ornithine decarboxylase, [3H] thymidine incorporation, nuclear factor kappa B cell

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Abbreviation

RCC = Renal cell carcinoma
NF-κB = Nuclear factor-kappa B
SIP = Siddhartha Institute of Pharmacy
CPCSEA = Committee for the Purpose of Control and Supervision of Experiments on Animals
ABTS = 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
DPPH = 2, 2-diphenyl-1-picrylhydrazyl
DEN = Diehtylnitrosamine
Fe-NTA = Ferric nitrilotriacetate
NTA = Nitrilotriacetic acid
MDA = Malondialdehyde
PBS = Phosphate buffer saline
I.P. = Intraperitoneal injection
TBA = Thiobarbituric acid
XO = Xanthine oxidase
N= Necrosis
AC = Adenocarcinoma
G = Alomerulus
LI = Leucocytic infiltration
DT = Distal tubule
NT = Necrotic tissue
PT = Proximal tubule
TE = Tubular epithelium
HC = Hyperchromatism
GT = Ghost tissue
SOD = Superoxide dismutase
CAT = Catalase
GSH = Reduced glutathione
GPx = Glutathione peroxidise
G6PD = Glucose-6-phosphate dehydrogenase
GR = Glutathione reductase
GST = Glutathione S transferase
QR = Quinine reductase
LPO = Lipid peroxidation (LPO)
PGE_2 = Prostaglandin - 2
G-6-P= Glucose-6-phosphate
GSSG= Oxidized glutathione
6-PG= 6-phosphogluconate
R= Xenobiotics
O_2•= Superoxide radical
H_2O_2= Hydrogen peroxide
R-SH= Thiol conjugated xenobiotics.
RNS = Reactive nitrogen species
ROS = Reactive oxygen species
TNF – α = Tumor necrosis factor – α
IL-6 = Interleukin-6
IL-1β = Interleukin-1β

1. Introduction

Renal cell carcinoma (RCC) indicates the most common toxic malignancy of adult kidney, but it is not well understood that 3% of all adult malignancies, comprises 90% of kidney neoplasm.[1,2] World widely, 2,09,000 cases of RCC has been estimated and 1,02,000 deaths also estimated per year.[3] The average endurance rate of RCC patient is about 4 month and only 10% of RCC patient endure for 1 year.[4] Renal system is extremely susceptible to toxicants for two reasons; firstly, high volume of blood flow via renal and secondly, it filer the large amount of toxins which can concentrate in renal tubules.[5] Its also start the systemic damage via inhibit the excretion of body waste, incapability of electrolyte balance, upholds body fluid and also inhibit the synthesis of essential hormones.[6] RCC is a histological diverse disease with an unpredictable lessons, RCC is complex to diagnosis at early stage because it's generally asymptomatic and metastatic disease. RCC could be increase via altering the pathophysiology process of promotion and/or initiation, which shows the necessary axis of different anticancer therapeutics.[7]

Nitrosamine is common ecological carcinogens, frequently present in baby feeder bottle, rubber products, pesticide, latex products, and various ranges of cosmetics. It has been provoked the generation of free radical/ROS/RNS, which consequence increase the oxidative stress and alter the cellular and tissue mediated antioxidant defense system. Thus, it may be believe as major factor in the etiology of cancer, it's also inducing a wide range of tumors in different organs especially liver and kidney in all species of animals.[4,8] Diethylnitrosamine (DEN), also recognized as N-Nitrosodimethylamine, is an extremely toxic chemical which is commonly caused the carcinogenic at diverse dose.[6]

Nitrilotriacetic acid (NTA) is commonly found in water, various domestic and hospital detergents.[9] The excess iron start the generation of free radicals and the excess storage of iron may elevate the risk of cancer in human.[10] NTA make a complex with iron and formed the ferric nitrilotriacetic acid (Fe-NTA), which is a potent nephrotoxic agent behave as tumor promoters. Its mainly act on renal system through the formation of free radical and alter the ornithine decarboxylase (ODC). ODC is a rate limiting enzymes basically found in polyamine biosynthesis and its increasing the rate of DNA synthesis with concurrent reduces the antioxidant defense system. Fe-NTA enters into lumen of renal proximal tubule via glomeruli, where the Fenton chemistry arises and starts the damage of oxidative DNA and lipid peroxidation (LPO).[11] Oxidative stress initiate the alteration in DNA bases including thymine-tyrosine, 8-oxoguanine,

thiobarbiturate acid-reactive substances, cross links, malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE) and MDA-modified proteins in Fe-NTA induced carcinogenesis. The involvement of LPO and oxidative stress in renal carcinogenesis is also the evident from the various studies, where the antioxidant and antiinflammatory therapies attenuate the Fe-NTA induced renal toxicity.[12]

Peroxynitrite (ONOO−), generate due to the interaction between the superoxide ($\bullet O_2$) and nitric oxide (NO•), which is a potent pro-inflammatory and cytotoxic RNS, involved in the enhancement of acute and chronic inflammation. RNS, also involved in the activation of redox sensitivity including nuclear factor-kappa B (NF-κB) and proinflammatory transcription factor.[13] NF-κB, regulates the various genes, which are involved in the protection of inflammatory and immune response. Various evidence claims the connection between renal carcinogenesis and NF-κB. NF-κB is an important redox-sensitive transcriptional factor which regulates the gene encoding chemokines, adhesion molecules and inflammatory cytokines.[14]

Several experimental (*invitro* and *invivo*), clinical trial and epidemiological data showed that the free radical scavenger rich diet can inhibit the condition of chronic diseases, especially cancer.[15] Umbelliferone (7-hydroxycoumarin), has received a lot of attention due to their antidiabetic, antihyperlipidemic, antioxidant,[16] antiinflammatory and antinociceptive,[17] allergic airway inflammation,[18] arthritis[19] and inhibition the laryngeal cancer cells[20] etc. These activities of umbelliferone have been recognized to their ability to act as antiinflammatory, anticancer and antioxidant. There are no previously published reports about the chemopreventive potential of UFG on renal carcinogenesis. Therefore, the current investigation was designed to scrutinize the efficiency of UFG against the renal carcinogenesis in rat model via two stages and find out the probable mechanism of action that might be showed the its anticancer effect. The effect of UFG was explored on key attribute of carcinogenesis with a major locus on tumor promotion and inflammation.

2. Material and methods

2.1. Chemical and reagents

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), 2, 2-diphenyl-1-picrylhydrazyl, Diehtylnitrosamine (DEN), Nitrilotriacetate (NTA), sodium nitropreusside, sodium bicarbonate, thiobarbituric acid nitrilotriacetic acid, deoxyribose, dithionitrobenzene (DTNB), glutathione reductase, H_2O_2, 1-chloro-2,4-dinitrobenzene (CDNB), Oxidized and reduced glutathione,

reduced nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and other chemical used in the experiment was purchased from the approved vendor.

2.2.Animals

Swiss albino Wistar strain (150-180 gm, male) rats were used for the experimental study. The experimental rats were procured from the Siddhartha Institute of Pharmacy (SIP), Dehradun, Uttarakhand animal house. All the rats were kept in polypropylene cages in animal house of SIP. Animals were kept in the animal house under the standard experimental condition (22±2^{o}C temperature, relative humidity 30-50% and 12 h light/dark cycle) with standard pellet diet (Lipton Rat Feed Ltd. Pune) and water *ad libitum.* The animals were acclimatized for 7 days before the experimentation. All the experimental procedure was followed according to the approved protocol by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) animal ethical committee of SIP, Dehradun. The experimental protocol was approved by Institutional Animal Ethical Committee of SIP, Dehradun (SIP/IAEC/05(B)/Sep-2012).

2.3.Invitro antioxidant activity

2.3.1. Free radical scavenging activity of UFG on 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

The free radical scavenging activity of UFG was estimated by using the DPPH radical scavenging method of Kumar et al. with minor modification.[21] In brief, prepared the solution of DPPH (0.1 mM soluble in 4 mL methanol) and prepared solution of DPPH was mixed with different concentration of UFG (12.5-200 µg/mL) and the sample was left for 30 min at the temperature 27°C for incubation. After incubation, the sample was subjected for the estimation of the absorbance of prepared sample at 517 nm. The free radical scavenging activity of UFG was estimated using the following formula.

$$(\%) = (\quad - \quad) \quad /$$

2.3.2. Free radical scavenging activity on 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals

The free radical scavenging capacity of UFG was estimated using the $ABTS^{+}$ free radical scavenging method as previously described method with minor modification.[21] Briefly, ABTS (7mM) solution mixed with the 2.45 potassium persulfate (4.95 mM) and incubated the solution

12-16 hr at room temperature; after 12-16 hr blue green colour of the ABTS solution was persist and maintained absorbance of the ABTS 0.7 ± 0.02 at 734 nm with diluted the ethanol. The test sample (0.1 mL) mixed with the ABTS solution (3.9 mL) and incubated at room temperature (37°C) for 10 min. The percentage of inhibition and EC_{50} value was estimated using the formula expressed for DPPH method.

2.4.Scavenging of RNS and ROS

The scavenging of free radicals such as •OH, NO, O_2•− and H_2O_2 was estimated. Scavenging activity of •OH was measured using the reported method of Aruoma and Halliwell with minor modification depended on the degradation of deoxyribose by •OH.[22] The NO scavenging was estimated by incubating the UFG with NO donor, preparation of 5 mM solution of sodium nitropreusside and incubated at 25°C for 2 h and after that mixed with the Griess reagent for estimating the NO according to the reported method.[23] The scavenging of O_2•− was determine by reported method of Yen and Chen with minor modification.[24] On the basis of the inhibition of NDT decline via the O_2•− (created via PMS-NADH system). Scavenging of H_2O_2 was determined via alteration the absorbance of H_2O_2 (230 nm) with minor modification of reported method of Kaur et al.[23].

2.5.Preparation of DEN

Preparation of DEN carcinogen was completed using the method given by Kazmi et al., 2014 as modified by Afzal et al.,[25] DEN (200 mg/kg) was dissolved in the phosphate buffer saline (PBS) (pH=4.5).

2.6.Preparation of Fe-NTA

The solution of Fe-NTA was prepared by using the reported method of Jahangir and Sultana.[26] Briefly, the solution of Fe-NTA (0.16 mM) was mixed with 4 fold molar of disodium salt of NTA (0.64 nM) and adjust the pH 7.4 using the sodium bicarbonate. Solution of Fe-NTA was ready freshly before the treatment.

2.7.Acute nephrotoxicity study

Male Wistar rats (150-180 kg, b.w. male) were randomly divided into five groups and each group contains 15 rats. The rats treatment showed in table 1. Single intraperitoneal injection (i.p.) of DEN (200 mg/kg, b.w.) and after 14 days, 2 doses of i.p. injection of Fe-NTA (9 mg/kg, b.w.) 2 days apart.

2.8.Estimation of biochemical parameters

The estimation of biochemical parameter such as blood urea nitrogen (BUN),[27] creatinine,[28] λ-glutamyl transpeptidase,[29] xanthine oxidase (XO),[30] Lactate dehydrogenase (LDH)[31] was estimated by using the reported method with minor modification.

2.9.Estimation of antioxidant markers

The antioxidant marker such as superoxide dismutase (SOD),[32] glutathione reductase,[33] quinine reductase (QR),[35] glutathione-S-transferase,[36] reduced GSH,[37] lipid peroxidation (LPO),[38] catalase (CAT).[39]

2.10. Estimation of cytokines

The serum level of proinflammatory cytokines such as IL-6 and IL-1β and TNF-α, NFκB and PGE_2 were analyzed. The estimation of the above mention cytokines were estimated following the given instruction of manufacture using the ELISA reader.

2.11. Histological examination

For histological analysis, all group rats kidneys were fixed in formalin (10%) paraffin embedded using the standard tissue processing and sections of 5µm of thickness were obtained. Eosin and hematoxylin stained was used for the staining of the kidney samples.

