

***Pueraria montana* (Kudzu vine) Ameliorate the Inflammation and Oxidative Stress against Fe-NTA Induced Renal Cancer**

Fan Yang[#], Xiaoqiang Shi[#], Weidong Yang, Chao Gao, Zhenyu Cui^{*}, and Wentao Wang^{*}

Department of Urology Surgery, Affiliated Hospital of Hebei University, Baoding, 071000, CHINA

Abstract: Renal tissue plays a crucial function in maintaining homeostasis, making it vulnerable to xenobiotic toxicity. *Pueraria montana* has more beneficial potential against the various diseases and has long history used as a traditional Chinese medicine. But its effect against the renal cancer not scrutinize. The goal of this study is to see if *Pueraria montana* can protect rats from developing kidney tumors caused by diethylnitrosamine (DEN) and ferric nitrite (Fe-NTA). Wistar rats was selected for the current study and DEN (use as an inducer) and Fe-NTA (promoter) for induction the renal cancer. For 22 weeks, the rats were given oral *Pueraria montana* (12.5, 25, and 50 mg/kg) treatment. At regular intervals, the body weight and food intake were calculated. The rats were macroscopically evaluated for identification of cancer in the renal tissue. The renal tumor makers, renal parameters, antioxidant enzymes, phase I and II enzymes, inflammatory cytokines and mediators were estimated at end of the experimental study. *Pueraria montana* treated rats displayed the suppression of renal tumors, incidence of the tumors along with suppression of tumor percentage. *Pueraria montana* treated rats significantly ($p < 0.001$) increased body weight and suppressed the renal weight and food intake. It also reduced the level of renal tumor marker ornithine decarboxylase (ODC) and [3H] thymidine incorporation along with suppression of renal parameter such as uric acid, blood urea nitrogen (BUN) and urea and creatinine. *Pueraria montana* treatment significantly ($p < 0.001$) altered the level of phase enzymes and antioxidant. *Pueraria montana* treatment significantly ($p < 0.001$) repressed the level of tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and improved the level of interleukin-10 (IL-10). *Pueraria montana* treatment suppressed the level of prostaglandin (PGE₂), cyclooxygenase-2 (COX-2), nuclear kappa B factor (NF- κ B) and transforming growth factor beta 1 (TGF- β 1). *Pueraria montana* suppressed the inflammatory necrosis, size the bowman capsules in the renal histopathology. *Pueraria montana* exhibited the chemoprotective effect via dual mechanism such as suppression of inflammatory reaction and oxidative stress.

Key words: *Pueraria montana*, renal cancer, inflammation, renal biomarker, oxidative stress

1 Introduction

Renal cancer is having 3rd rank among all types of cancer and approximately 1,30,000 men cases and 63,000 deaths found from this cancer every year¹. The kidney is more susceptible to the toxicant due to 2 main reasons such as high volume of blood flowing via it and filter the huge volume of toxins which can deposit in the kidney tubules^{2,3}. Due to this systemic toxicity occurred: suppressed the capability to evacuate body wastes, inability to balance the electrolyte and body fluid and reduced the synthesis of essential hormones⁴. Various medicine and toxic chemical can induce the renal injury, are recognized as the toxico-

Abbreviations: DEN; Diethylnitrosamine, Fe-NTA; ferric nitrite, ODC; ornithine decarboxylase, BUN; blood urea nitrogen, TNF- α ; tumor necrosis factor- α , IL-1 β ; interleukin-1 β , IL-6; interleukin-6, IL-10; interleukin-10, PGE₂; prostaglandin E₂, COX-2; cyclooxygenase-2, NF- κ B; nuclear kappa B factor, TGF- β 1; transforming growth factor beta 1, ROS; reactive oxygen species, O₂⁻; superoxide radical, OH; hydroxyl radical, PBS; phosphate buffer saline, LPO; lipid peroxidation, GSH; glutathione, SOD; superoxide dismutase, GPx; glutathione peroxidase, CAT; catalase, GR; glutathione reductase, H₂O₂; hydrogen peroxide, GST; glutathione S-transferases

[#] Both equally contributed

^{*}Correspondence to: Zhenyu Cui, Department of Urology Surgery, Affiliated Hospital of Hebei University, Baoding, 071000, CHINA. Wentao Wang, Department of Urology Surgery, Affiliated Hospital of Hebei University, Baoding, 071000, CHINA

E-mail: hdfycuizhenyu@sina.com (ZC), 17732219385m@sina.cn (WW)

Accepted June 25, 2022 (received for review April 21, 2022)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

http://www.jstage.jst.go.jp/browse/jos/ http://mc.manuscriptcentral.com/jjoes



logical problem. Renal dysfunction reduces bodily waste elimination, disrupts electrolyte balance, blocks bodily fluid movement, and inhibits the production of vital hormones^{1,5}.