2.12. Staging and grading of RCC

Staging of RCC was done on the basis of tumor size. The size of tumors was determined by assigning them on scale from 1 to 5. Scale 1=maximum diameter 0.5 to 2 mm, scale 2=2.5-3 mm diameter, scale 3=4 to 5 in diameter, scale 4= 6 mm in diameter and scale 5= 15-45 mm in diameter, respectively. The RCC grading was performed according to Fuhrman grade method which dependent solely on content, prominence of nucleoli and nuclear morphology.[40]

3. Statistical analysis

All the data were expressed as the mean±SEM. and analysis of variance (ANOVA) was used for the statistical analysis using Graph Pad Prism version 5.0. The values were considered to be significant when the P value was $p<0.05$, $p<0.01$ and $p<0.001$.

4. Result

4.1.Free radical scavenger activity of UFG

UFG significantly showed the 99.6% and 90% inhibition of DPPH and ABTS free radical at a concentration of 100 µg/mL (figure 1a,b) and showed the 42 µg/mL and 64 µg/mL IC_{50}

value of UFG for DPPH and ABTS free radicals. H_2O_2 radical scavenge capacity of UFG was estimated and it IC_{50} 53 µg/mL was found (figure 2a).

The UFG considerably scavenged the O_2 and IC_{50} value was 65 µg/mL (figure 2b).

The scavenging activity of NO was estimated by using SNP (NO indicator). The incubation of PBS with the SNP at 25°C for 2 hours resulted in the increase the generation of NO radical (figure 2c). The UFG successfully scavenge the NO radical with IC_{50} value 48 µg/mL.

The UFG scavenge the OH radical, which was generated through Fenton reaction and determined through their capacity to break the deoxyribose sugar into the different portion that react with thiobarbituric acid (TBA) and produced a pink chromogen. The UFG scavenge the OH radical with IC_{50} 105 µg/mL (figure 2d).

4.2.Effect of the UFG on DEN+Fe-NTA induced tumor

Macroscopic evaluation of rat kidneys was show in figure 3. Macroscopically study of normal control and normal control received UFG (20 mg/kg) rats did not show any visible tumor in kidney portion. DEN+Fe-NTA induced group rats macroscopically showed the development of tumor, which was expand in kidney. Table 2 summarized the number of rats with RCC, total number of rats, number of unilateral and bilateral tumors with percentage of tumor incidence in DEN+Fe-NTA group rats. The oral administration of UFG considerably repressed the renal tumor in both kidneys. Table 3 showed the size of tumor in both kidney and metastasis in DEN+Fe-NTA induced rats. DEN+Fe-NTA showed the 0.5 – 45 mm diameter of tumor in kidney, which was significantly ($P<0.001$) restrained by UFG at dose (10 and 20 mg/kg, b.w.).

4.3.Effect of UFG on body weight

The data from the current experiment clearly indicated that the all group rats increased the body weight till end of study, in comparison with initial body weight. As showed in figure 4, normal control group rats augmented body weight from 147±1.77 to 396.4±4.49 with growth gain rate of 1.61±0.84 per day and normal control treated with UFG (20 mg/kg, b.w.) increased body weight from 153.2±2.73 to 401.6±5.98 with growth gain rate 1.62±0.73. DEN+Fe-NTA treated control group rats showed the less improved body weight from 153.8±1.83 to 366±4.83 with growth grain rate 1.37±0.11 per day, in comparison with normal control. DEN+Fe-NTA rats received UFG (10 and 20 mg/kg, b.w.) significantly increased the body weight 154.4±2.76 to 393±4.39

with growth gain rate per day 1.54±0.21 and increased the body weight 153.4±3.43 to 408.8±5.43 with growth rate per day 1.66±0.87, respectively.

4.4.Effect of UFG on the renal histopathology

Table 4 showed the histopathological changes, which were seen in normal control and experimental rats. Renal histopathology of normal control and normal control received UFG (20 mg/kg, b.w.) did not exhibit any morphological changes at end of the experiment. Figure 13 showed the remarkable toxicity induced by DEN and promoted by Fe-NTA in renal histopathology. Disease induced rats histopathology showed the hydropic deterioration (degenerative and strophic alteration), deformation of renal cortical structure (cyto-architecture), tubular cost, less identifies corpuscles and enhance the size of bowmen capsules as compared to normal control rat histopathology. By contrast, DEN+Fe-NTA induced rats kidney histopathology showed the remarkable pathological changes including swelling in the kidneys, flattened epithelium, inflammatory blood vessels in kidney glomeruli, congested blood vessels and necrosis of proximal tubules. Another observation in histopathology is hyperplastic glomeruli, lumina, swelling lining epithelium, prominent adenocarcinoma, tubular dilation, hyperchromatism, leucocyte cell infiltration and tubular brush margin loss through the existence of ghost tissue. Pretreatment with UFG at both doses resulted in alleviation of DEN+Fe-NTA induced renal pathological deterioration as evident from the figure 14.

4.5.Effect of UFG on DEN+Fe-NTA induced acute nephrotoxicity and tumor promotion

4.5.1. Effect of UFG on renal marker

Treatment with UFG exhibited momentous inhibition of renal marker on week 22 in DEN+Fe-NTA induced renal carcinogenesis rats. UFG evidenced 44.59, 68.05, and 48.67 % inhibition of urea, creatinine and BUN level, respectively, in comparison to DEN+Fe+NTA control (figure 5). The results indicate the abeyant renal parameter abnormability protection against DEN+Fe+NTA induced renal carcinogenesis, which is more akin to clinical renal damage conditions. From the figure 6, DEN+Fe-NTA induced RCC rats showed the increased level of Xanthine oxidase (XO), -glutamyl transpeptidase, LDH and decreased level of QR compared to normal control group rats. Pretreatment with UFG, significantly altered the renal serum level induced by DEN+Fe-NTA as comparison to DEN+Fe-NTA control group. The alteration in serum parameter level might be due to potent antioxidant effect of UFG and it's an indication of reverse the injury induced by DEN+Fe-NTA. Normal control group rats received UFG (20 mg/kg b.w.) did not

prove any alteration in the level of serum parameters. The current result showed the protective nature of UFG against the chemically induced RCC and its nontoxic in nature.

Figure 7 showed the effect of total sulphydryl group and vitamin C in the normal control and DEN+Fe-NTA experimental rats. Figure 7 showed the declined level of total sulphydryl and vitamin C in DEN+Fe-NTA induced rats as compared to normal control. More importantly, UFG treated rats significantly ($P<0.001$) restored the level of total sulphydryl and vitamin C near to normal control.

4.5.2. Effect of UFG on induction of renal ODC activity and renal DNA synthesis

Figure 8A showed the effect of ODC and thymidine activity of normal control and experimental rats. DEN+Fe-NTA induced rats caused 556% induction in the activity of ODC as compared to normal control. Pretreatment of rats with UFG at a dose 10 mg/kg b.w. caused the 155% and dose 20 mg/kg b.w. caused the 426% inhibition of ODC activity as compared to DEN+Fe-NTA induced rats.

Figure 8B showed consequence of increased incorporation of [^{3}H] thymidine into the renal DNA of DEN+Fe-NTA induced carcinogenesis rats. DEN+Fe-NTA induced rats showed the 226% enhancement of [^{3}H] thymidine into renal DNA as compared to normal control, which was significantly ($P<0.001$) inhibited by UFG. The dose of UFG 10 mg/kg, b.w. showed the inhibited 134% and 300% inhibition of [^{3}H] thymidine into the renal DNA, confirmed by the UFG dose 20 mg/kg, b.w. as compared to DEN+Fe-NTA control group rats.

4.5.3. Effect of UFG on antioxidant marker

UFG pretreatment of DEN+Fe-NTA induced rats showed the alteration the activities of antioxidant marker and phase II enzymes (figure 9, 10). DEN+Fe-NTA induced rats showed the retained activities of endogenous antioxidant enzymes such as SOD, GPx, CAT and GR as compared to normal control group rats. There was a concomitant and substantially increase the activity of microsomal membrane of LPO in the DEN+Fe-NTA induced rats. However, UFG (10 and 20 mg/kg, b.w.) pretreatment significantly suppressed the enhancement. DEN+Fe-NTA induced rats received UFG significantly ($P<0.001$) recover the activity of GSH and all other enzymes. DEN+Fe-NTA induced rats showed the declined activity of phase II metabolizing enzymes such as QR and GST. But, this declined enzymes activity was attenuating considerably by the treatment of UFG. The increased level of glutathione-S-transferase, glutathione reductase,

reduced glutathione and glutathione peroxidase were significantly (P<0.001) reduced to near normal control level when treated simultaneously treated with UFG.

4.5.4. Effect of UFG on proinflammatory cytokines of DEN+Fe-NTA induced RCC

The level of proinflammatory cytokines including TNF-α, PGE_2 and IL-6 was increased in DEN+Fe-NTA rats and confirm the considerably role in the expansion of carcinogenesis. DEN+Fe-NTA induced rats treated with UFG significantly (P<0.001) restored the elevated level of proinflammatory cytokines near to normal control (figure 11). In the current study, we found the increased level of NFkB in DEN+Fe-NTA induced rats, which was significantly (P<0.001) restored by UFG as compared to normal control (figure 12).

5. Discussion

Chemoprevention includes the multiple intervention mechanism and methods, either use of medicinal plants or pharmacological agents to reverse, inhibit or seize the carcinogenesis effect at various stages of cancers. The medicinal plants which have more value to inhibit the expansion of invasive cancer due to their low toxicity and common acceptance with high safety. Most of epidemiological studies showed that the regular intake of vegetables and fruits inhibit the incidence of multiple cancers.[41-42] Most of the researchers are exploring the new classes of chemical constituents which are being scrutinize the clinical trials as cancer prevention agents for various types of malignancies especially renal cancers.

The result revealed that the UFG potentially quenched the free radical such as •OH, NO, O_2•− and H_2O_2 and claim the inhibition of RNS and ROS. The interaction between the RNS, ROS and other products are extremely toxic, proficient of functional damage and inflicting the structural damage to approximately all cellular molecules. UFG inhibit the ROS, RNS and claim the sturdy free radical extinguish activity, was foreseeing to protect the bimolecules from cellular and oxidative damage.[43] Oxidative stress very commonly damages the lipids, DNA and increases the significance of carcinogenesis. LPO and DNA damage is consider as an important indicator of early stage of carcinogenesis and toxicity. Several evidences showed the connection between the oxidatively modified DNA bases (8-oxodGuo), HNE and LPO (MDA, malonaldehyde) product along with close relationship between LPO and DNA mutilation in DEN+Fe-NTA induced renal carcinogenicity. Potentially, a toxic LPO product MDA and HNE has been exposed to cross-link membrane, causes mutations and inflicts the DNA damage.[44] Therefore,

reserve of DNA and LPO damage by UFG seems to be prominently concerned in its preventive capacity against renal carcinogenesis.

Fe-NTA promotes the renal carcinogenesis, it generates the ferrous ions, and ferrous ions start the generation of free radicals. It was relevant to estimation the ROS, UFG showed invitro antioxidant activity against the superoxide scavenging activity.[45] DEN+Fe-NTA induced rats showed the proteinaceous casts and necrosis in renal histopathology, which matches with other, reports.[46] The current study, we have observed that the oxidative stress and inflammation play an important role in pathophysiology of nephrotoxicity induced by DEN+Fe-NTA. It also induced the oxidative stress and tumor expansions which were intricately associated with inflammation as well.[47] Distorted level of iron showed the increased level of neutrophil and augmented condition of macrophage activity, which is confirmed inflammation condition. Neutrophil is important factor for increasing the inflammatory reaction, it also increase the activity of MPO due to damage of organ.[48] Some of the reported claim that the increased level of nitrite, distinguished the pathological situation of oxidant. NO react with superoxide (O_2^-) radical and generated the cytotoxic peroxynitrite during the renal injury.[49-50] DEN+Fe-NTA induced rats treated with UFG considerably altered the histopathology changes of renal due to its antioxidant and antiinflammatory potential as previously reported.[16,19,51]

ODC is a rate limiting enzyme use in polyamine biosynthesis and provoke the large number of tumor promoters. Thymidine and ODC incorporation are extensively used as the biochemical marker for assessment of tumor expansion and hyperproliferative agents.[52] Increase the fold of renal ODC and thymidine activity by DEN+Fe-NTA suggests that the both of agents are potent renal carcinogenesis agents.[53] ODC catalyses the decarboxylation of ornithine to putrescine, which again converted to higher polyamines, necessary for duplication of DNA. The above discussed pathway is confirming the role of ODC as key enzyme of polyamine biosynthesis.[54] ODC and polyamine take part in the expansion of tumor, cellular proliferation, cell transformation and tumor promotion. Further, DEN+Fe-NTA showed the effect on DNA synthesis, which is akin effect like the other potent carcinogenesis in different organs; also suggest a proliferative effect of DEN+Fe-NTA in tumor induction and expansion. The increased level of free radical inhibited the activities of endogenous antioxidant enzymes after DEN+Fe-NTA treatment communicated with the time of maximum ODC initiation, suggesting a role of oxidant production in initiation of ODC activity.[55] UFG significantly ($P<0.001$) inhibited the

activity of ODC and suggesting, its portentous antitumor effect. UFG showed the marked reduction in the level of kidney makers including BUN and serum creatinine showing the prophylaxis of UFG and improved the kidney function in DEN+Fe-NTA treated groups. UFG showed the free radical scavenging activity and inhibiting the functions of arachidonic acid and polyamines metabolites via PGE_2 and ODC. The possible mechanism of action of UFG may be involved the interaction of electrophilic species and inhibition of excessive oxidant formation, including free radicals with macromolecules.