Because of the heat impact and the presence of gastric acid, nitrates are utilized as a food preservative, and this chemical could transform to nitrosamine^{6,7}. It is absorbed in the colon and reaches the hepatic tissue via the portal venous system, altering the liver tissue's detoxification system by interfering with cytochrome P450 enzymes, and causing toxicity in many organs⁸. One of the major environmental carcinogens, nitrosamine, has been linked to the generation of reactive oxygen species (ROS), which produces oxidative stress, altered the level of endogenous antioxidants, and enhanced oxidative stress in the tissues⁴. Previous literature suggests that this is the one of the etiology of cancer, inducing the variety of tumors in all animal species and all animal organs especially liver and kidney^{3,9}. DEN is the most frequent hepatotoxin and hepatocarcinogen among all nitrosamines. It is produced endogenously and can be found in cheese, processed meats, soybeans, tobacco smoke, and a variety of other foods, as well as during the metabolism of numerous medicines^{7,10}. DEN induces the cellular injury and oxidative stress due to involvement of free radicals and it induces the mutagenicity, carcinogenicity and cytotoxicity^{4,5}. During the DEN induce organ toxicity, huge number of free radicals generated and start the induction of tissue toxicity via suppression the antioxidant defence system^{3,11}. According to prior studies, free radicals and associated lipid peroxides have a role in the pathogenesis of age-related chronic degenerative disease and exogenous oxidative stress⁸. Thus, the antioxidant therapy is more effect to suppress the vulnerability of the renal to oxidative challenges.

Iron is a vital nutrient for all living organisms. But the overload of iron may also cause the numerous diseases¹². The iron produces a complex with the chelating agent (nitrilotriacetic acid) and produce the Fe-NTA¹³. Fe-NTA is absorbed through the mesothelium into the portal vein and then circulated into liver tissue¹². Fe-NTA has a low molecular weight and is easily filtered into the renal proximal tubule lumen via glomeruli, where glutathione breakdown products such cysteinylglycine or cysteine decrease Fe³⁺ NTA to Fe²⁺ NTA^{12,14}. γ -Glutamyl transpeptidase hydrolyzes glutathione to cysteinylglycine, which is promptly reduced to glycine and cysteine via dipeptide bonding at the brush border surface of the renal proximal convoluted tubules¹⁵. Cysteine and cyteinylglycine both are the thiol reductant which suppress the Fe³⁺ to Fe²⁺ NTA. The Fe²⁺ autooxidation generate the free radical such as superoxide radical (O₂⁻) which subsequently potentiate the iron catalyzed Haber-Weiss reaction to generate the hydroxyl radical (OH), leading to inducing the DNA injury and start the lipid peroxidation reaction^{12,16,17}. Oxidative stress was increased the DNA mutation point, break the DNA strand

and DNA cross linking and finally alter the normal cells¹².

Kudzu (*Pueraria montana*) is native to the China, Korea, India and Japan and last few decades it grows whole over the world^{18,19}. Its root as used as a source of starch in Japan and China and it also used as a vegetable and actively use in various cooking recipe²⁰. Kudzu is also used as traditional Chinese medicine to treat the various disease condition such as headache, common cold, heart disease and diarrhea and it also showed the various health benefit effects. Kudzu is the rich source of various phytoconstituents such as phenolic, terpenoids, alkaloids and tannins. Kudzu possess the various biological actions such as osteoporosis, gynecological, diabetic nephropathy, anti-inflammatory, cognitive capability and antioxidant effect²⁰⁻²². As prior literature reveals it has a significant antioxidant and anti-inflammatory activity. In this study, we aimed to see if *Pueraria montana* could protect rats from Fe-NTA-induced kidney cancer.