DEN+Fe-NTA induced rats showed the declined glomerular filtration which was indicated the elevated level of creatinine, uric acid and increases the LDH activity. Creatinine is excreted through glomerulus filtration and tubular secretion. During the RCC, reduction in the glomerulus filtration rate increased the level of creatinine. Creatinine and urea both value commonly used for the evaluation of kidney blood flow, acute renal failure, accumulation in circulating blood and decreased renal excretion.[7] The serum creatinine and urea level are usually scrutinized together due to acute kidney failure and related blood flow of kidney, accumulation in the circulating blood and decreased the renal excretion. Urea is generating, when the body degrades the purine nucleotides; it's a sign of nucleoprotein metabolism and finally starts the excretion of urea from kidney. On the other hands, renal dysfunction is followed by increased the serum enzymes level indication the loss of functional integrity and cellular leakage of renal membrane. Both levels were comparing with our results, which indicate the elevated serum level of creatinine, LDH and BUN in DEN+Fe-NTA induced rats. DEN+Fe-NTA induced rats treated with UFG considerably declined the serum creatinine, LDH and BUN level. The result clearly indicated the protective effect of UFG against the DEN+Fe-NTA induced renal damage. Uric acid is formed when the body breakdown to purine nucleotides; it is the end product of nucleoprotein metabolism which is excreted from kidney. The increase level of uric acid was evaluate during the renal damage that result in high cellular turnover release nucleic acids into the circulating blood which convert into uric acid by liver.[56] The significantly changes of renal parameters occurs during DEN+Fe-NTA, reflects the severity of renal function deficiency, which occurred in association with suddenly decline the glomerular filtration rate in acute tubular necrosis. DEN specifically enters into the proximal tubular epithelial cells and binds to the anionic phospholipids and targeting the cells to inducing the deformity especially renal function,

organelles and metabolism of multiple intracellular membranes, which are developed during the renal injury in proximal tubular epithelial cells and caused the acute renal failure.[6,57,58]

UFG treated rats showed the increased body weight at end of the study as compared to DEN+Fe-NTA induced group rats and claim the nutritional effect of UFG. On the contrary, DEN+Fe-NTA induced group rats showed the declined body weight as compared to normal control, which was recommend the health deterioration of rats and oral administration of UFG claims the preventive effect in the deterioration of rats health.

LPO, is a chemical mechanism which capable to disrupting the structure and function of biological membrane. Cell membranes are phospholipids bilayers with extrinsic proteins, directly target to LPO, which lead to generation of number of deleterious effects such as increased osmotic fragility, membrane rigidity, cell damage and cell membrane destruction. Furthermore, the increased level of LPO contributes to a crucial mechanism of renal injury. The current study showed the obvious increased LPO level, which could be due to escalation generation of free radicals as a consequence of DEN+Fe-NTA induced renal damage. The increased level of serum parameters and LPO lead to modulation in the endogenous antioxidant enzymes including SOD, CAT, GR, GPx and reduced thiol pool, which play an important role in the defense of renal damage by quenching the free radicals. A lot of researchers showed that the Fe-NTA downregulated the all antioxidant enzymes as well as concentration of ~~the~~ antioxidant enzymes.[59] DEN+Fe-NTA induced group rats showed the increased level of LPO, significantly ($P<0.001$) attenuated by UFG. The outcome harmonize with UFG can prevent LPO by quenching the LPO chain and directly scavenging free radicals. UFG successfully play down the LPO in biological systems.

DEN is the most important environmental carcinogen, mostly found in food as preservative, induced the two major tubular abnormalities such as back leak of glomerular filtrate and obstruction, which could be involved in the inhibition of glomerular function. DEN alterated of glomerular function, may be due to generation of ROS which induce the modulation of filtration area, cellular injury, modifying the ultrafiltration coefficient, mesangial cells contraction and oxidative stress, which inhibit the glomerular filtration rate.[60-62] In pharmacokinetic studies, DEN degraded and attracts the tubular epithelium and transformed to electrophilic species following the α and β hydroxylation, resultant generation of unsteady hydroxyalkyl compounds that are consequently transformed to alkyl carbonium ions. DEN

generated free radical and inhibits the action of endogenous antioxidant including G-6PD, CAT, GPx (Phase I) and GST, GR and QR (Phase II) enzymes. The generated free radicals can cause the oxidative stress in lipids, DNA and proteins.[63-64] Firstly, carcinogenesis need to required metabolism before being to transfer their fully carcinogenesis effect through the phase I and phase II enzymes, which initiate the metabolism of carcinogenesis. DEN metabolized by phase I enzymes like cytochrome P450 and catalyzes free radicals reaction, while phase II enzymes detoxify the oxidized metabolic products to extractable and inert form.[59,61] GR, GST and QR are an important phase II enzymes and actively take part in the detoxification of several carcinogens. GST enzyme detoxifies the metabolites of carcinogenesis by thiol conjugation, although GR support the maintenance of GSH pool. QR, take part in the reduction of enormous range of substrates by catalyzing the two electron inhibition and defends against the carcinogenicity or toxicity induced by free radicals. Several researchers established a relation between the alterations of these Phase I and Phase II enzymes and chemopreventive effect of anticancer agents.[65] The substrate including O_2 and H_2O_2 (due to their generation by overproduction) may reduce the activities of antioxidant enzymes such as CAT, GPx and SOD involved in their dissipation. Under these circumstances, UFG possessing persuasive H_2O_2 and O_2 scavenging activity may alter the level of these ROS and restoring enzymes activities. The inhibition of free radical by UFG may be due to inducing the de nova synthesis of antioxidant enzymes. Another effect of UFG to restoring the increased level of XO near the normal control and declined the level of H_2O_2, claim the antitumor effect. The result showed the potent free radical scavenging activity of UFG appears probable to be responsible for refill the renal antioxidant arsenal.

DEN+Fe-NTA ~~also~~ reduce the enzymatic and non enzymatic antioxidant makers in kidney including GPx, CAT, GSH and G6PD. The turnover of ~~the~~ antioxidant enzymes such as GSH depends upon the activities of various GSH metabolism enzymes.[66,67] DEN+Fe-NTA induced rats showed the depleted level of GSH due to augmented activity of λ-glutamyl transpeptidase and reducted ~~in~~ the activity of oxidized glutathione and may lead to accession of peroxides, which lead the enhancement of oxidative stress.[76] Accumulation of λ-glutamyl transpeptidase, start the increased level of λ-glutamyl transpeptidase fixes the degradation of GSH, which start the accumulation of cysteine and cysteinyl glycine. The enhance level of cysteine and systeinyl glycine, increase the reduction of Fe-NTA to ferrous complex, which in turn to increase the peroxidative damage to tissue/membrane.[68] GSH (tripeptide), play a crucial

role in the balancing of cellular redox status and considered a significant indicator of oxidative stress. GSH present 90% of the total non protein sulphur and mammalian tissues at millimolar concentrations.[69] The intracellular level of GSSG (oxidised GSH) increased from the metabolism of H_2O_2 ~~by~~ through GPx, declined the GSSG from the cell, glutathione reductase and NADPH mediated recon-version of GSSG to GSH.[70] Another mechanism of Fe-NTA to induce the renal oxidative stress by potentiates the LPO in renal, which may be diminished the activities of endogenous antioxidant and declined the GSH level.

Various published data has been showed that the oxidative stress contribute a significant role in boost the inflammatory reaction and release the proinflammatory cytokines. Oxidative stress also influences the regulation of NF-kB and other genes which involve in the proliferation, angiogenesis and cell transformation. Although, a connection between the NF-kB and oxidative stress is very complex, oxidative stress are believed that it act as secondary messenger in trigger to NF-kB via TNF- α and other proinflammatory mediators (figure 15).[71] NF-kB plays an imperative role in the expansion of all types of cancers including RCC.[72] Activation of NF-kB connected to regulatory pathways usually underlies inflammatory processes. NF-kB readjusts the growth of several genes during the inflammation and is concerned in various other facets of oncogenic process like angiogenic ability, preclusion of apoptosis, conferring the tumor cells and cellular proliferation.[73] The various cell stresses induced by NF-kB, which showed the effect on the cytokines, growth factors, oxidative stress and vasoactive agents. The increase level of NF-kB regulated the various genes encoding proteins including inflammatory and immune response (i.e. chemokines, cytokines, immune response, growth factors, adhesion molecules and cellular legends). The inhibit level of NF-kB, now a day used as a valuable indicator to control the carcinogenesis expansion. Some evidence claimed that the COX-2 play a significant role in the transcriptional regulation of NF-kB and are reported to be connected with renal inflammation and tumor expansion. DEN and Fe-NTA increase the expression of COX-2 and tumor promotion through NF-kB activation.[74,75] Restrained the NF-kB, is an excellent approach to control the tumor expansion and carcinogenesis. Additionally, there are sufficient facts that the inhibition of COX-2 used as a suitable target for prevention of various proliferative disease like cancer. Our data showed that UFG inhibited the COX-2 (Invitro) and claims its pivotal role in the inhibition of RCC. Consequently, inhibition of DEN+Fe-NTA induced COX-2 by UFG may also probably be implicated in the defense against RCC.[77]

Proinflammatory cytokines including TNF-α, IL-6, IL-β and inflammatory mediator PGE_2 play a crucial role in the proliferation, vascular permeability as well as inflammation.[77] DEN+Fe-NTA induced the enhance level of proinflammatory cytokines as well as inflammatory mediators which are under the direct transcriptional regulation of NF-kB. Our result showed the significantly inhibition of the proinflammatory cytokines and inflammatory mediator by UFG and its claim a preventive role against the RCC.

Conclusion

Clear cell histology was notorious in this DEN initiated and Fe-NTA promoted renal cell carcinogenesis model, which is akin to human cancer. A number of investigations have established that inflammation and oxidative stress play an important role in the promotion, progression and initiation of RCC. UFG treatment resulted in notoriously declined the renal ODC, thymidine incorporation, hyperplasia and proinflammatory cytokines, all of which are the traditional markers of tumor promotion and inflammation. This preventive effect of UFG may be enlighten, at least in part, by modulating the antioxidant arsenal and restrain the activity of NFkB (figure 15). These results supported that the UFG to be a potential candidate for RCC, since it inhibits the several biomarkers of tumor initiation, antioxidants and inflammation in rat model of RCC.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Table 1: Different group of rats

S. No	Group	Treatment
I	**Normal Control**	Received (0.9% normal saline) only
II	**Normal Control**	Received UFG (20 mg/kg. b.w.)
III	**DEN Control + Fe-NTA Control**	Received only single intraperitoneal injection of DEN (200 mg/kg) + intraperitoneal injection of Fe-NTA (9 mg/kg) twice a week
IV	**DEN Control + Fe-NTA Control**	Received UFG (10 mg/kg, b.w.)
V	**DEN Control + Fe-NTA Control**	Received UFG (20 mg/kg, b.w.)