2 Material and Methods

2.1 Rodent

This study used Swiss Albino Wistar rats aged 12-14 weeks and weighing 150 ± 20 g. The rats were taken from the animal house and housed in a single cage under laboratory conditions. During the storage the temperature 22-28°C, relative humidity 70% and 12 h day and 12 h dark cycle. During the whole experimental period rats were received the standard pellet diet with water *ad libitum*. The Affiliated Hospital of Hebei University's animal ethical committee authorized this study (HUAH-34555).

2.2 Preparation of *Pueraria montana* extract

The powdered *Pueraria montana* extract was extracted for 72 hours utilizing continuous extraction with ethanol using a Soxhlet apparatus. After the extraction process, the extract was concentrated to dryness under reduced pressure using a rotary evaporator and maintained at a low temperature (4°C).

2.3 Preparation of toxicant

DEN (200 mg/kg) was injected intraperitoneally to induce kidney cancer in this study^{2,11}. DEN (200 mg/kg) was dissolved in phosphate buffer saline (PBS) at pH = 4.5 for a few minutes¹. Fe-NTA (9 mg/kg) was used for promotion of renal cancer. The Fe-NTA solution was made by dissolving 0.16 mmol/kg with a 4x molar excess of 0.64 nmol disodium salt of NTA and keeping the pH at 7.4 with sodium bicarbonate¹².

2.4 Experimental groups

The rats were separated into the groups shown in Fig. 1 after being infected with kidney carcinoma. For 22 weeks,

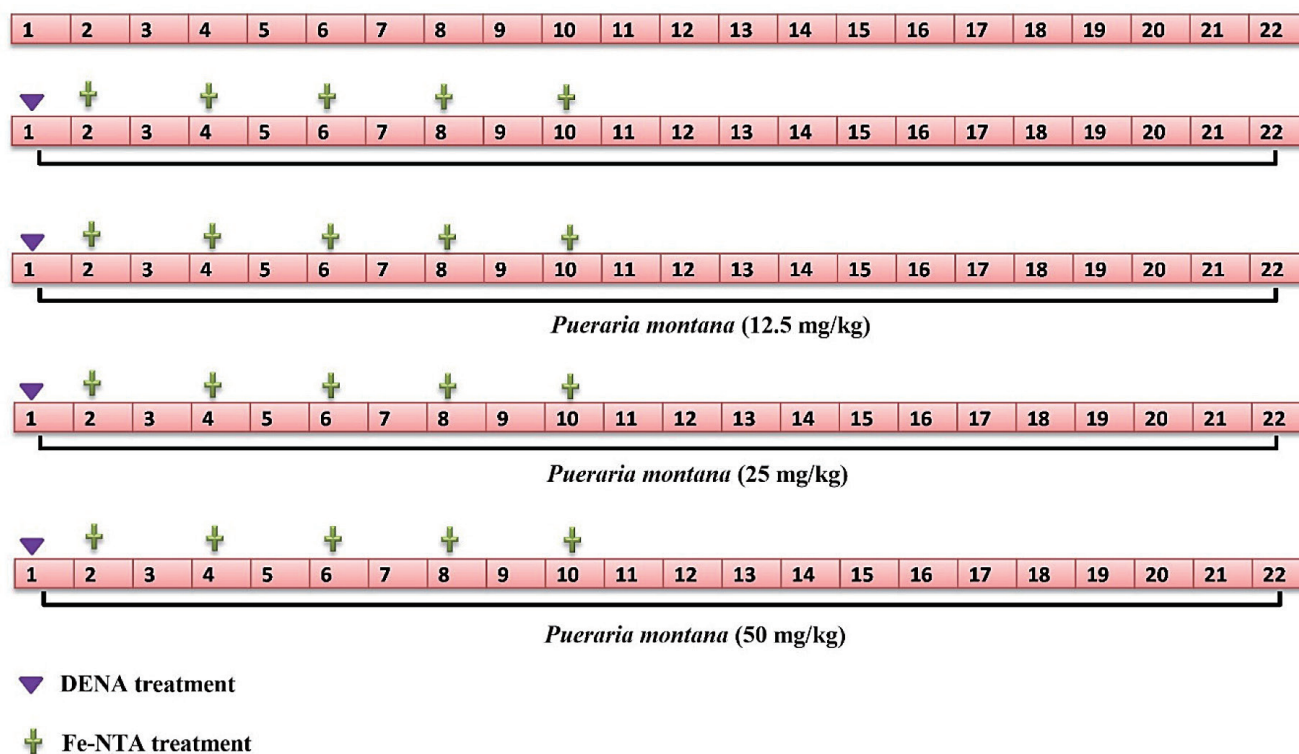


Fig. 1 Treatment and duration of the different groups.

all of the rats were given the same treatment. At regular intervals, the body weight was measured.