Table 2: Renal cell carcinoma incidence in DEN+Fe-NTA induced carcinogenesis rats

S. No	Groups	No. of rats	No. of rat with RCC	No. animals with unilateral tumors	No. animals with bilateral tumors	Total no. of tumor	Incidence of tumors (%)
1	**DEN+Fe-NTA**	15	14	9	5	36	93.3
2	**DEN+Fe-NTA received UFG (10 mg/kg)**	15	10	6	4	19	67
3	**DEN+Fe-NTA received UFG (20 mg/kg)**	15	5	4	1	7	33

Table 3: Features of renal cell carcinoma tumors

S. No	Groups	Animals Identification Number	Number of Tumor	Tumor Size (mm)		Metastasis
				Right Kidney	Left Kidney	
1	DEN+Fe-NTA	1	2	8(4)	2 (1)	No detected
		2	3	6(5)	6(3)	No detected
		3	3	4(2)	-	Lung
		4	2	-	3(2)	Lung
		5	4	15(6), 4(2)	5(2), 6(4)	Lung
		6	3	45(5)	2(1), 4(3)	Lung
		7	2	6(4)	4(2)	Lung
		8	1	8(3)	-	Lung
		9	4	4(3)	7(3), 4(2)	Lung
		10	2	5(2)	3(2)	Lung
		11	4	4(3)	-	No detected
		12	1	3(2)	-	No detected
		13	3	6(2)	5(2)	No detected
		14	2	2(1)	4(3)	No detected
2	DEN+Fe-NTA received UFG (10 mg/kg)	1	1	4(2)	3(2)	No detected
		2	2	-	5(4)	No detected
		3	4	6(3), 4(2)	4(2)	Lung
		4	3	11(4)	6(4)	Lung
		5	3	8(5)	-	Lung
		6	1	3(2)	4(3)	No detected
		7	1	4(2)	-	No detected
		8	1	-	4(2)	No detected
		9	2	11(5)	-	Lung
		10	1	-	5(3)	No detected
3	DEN+Fe-NTA received UFG (20 mg/kg)	1	2	6(4)	-	No detected
		2	1	-	6(3)	No detected
		3	1	5(4)	-	No detected
		4	2	-	5(2)	Lung
		5	1	-	3(2)	No detected

Table 4: Effect of UFG on DEN+Fe-NTA induced carcinogenesis in rats.

S. No	Histopathology	Normal Control	Normal Control + UFG (20 mg/kg)	DEN+Fe-NTA	DEN+Fe-NTA + UFG (10 mg/kg)	DEN+Fe-NTA + UFG (20 mg/kg)
1.	Inflammatory cells	-	-	+	+	-
2.	Glomerular congestion	-	-	+	+	-
3.	Peritubular congestion	-	-	+	-	-
4.	Tubular casts	-	-	+	+	-
5.	Blood vessel congestion	-	-	+	+	-
6.	Necrosis	-	-	+	+	-
7.	Fat deposition	-	-	+	-	+
8.	Interstial edema	-	-	+	+	-
9.	Vacuolated tubules	-	-	+	-	-
10.	Cell debris	-	-	+	-	-
11.	Medullary ray	-	-	+	-	-
12.	Hyperplatic glomeruli	-	-	+	+	-
13.	Renal swollen tubules	-	-	+	-	-
14.	Fibroblasts	-	-	+	+	-
15.	Mononuclear cell infiltration	-	-	+	-	-
16.	Damaged macula densa	-	-	+	+	+
17.	Albuminious material	-	-	+	-	-
18.	Epithelial desquamation	-	-	+	+	-
19.	Dilation in US	-	-	+	+	-
20.	Atrophied glomeruli	-	-	+	+	-

+=present, -=absence

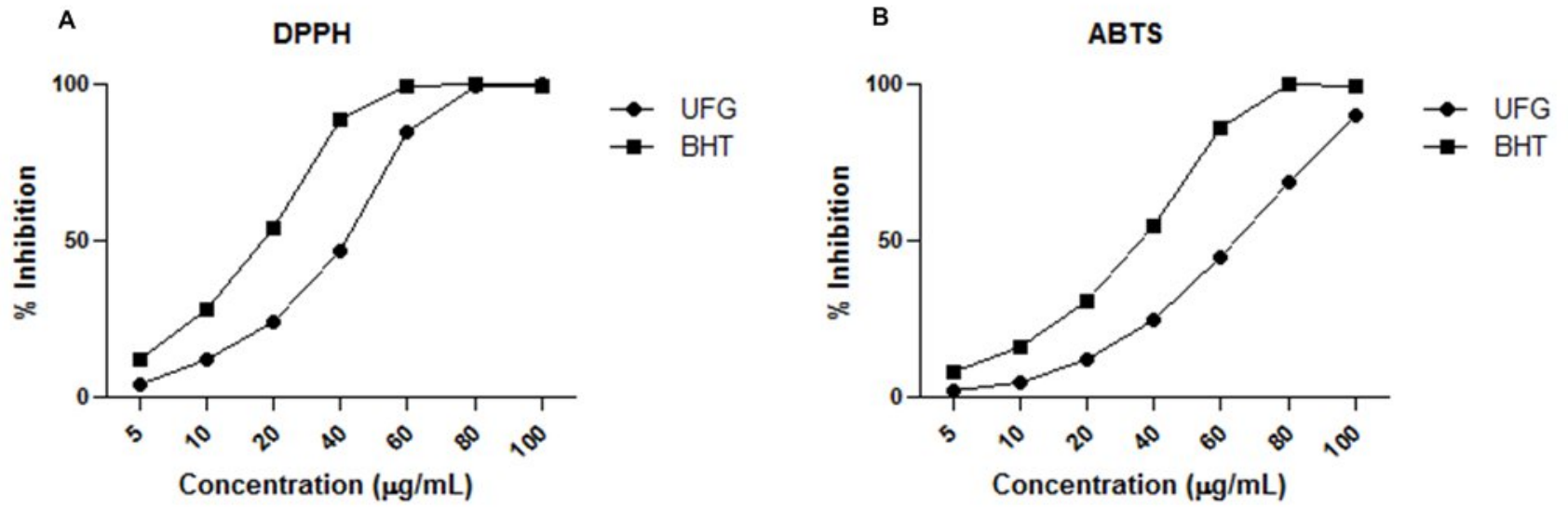

Figure 1: Effect of Umbelliferone β-D-galactopyranoside (UFG) on free radical scavenging activity. Free radical scavenging activity was assessed in terms of (A), Scavenge of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (B) and ABTS as described in Materials and methods. Butylated hydroxy toulene (BHT) was used as a positive control.

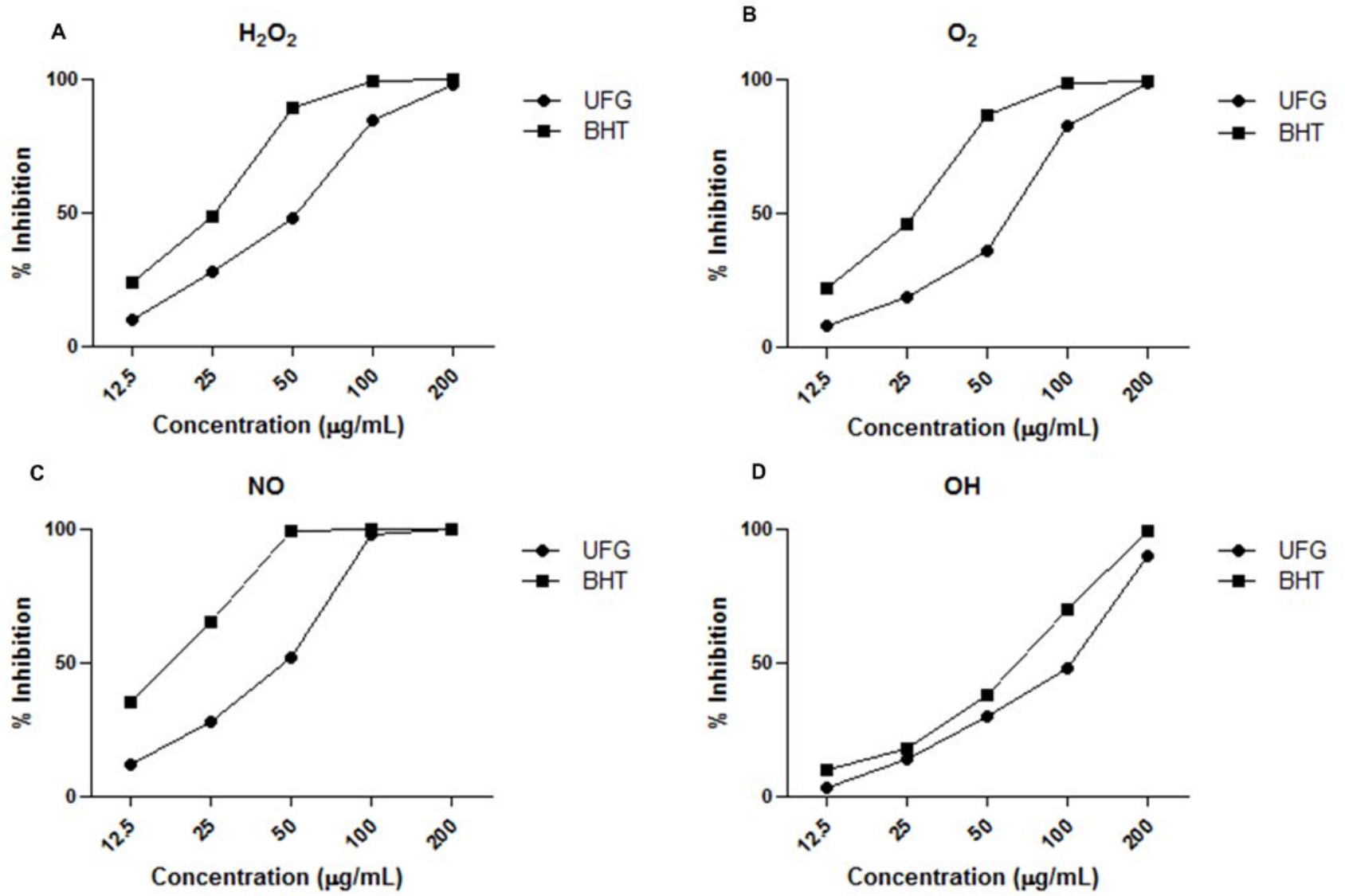

Figure 2: Effect of Umbelliferone β-D-galactopyranoside (UFG) on free radical scavenging activity. Free radical scavenging activity was assessed in terms of (A), Scavenge of hydrogen peroxide (H_2O_2) (B) superoxide (O_2) (C) nitric oxide (NO) (D) and hydroxyl (OH) as described in Materials and methods. Butylated hydroxy toulene (BHT) was used as a positive control.

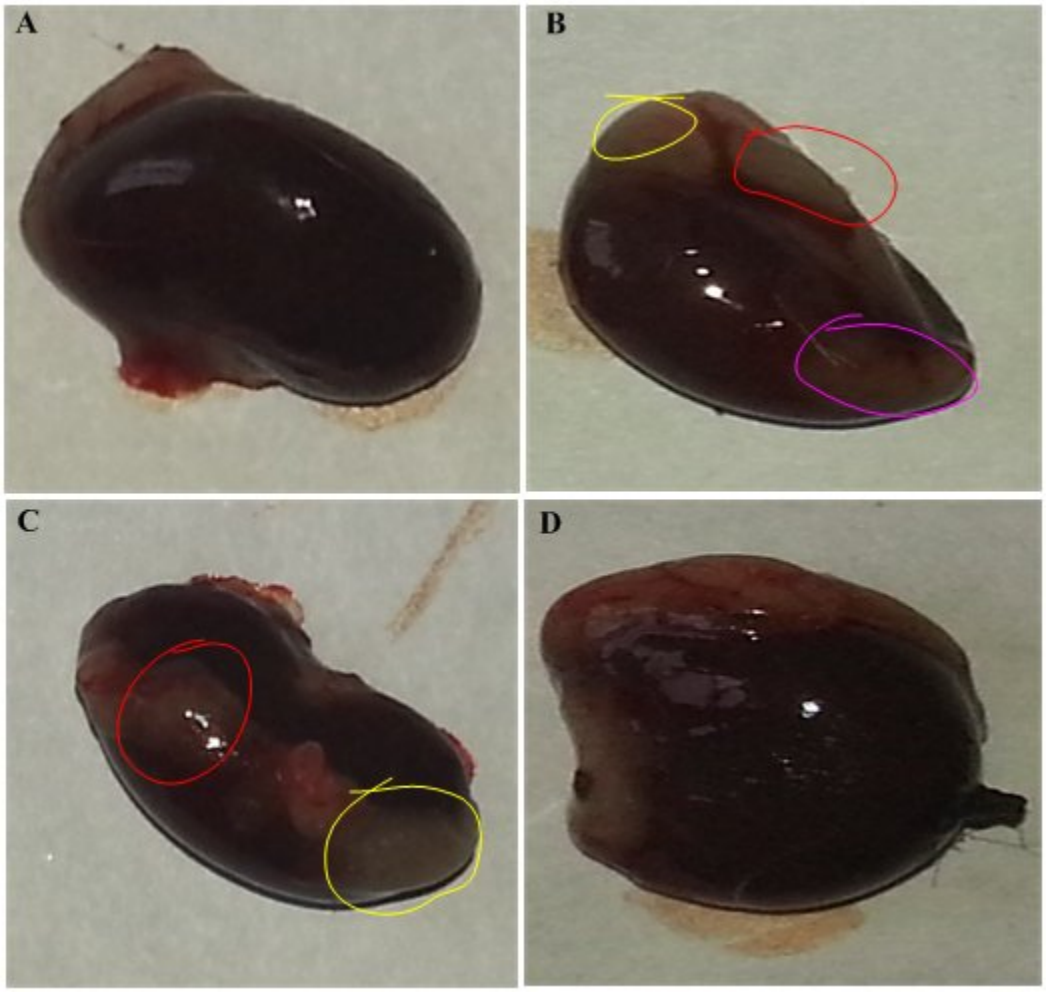

Figure 3: Representative photographs of normal control, normal control+ UFG, DEN+Fe-NTA and DEN+Fe-NTA+UFG induced kidneys at the end of experimental periods. (A) Normal control kidney did not show the any morphological change (B) DEN+Fe-NTA photograph represents tumors by red, yellow and purple colour circles (C) DEN+Fe-NTA+UFG (10 mg/kg) photograph represents the tumor with red and yellow circles and (D) DEN+Fe-NTA+UFG (20 mg/kg) photograph represents the less tumor formation.

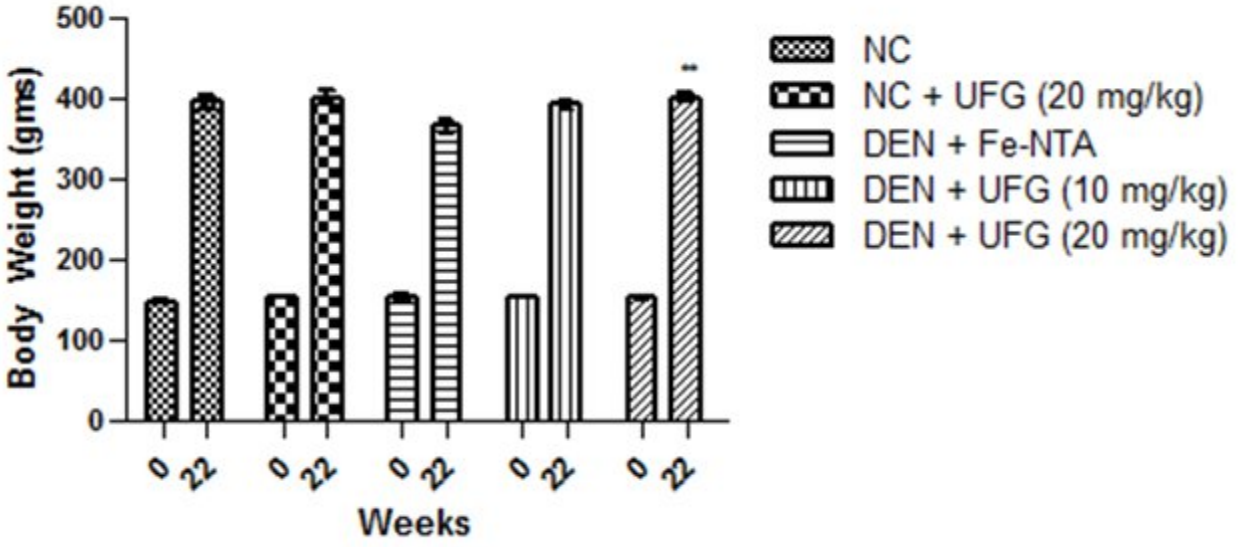

Figure 4: Effect of Umbelliferone β-D-galactopyranoside (UFG) on the body weight of DEN+Fe-NTA induced renal tumorigenesis rats. ns = non-significant, DEN = Diethylinitrosamine, Fe-NTA= Ferric nitrilo tetra acetic acid. The comparisons were made by ANOVA followed by Dunnett's test. *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

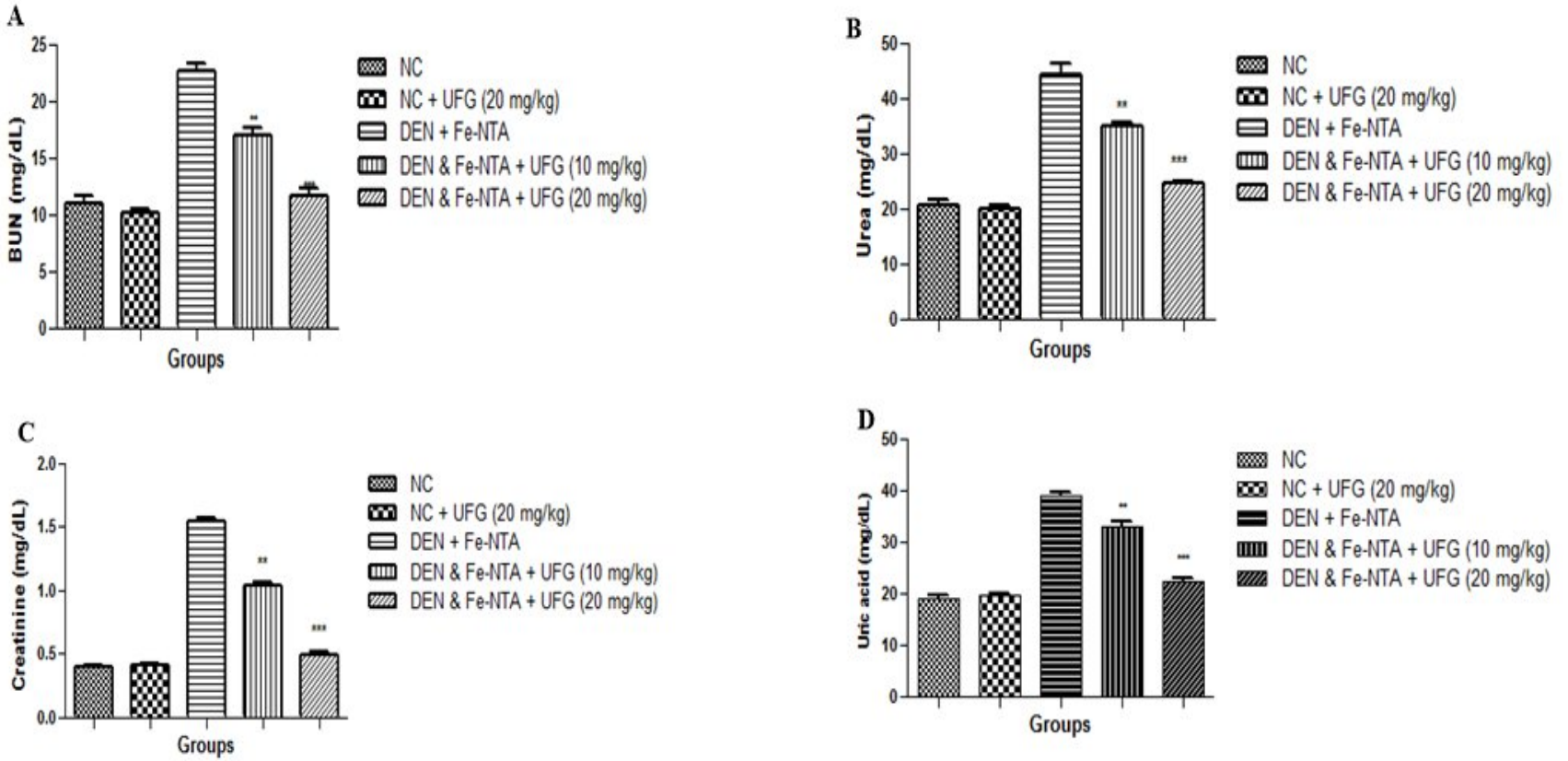

Figure 5: Effect of Umbelliferone β-D-galactopyranoside (UFG) on DEN+Fe-NTA induced renal tumorigenesis in rat. Renal marker was assessed in terms of enhanced of (A) blood urea nitrogen (BUN), (B) Urea level, (C) Creatinine and (D) Uric acid as described in Materials and methods. ns = non-significant, DEN = Diethylinitrosamine, Fe-NTA= Ferric nitrilo tetra acetic acid. The comparisons were made by ANOVA followed by Dunnett's test. *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

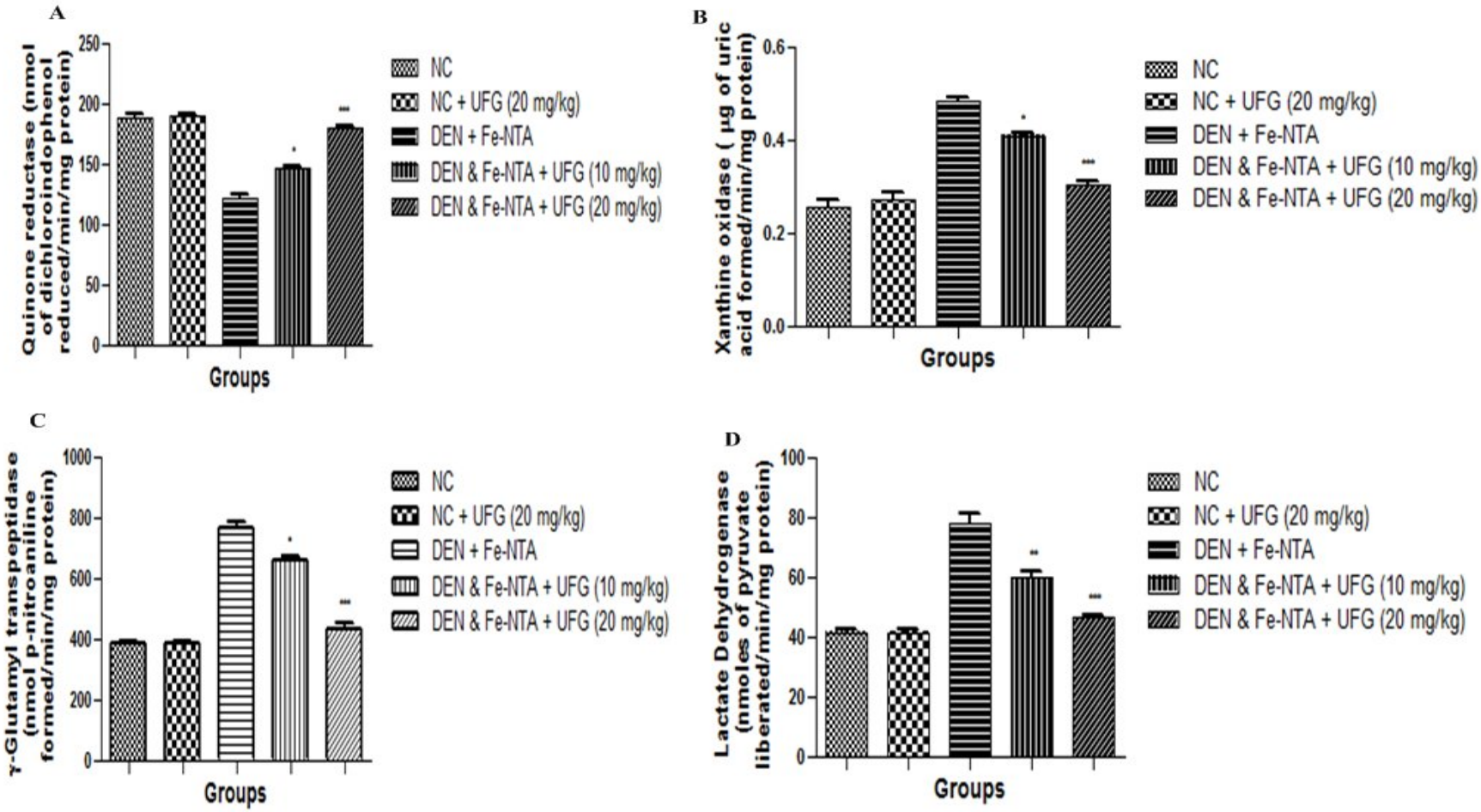

Figure 6: Effect of Umbelliferone β-D-galactopyranoside (UFG) on DEN+Fe-NTA induced renal tumorigenesis in rat. Renal marker was assessed in terms of alteration of (A) Quinone reductase (QR), (B) Xanthine oxidase (XO), (C) λ-Glutamyl transpeptidase and (D) Lactate dehydrogensae (LDH) as described in Materials and methods. ns = non-significant, DEN = Diethylinitrosamine, Fe-NTA= Ferric nitrilo tetra acetic acid. The comparisons were made by ANOVA followed by Dunnett's test. *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