At the end of experiment, the rats were anesthetized with diethyl ether, and blood was obtained by puncturing the retro orbital plexus. The obtained blood was centrifuged at 12000 *g* for 15 minutes to separate the serum. For further examination, the serum was kept at -20°C .

2.5 Renal tumor

Renal tumor markers such as thymidine[3H]incorporation and ODC were determined using a modified version of a previously published method^{11, 23, 24}.

2.6 Renal parameters

Renal parameters include uric acid, BUN, urea and creatinine were analyzed using available kit (Friedrichsring 4, Mannheim, Germany) following the manufacture instruction.

2.7 Antioxidant parameter

Thiobarbituric acid (TBA) reaction procedure was used for the estimation of LPO²⁵. The tested samples were mixed with the TBA reagent, which contains the TCA and TBA and kept the sample for 30 min in the boiling water bath. After that the sample was centrifuged at 2000 *g* for 10 min and finally estimated the absorbance at 535 nm. The content of SOD was estimated on the basis of photoreduction of NBT dye and finally estimated the absorbance

at 560 nm²⁶. The level of CAT was assessed using Sinha *et al.*, with minor modifications²⁷. Briefly, the samples mixed with the H_2O_2 solution and PBS (5 mL) in the Erlenmeyer flask and allow the reaction to carried out at room temperature. The 1 mL quantity of reaction mixture was withdrawn and added the reagent (dichromate/acetic acid) and estimated the absorbance at 570 nm. The content of glutathione (GSH) was estimated using the previous reported method of Sedlak and Lindsay with minor modification²⁸. The sample absorbance was estimated at 412 nm. Previous reported method was used for the estimation of Carlberg and Mannervik with minor modification²⁹. Briefly, the reaction mixture contains the PBS, EDTA, NADPH and added the GSSH and microsomal or cytosol suspension. The reaction was performed and added the enzyme for initiate the reaction. The activity of GR was estimated at 340 nm. Lawrence and Burk method was used for the determination of GPx activity with minor modification³⁰. Briefly, the sample contain the potassium phosphate buffer, glutathione reductase, NaNs, NADPH, enzyme source, GSH and either cumene hydroperoxide or hydrogen peroxide. The absorbance of sample reaction was estimated at 340 nm.

2.8 Inflammatory cytokines and inflammatory parameters

Inflammatory cytokines such as $\text{TNF-}\alpha$ (MBS508481), IL-1 β (MBS697369) and IL-6 (MBS8123859), IL-10 (MBS7720408) and inflammatory parameters like COX-2 (MBS7721379), PGE_2 (MBS7727909), TGF- β 1

(MBS8819920) were analyzed using the ELISA kits following the manufacture instruction (MyBioSource Ltd., San Diego, USA). The NF- κ B(ab288581) was estimated using the ELISA kits following the manufacture instruction (Abcam, USA).

2.9 Histopathology

At end of the study, the renal tissue isolated and cut into small pieces for prepared the histopathology.

2.10 Statical analysis

The GraphPad Prism software was used to analyze all of the data in the current experimental study (St. Louis, USA). The t-test by Dennett was used to compare the groups. The significance is determined by the $p < 0.05$ value.

3 Results

3.1 Renal tumor

The renal tumor nodules of several groups of rats were shown in Table 1. Normal rats did not show any signs or symptoms of a kidney tumor. Fe-NTA rats exhibited the development of the renal nodules in all rats. 6 rats contain the unilateral tumors and 4 rats contains the bilateral tumors with total number of 39 tumors. *Pueraria montana* treated rats (12.5 mg/kg) exhibited the 8 rats contains the renal tumors with 5 rats contains the unilateral tumors and 3 rats contains the bilateral tumors with total number of 21 tumors, 80% incidence of tumors. *Pueraria montana* (25 mg/kg) rats exhibited 5 rats contains the renal tumors, unilateral tumors (3) and bilateral (2) along with tumor incidence 50%. *Pueraria montana* (50 mg/kg) treated rats exhibited the maximum reduction in the renal tumors, its group rats showed the 2 rats contain renal tumor, unilateral tumors (1) and bilateral (1) along with tumor incidence 20%.

3.2 Body weight, renal weight and food intake

In this study, the body weight of all-group rats was assessed at regular intervals. In this experiment, we chose rats of almost equal weight, and during the initial weight, all of the groups showed no significant differences in weight. After completion of experimental study (22 weeks), the body weight of normal rats increased at normal pattern, but the Fe-NTA rats demonstrated the suppressed body weight as compared to initial and other group body weight. *Pueraria montana* (12.5, 25 and 50 mg/kg) treated rats significantly ($p < 0.001$) improved the body weight in comparison with the Fe-NTA group rats (Fig. 2a).

Due to the formation of renal tumors, the Fe-NTA group rats' renal weight increased. The weight of renal tissue in rats treated with *Pueraria montana* was significantly ($p < 0.001$) decreased (Fig. 2b).

Food consumption of experimental rats increased in certain cases during the sickness, and a similar effect was reported in this investigation. The food consumption of Fe-NTA-induced rats was higher, but the food intake of *Pueraria montana*-treated rats was lower (Fig. 2c).

3.3 Renal tumor marker

ODC and 3[H]thymidine incorporation is the biomarker of renal tumor and both marker level estimated for analyzed the servitiy of renal cancer. During the normal condition, the level of ODC and 3[H]thymidine incorporation remain within limit, but during the renal cancer, the level of both markers boosted more than 5-10 times, depend upon the stage of the renal cancer. The level of ODC (Fig. 3a) and 3[H]thymidine incorporation (Fig. 3b) increased more than 5 times in Fe-NTA rats compared to control rats, while *Pueraria montana* treated rats significantly ($p < 0.001$) lowered the level of both biomarkers, implying a renal protective effect.

3.4 Renal parameters

Renal parameters changed as the disease progressed throughout renal carcinoma. The level of BUN (Fig. 4a),

Table 1 Demonstrated the effect of kudzu vine extract on Fe-NTA induced renal tumor.

Group	No of rats	No of animals studies histopathologically	No of rats with renal tumor	No of rats with unilateral tumors	No of rats with bilateral tumors	Total no of tumors	Incidence of tumors (%)
NC	10	0	0	0	0	0	0
Fe-NTA	10	10	10	6	4	39	100
Fe-NTA+ Kudzu vine (12.5 mg/kg)	10	9	8	5	3	21	80
Fe-NTA+ Kudzu vine (25 mg/kg)	10	10	5	3	2	14	50
Fe-NTA+ Kudzu vine (50 mg/kg)	10	10	2	1	1	4	20