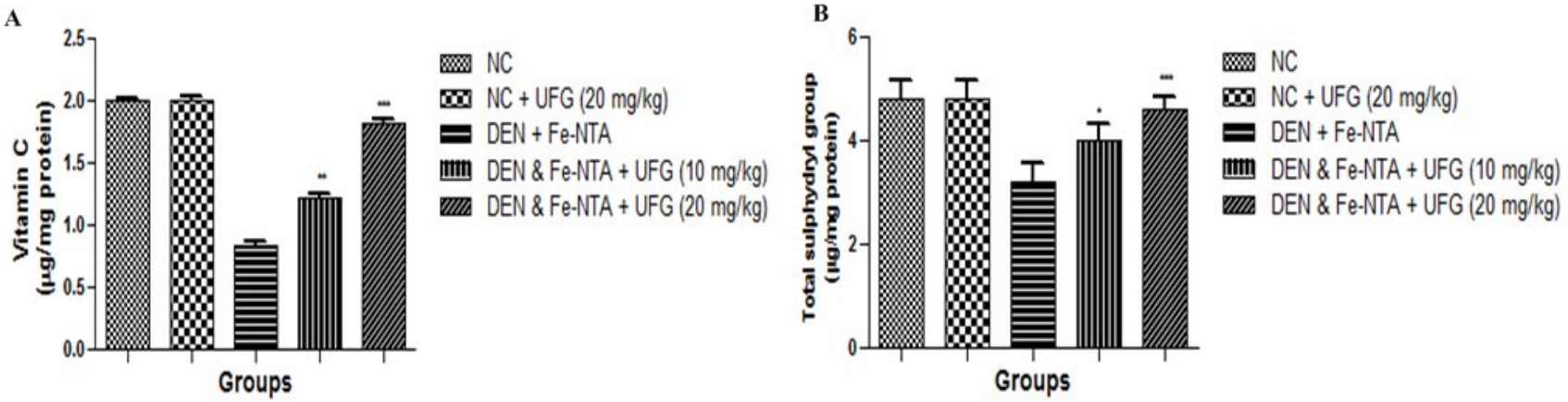

Figure 7: Effect of Umbelliferone β-D-galactopyranoside (UFG) on vitamin C and total sulphydryl group of DEN+Fe-NTA induced renal tumorigenesis rats. ns = non-significant, DEN = Diethylinitrosamine, Fe-NTA= Ferric nitrilo tetra acetic acid. The comparisons were made by ANOVA followed by Dunnett's test. *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

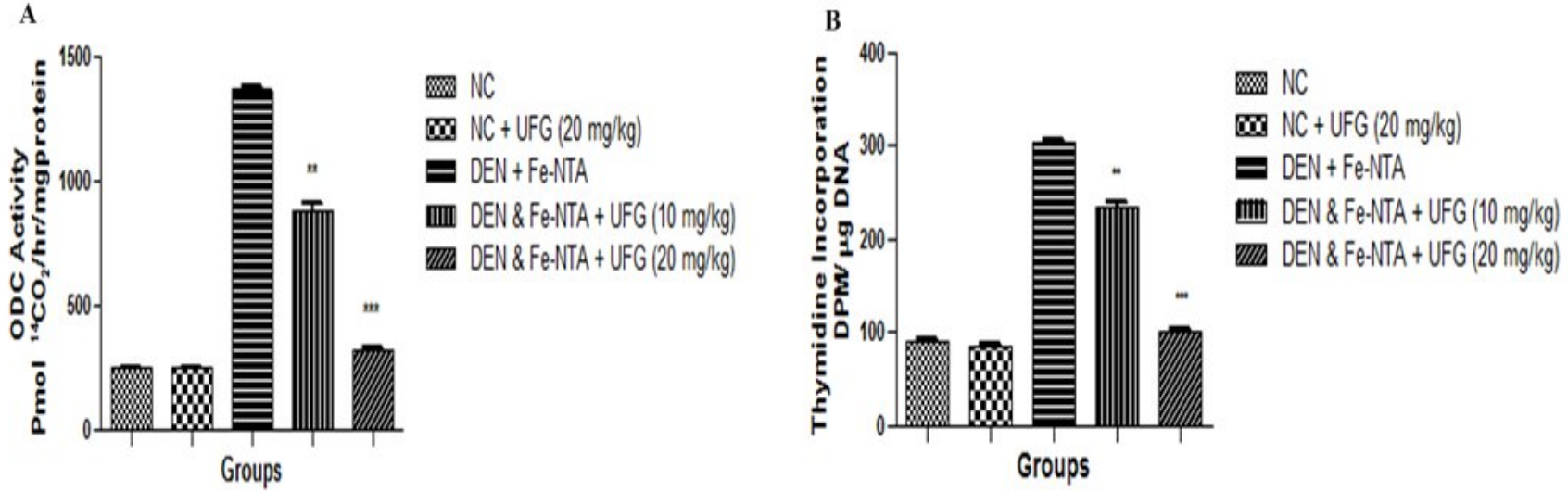

Figure 8: Effect of Umbelliferone β-D-galactopyranoside (UFG) on ODC and Thymidine incorporation of DEN+Fe-NTA induced renal tumorigenesis rats. Renal tumor marker was assessed in terms of alteration of (A) Ornithine decarboxylase (ODC), (B) Thymidine incorporation as described in Materials and methods. ns = non-significant, DEN = Diethylinitrosamine, Fe-NTA= Ferric nitrilo tetra acetic acid. The comparisons were made by ANOVA followed by Dunnett's test. *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

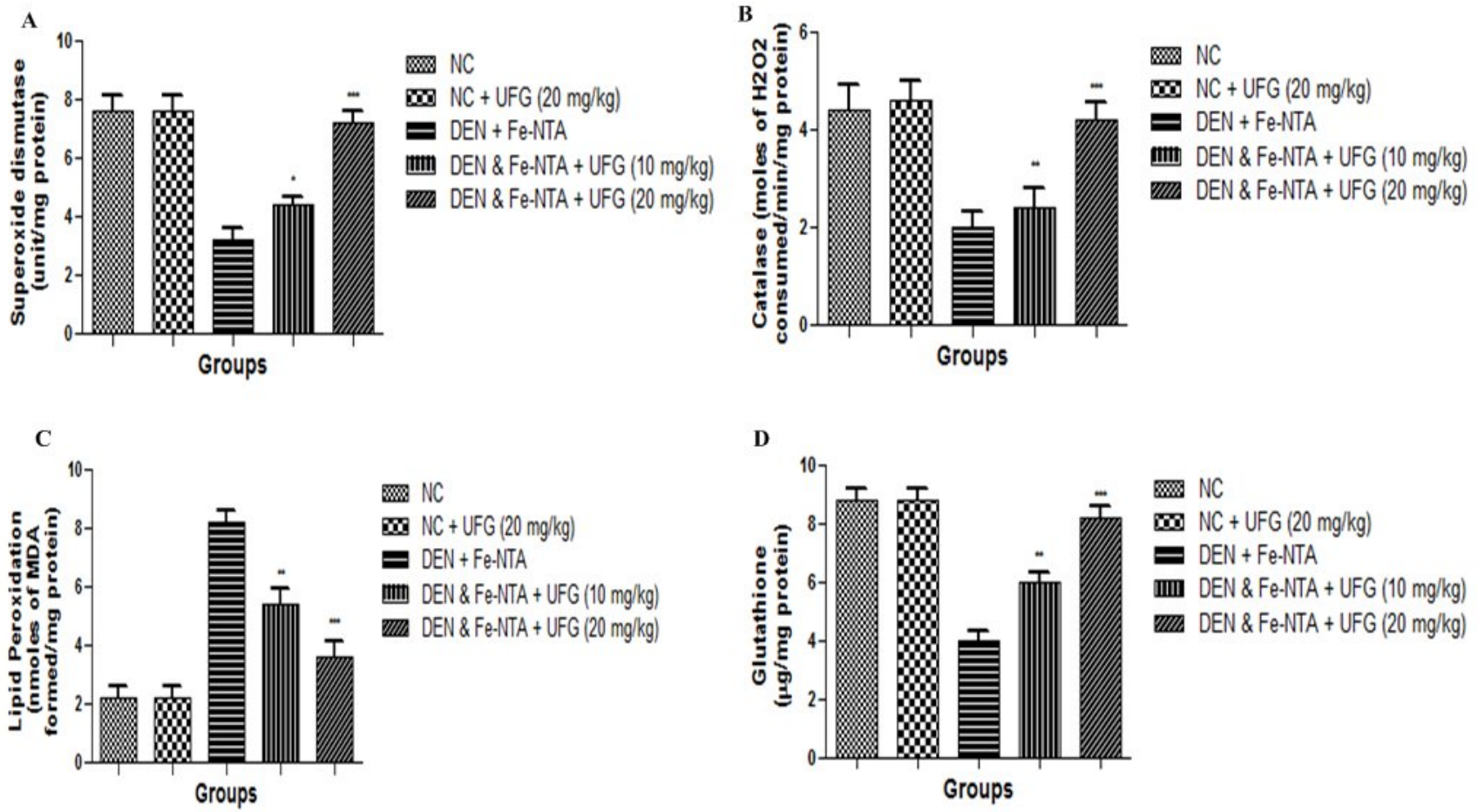

Figure 9: Effect of Umbelliferone β-D-galactopyranoside (UFG) on antioxidant markers of DEN+Fe-NTA induced renal tumorigenesis rats. Antioxidant markers were assessed in terms of alteration of (A) Superoxide dismutase (SOD), (B) Catalase (CAT), (C) Lipid peroxidation (LPO) and (D) as described in Materials and methods. ns = non-significant, DEN = Diethylinitrosamine, Fe-NTA= Ferric nitrilo tetra acetic acid. The comparisons were made by ANOVA followed by Dunnett's test. *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