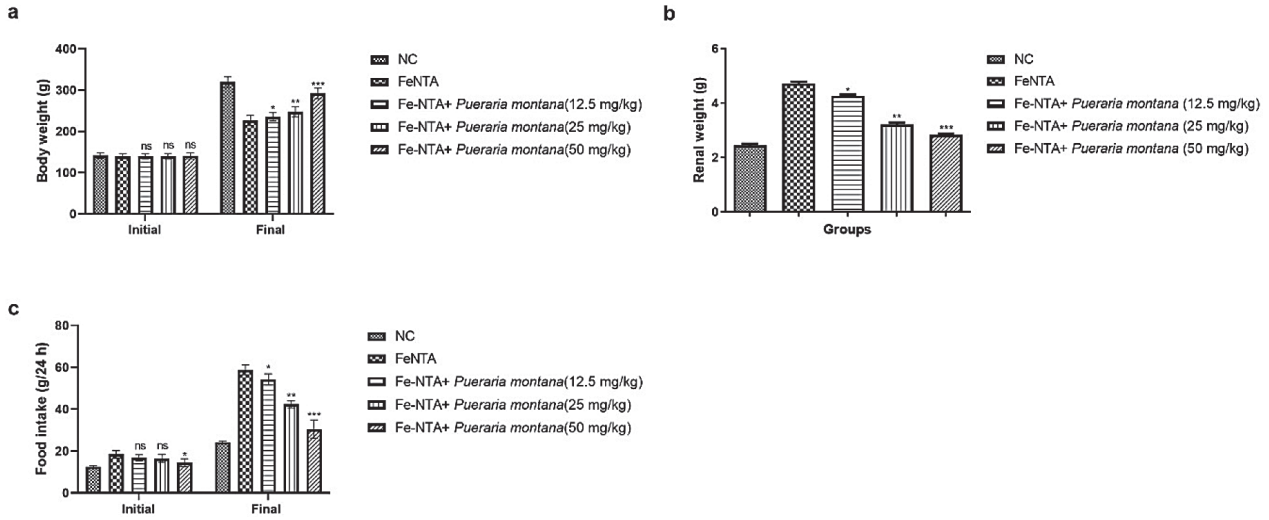


Fig. 2 Effect of *Pueraria montana* on the body, renal tissue weight and food intake of different group rats. **a:** body weight, **b:** renal weight and **c:** food intake. Dunnett's test was used for the statistical analysis between the disease control and tested group rats. Where $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ was consider as significant, more significant and extreme significant.

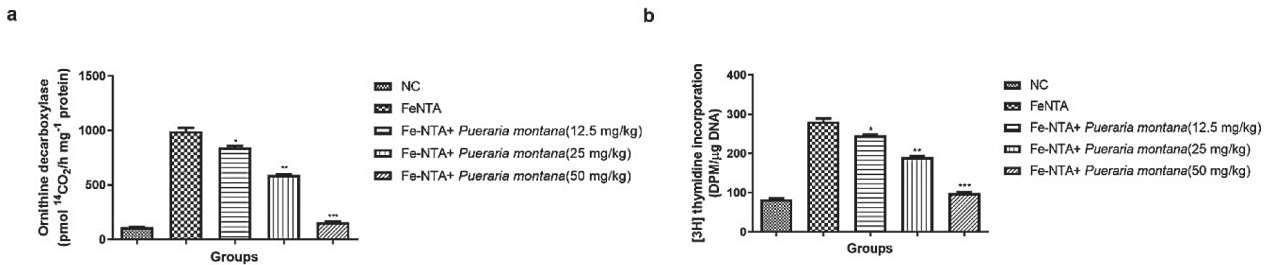


Fig. 3 Effect of *Pueraria montana* on the renal tumor markers of different group rats. **a:** ODC and **b:** [3H]thymidine incorporation. Dunnett's test was used for the statistical analysis between the disease control and tested group rats. Where $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ was consider as significant, more significant and extreme significant.