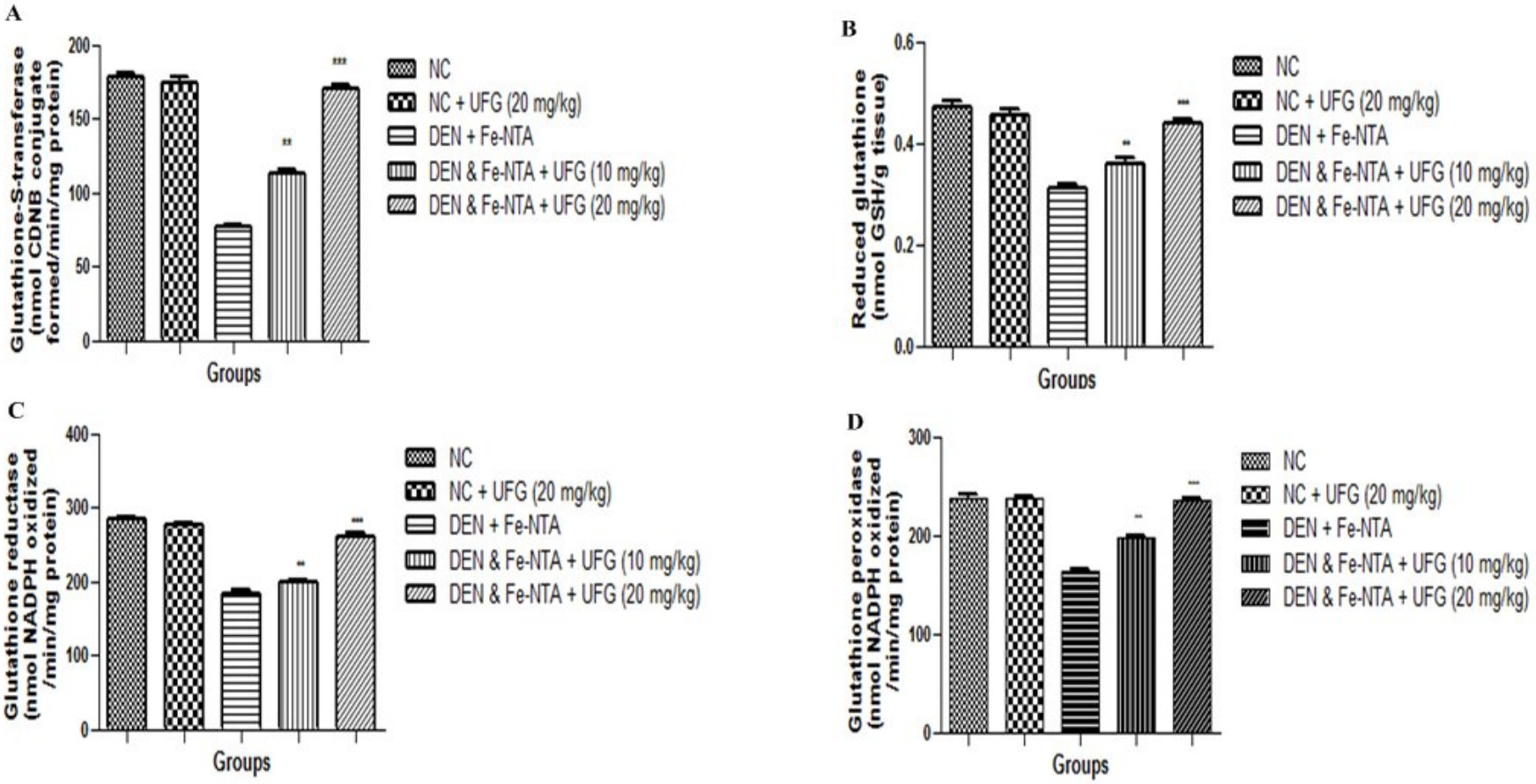

Figure 10: Effect of Umbelliferone β-D-galactopyranoside (UFG) on antioxidant markers of DEN+Fe-NTA induced renal tumorigenesis rats. Antioxidant markers were assessed in terms of alteration of (A) Glutathione-S-transferase, (B) Reduced glutathione, (C) Glutathione reductase and (D) Glutathione peroxidase (GPx) as described in Materials and methods. ns = non-significant, DEN = Diethylinitrosamine, Fe-NTA= Ferric nitrilo tetra acetic acid. The comparisons were made by ANOVA followed by Dunnett's test. *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

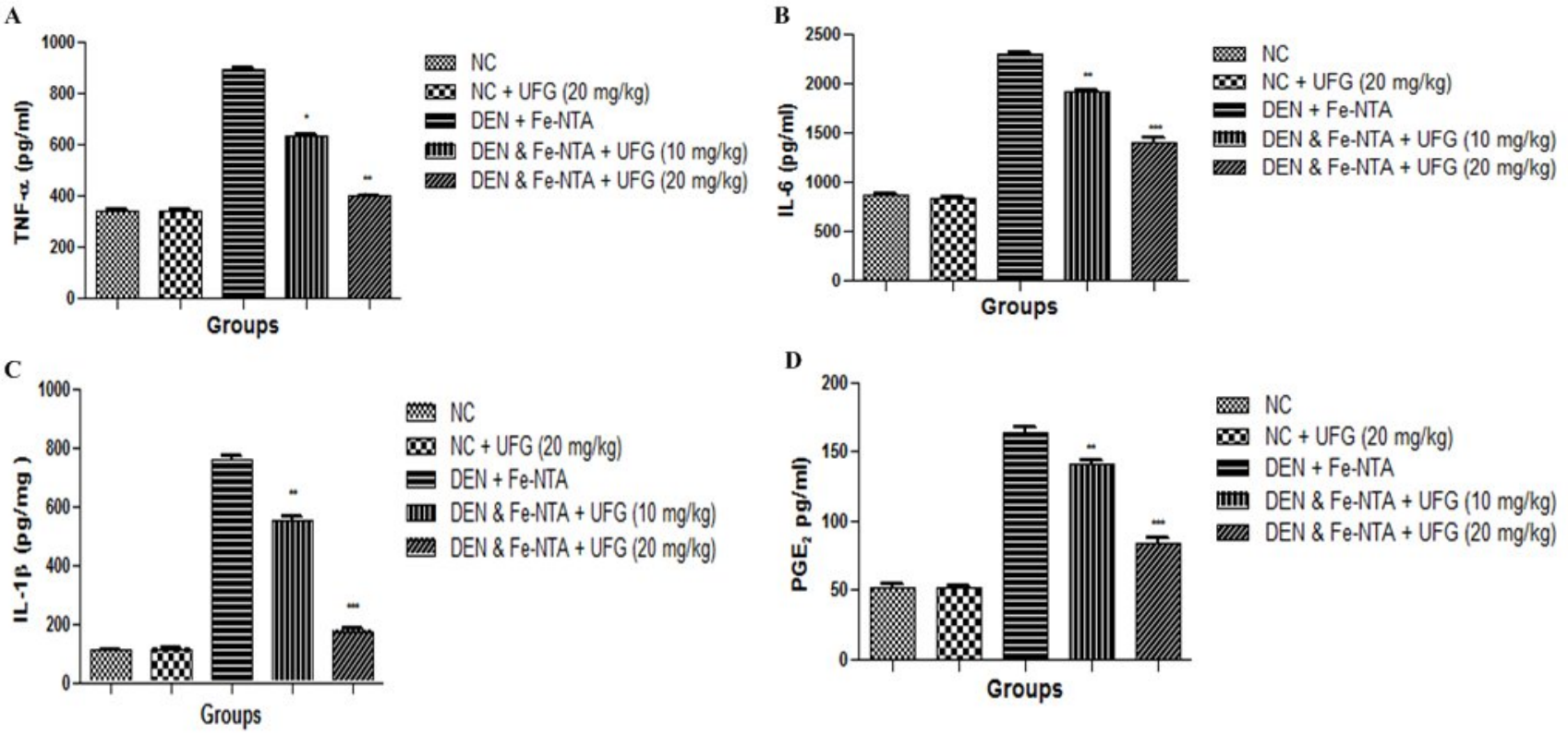

Figure 11: Effect of Umbelliferone β-D-galactopyranoside (UFG) on proinflammatory mediators of DEN+Fe-NTA induced renal tumorigenesis rats. Proinflammatory mediators were assessed in terms of alteration of (A) Tumor necrosis factor-α (TNF- α), (B) Interlukin-6 (IL-6), (C) Interlukin-1β (IL-1 β), and (D) Prostaglandin (PGE_2) as described in Materials and methods. ns = non-significant, DEN = Diethylinitrosamine, Fe-NTA= Ferric nitrilo tetra acetic acid. The comparisons were made by ANOVA followed by Dunnett's test. *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

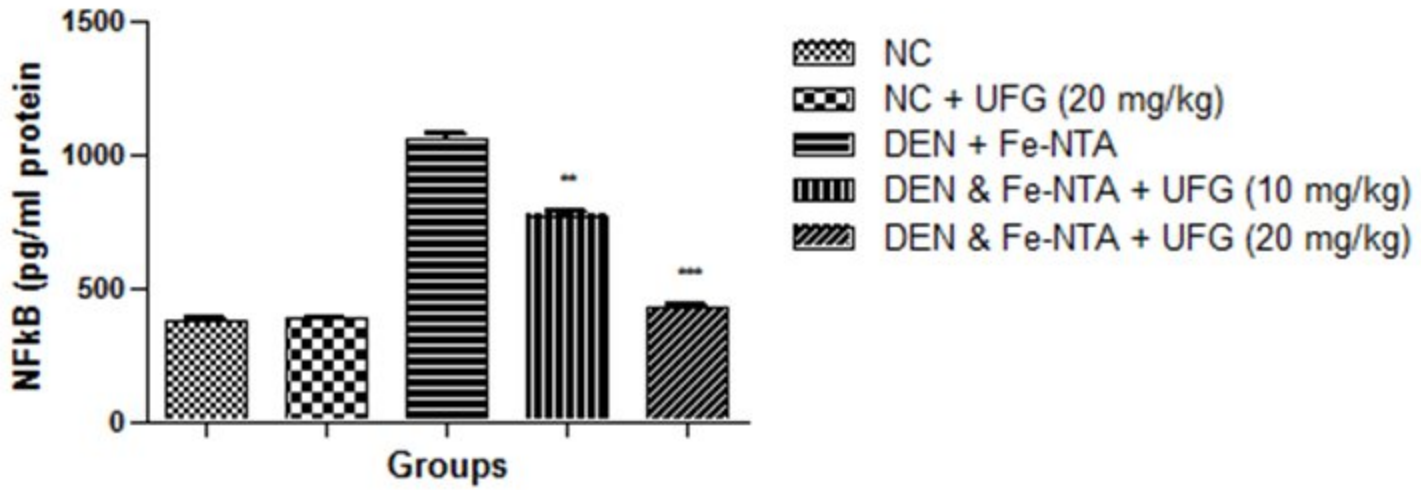

Figure 12: Effect of Umbelliferone β-D-galactopyranoside (UFG) on nuclear factor kappa-B cells (NFkB) of DEN+Fe-NTA induced renal tumorigenesis rats. NFkB was assessed as described in Materials and methods. ns = non-significant, DEN = Diethylinitrosamine, Fe-NTA= Ferric nitrilo tetra acetic acid. The comparisons were made by ANOVA followed by Dunnett's test. *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

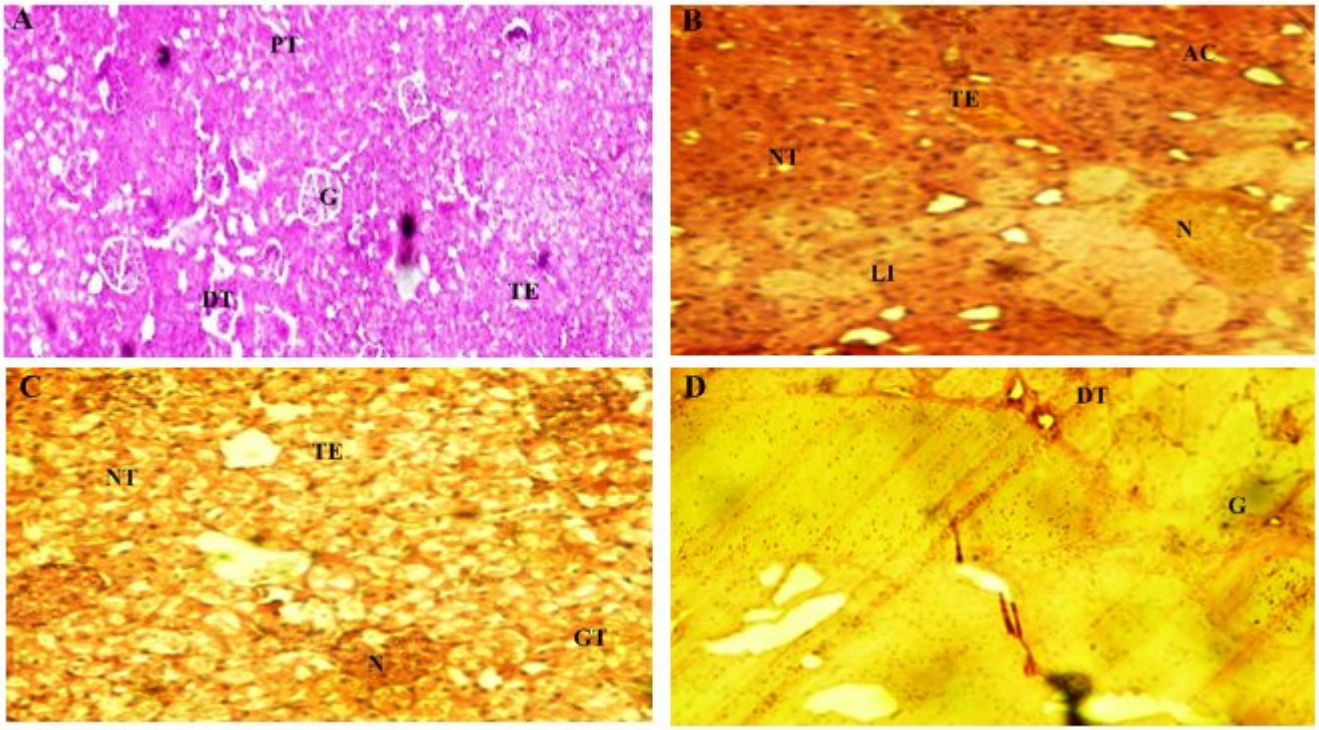

Figure 13: Effect of Umbelliferone β-D-galactopyranoside (UFG) on kidney histopathology study of DEN+Fe-NTA induced renal tumorigenesis rats. **(a)** Normal control, **(b)** DEN+Fe-NTA treated **(c)** DEN+Fe-NTA received UFG (10 mg/kg) **(d)** DEN+Fe-NTA received UFG (20 mg/kg)**.** All slides were stained with eosin and hematoxylin. (Original magnification 10×, DXIT 1200, Nikon, Japan). Dose of DEN =200 mg/kg b.w. and Fe-NTA = 9 mg/ kg b.w. dose N= necrosis; AC = adenocarcinoma; G = glomerulus; LI = leucocytic infiltration; DT = distal tubule; NT = necrotic tissue; PT = proximal tubule; TE = tubular epithelium; HC = hyperchromatism, GT = ghost tissue.

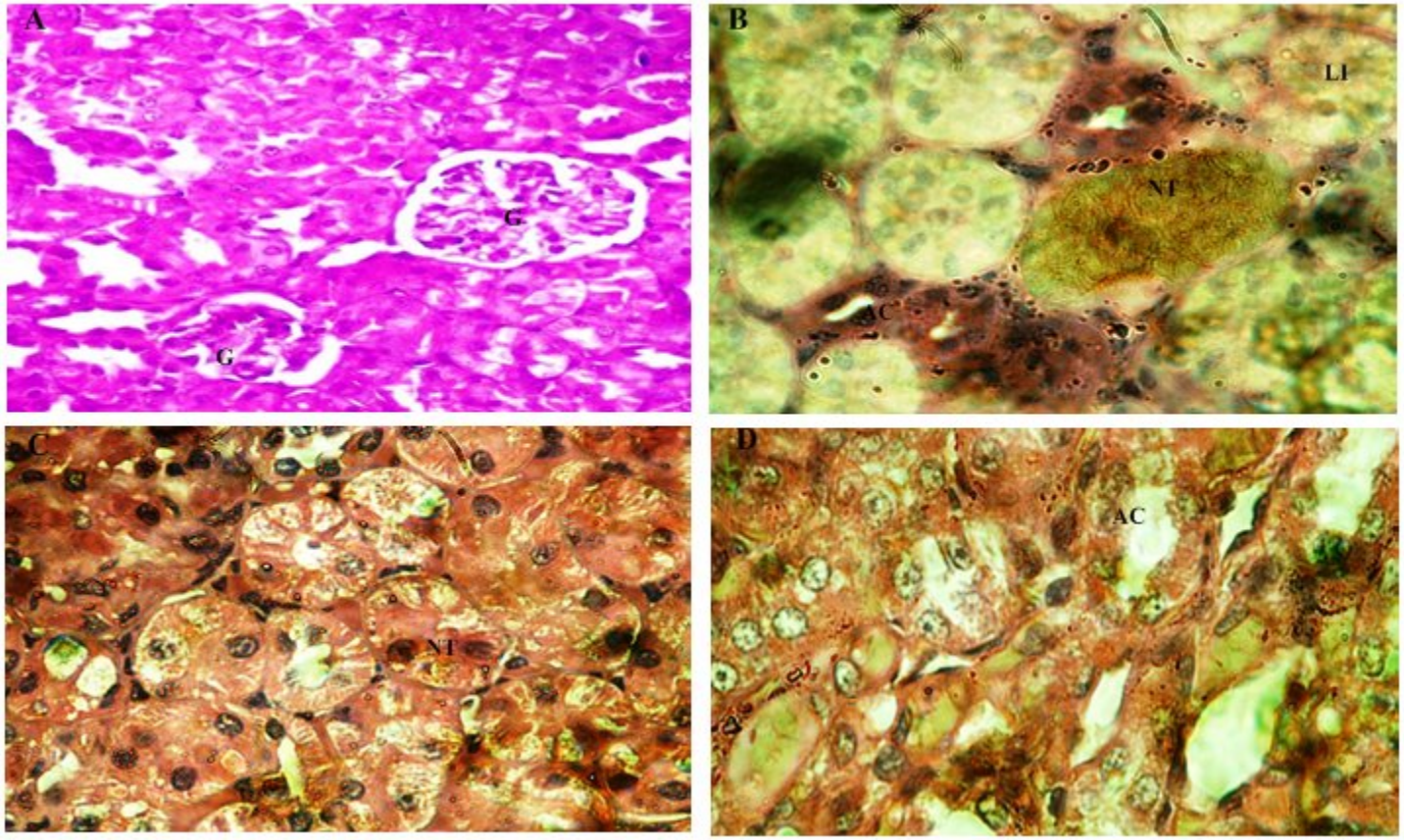

Figure 14: Effect of Umbelliferone β-D-galactopyranoside (UFG) on kidney histopathology study of DEN+Fe-NTA induced renal tumorigenesis rats. **(a)** Normal control, **(b)** DEN+Fe-NTA treated **(c)** DEN+Fe-NTA received UFG (10 mg/kg) **(d)** DEN+Fe-NTA received UFG (20 mg/kg)**.** All slides were stained with eosin and hematoxylin. (Original magnification 40×, DXIT 1200, Nikon, Japan). Dose of DEN =200 mg/kg b.w. and Fe-NTA = 9 mg/ kg b.w. N= necrosis; AC = adenocarcinoma; G = glomerulus; LI = leucocytic infiltration; DT = distal tubule; NT = necrotic tissue; PT = proximal tubule; TE = tubular epithelium; HC = hyperchromatism, GT = ghost tissue.

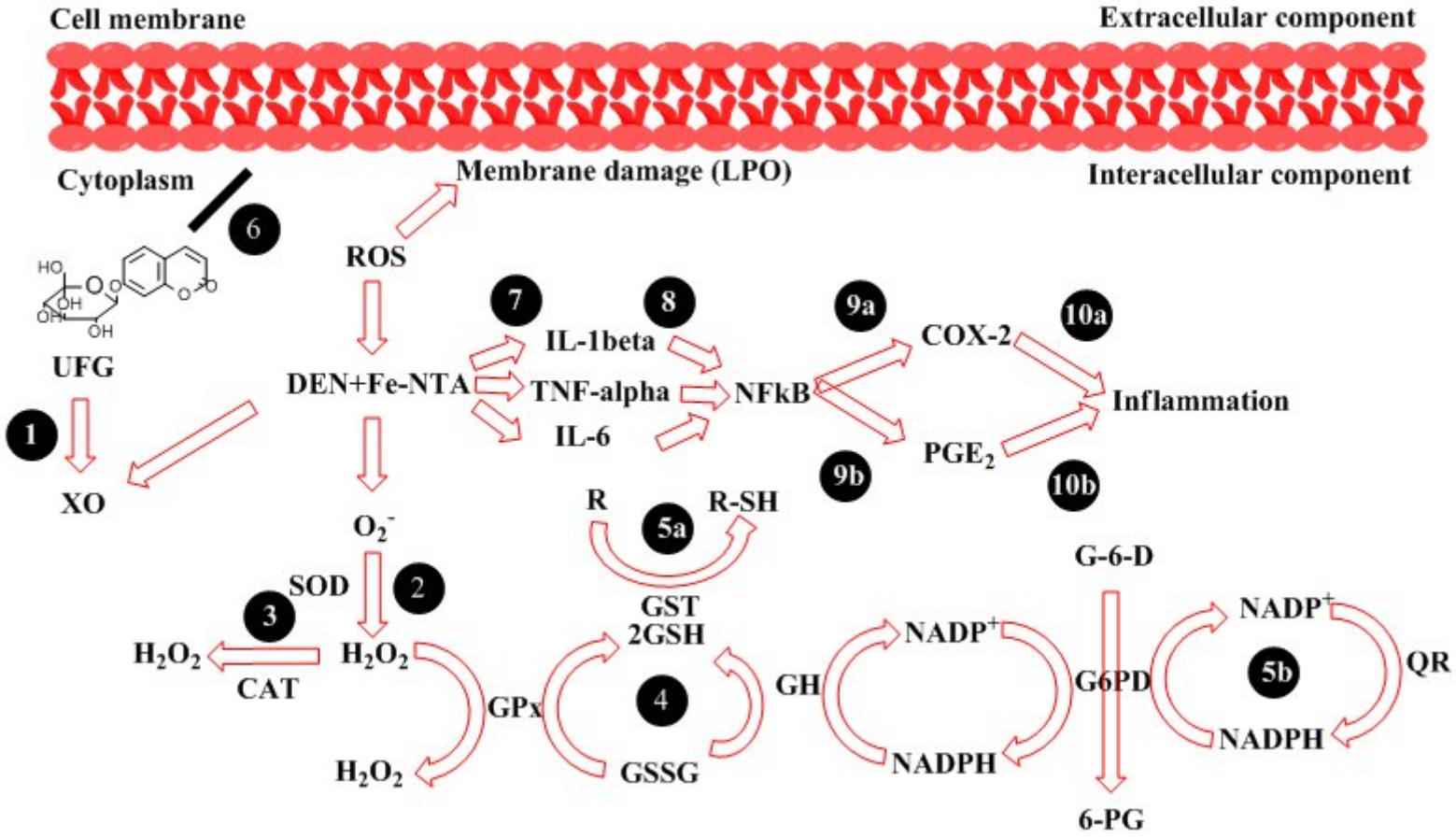

Figure 15: Effect of UFG against DEN+Fe-NTA induced debilities, in kidney of Wistar rats. DEN+Fe-NTA cause toxicity through generation of reactive oxygen species (ROS) and initiation of inflammatory response. Pretreatment of UFG confirm the reduction in the activity of xanthine oxidase (XO) (1) and start the reduction in the ROS formation. Further augmentation in antioxidant markers such as superoxide dismutase (SOD) (2), catalase (CAT) (3) and reduced glutathione (GSH) content and related redox cycle enzymes glutathione peroxidise (GPx), glucose-6-phosphate dehydrogenase (G6PD) and glutathione reductase (GR). (4) Potentiate its role against oxidants-induced damages. Furthermore, pretreatment of UFG also augmented phase-II metabolizing enzyme like glutathione S transferase (GST) and quinine reductase (QR) activities (5a and 5b). UFG also inhibit the lipid peroxidation (LPO) of cellular membrane (6). UFG proves the promising role against DEN+Fe-NTA-induced damage in kidneys by modulating the levels of TNF-α, IL-β, IL-6, PGE_2 and NFkB (7, 8, 9a, 9b, 10a and 10b). G-6-P= glucose-6-phosphate; GSSG= oxidized glutathione; 6-PG= 6-phosphogluconate; R= xenobiotics; O_2•= superoxide radical; H_2O_2= hydrogen peroxide; R-SH= thiol conjugated xenobiotics.