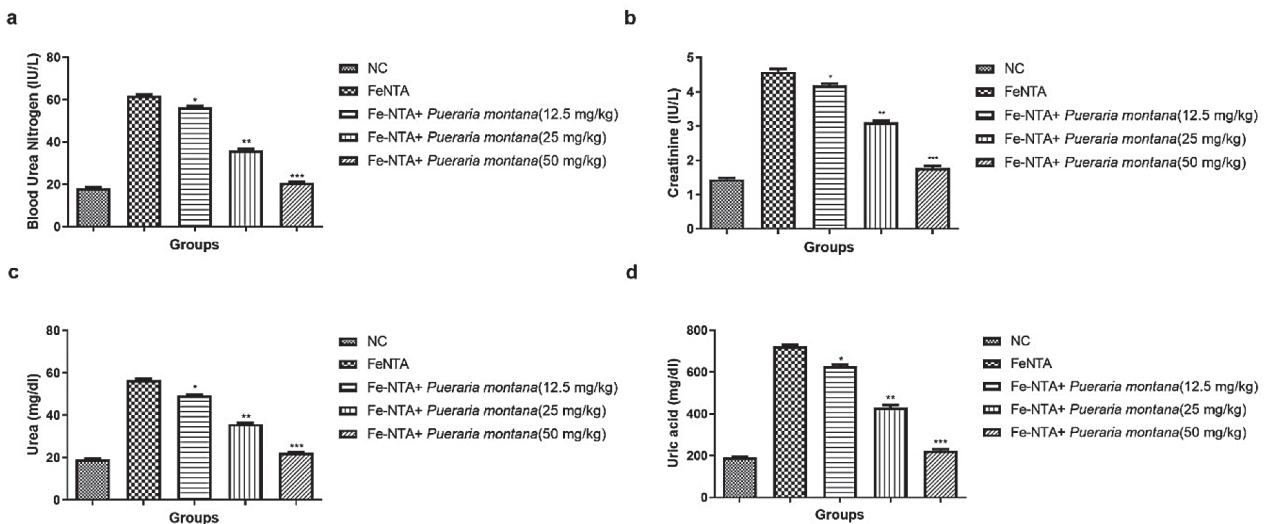


Fig. 4 Effect of *Pueraria montana* on the renal parameters of different group rats. **a:** blood urea nitrogen, **b:** creatinine, **c:** urea and **d:** uric acid. Dunnett's test was used for the statistical analysis between the disease control and tested group rats. Where $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ was consider as significant, more significant and extreme significant.

creatinine (Fig. 4b), urea (Fig. 4c), uric acid (Fig. 4d) was boosted in the Fe-NTA group rats and *Pueraria montana* treatment significantly ($p < 0.001$) repressed the level of renal parameters at dose dependent manner. *Pueraria montana* (50 mg/kg)-treated rats had the most significant reduction in renal parameters.

3.5 Antioxidant, phase I and II parameters

Free radical reactions have long been known to have a role in the progression of cancer. We estimated the level of different antioxidant parameters. The result showed that

Fe-NTA induced rats exhibited the boosted level of LPO (Fig. 5a) and repressed the level of CAT (Fig. 5b), SOD (Fig. 5c), GSH (Fig. 5d), GPx (Fig. 5e), GR (Fig. 5f). *Pueraria montana* treated rats exhibited the significantly ($p < 0.001$) suppression the LPO level and boosted the level of CAT (Fig. 5b), SOD (Fig. 5c), GSH (Fig. 5d), GPx (Fig. 5e), GR (Fig. 5f).

The effect of *Pueraria montana* on the levels of phase I and II enzymes was shown in Fig. 6. Fe-NTA rats had lower levels of QR, glucose-6-phosphatase dehydrogenase and higher levels of xanthine oxidase. *Pueraria montana*

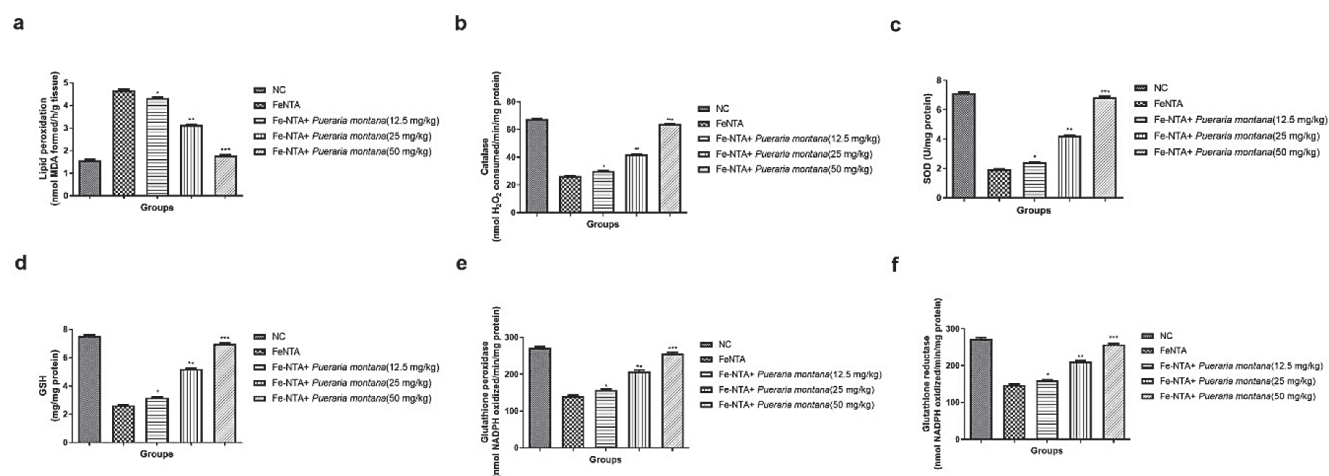


Fig. 5 Effect of *Pueraria montana* on the antioxidant parameters of different group rats. a: lipid peroxidation, b: catalase, c: SOD, d: GSH, e: glutathione peroxidase and f: glutathione reductase. Dunnett's test was used for the statistical analysis between the disease control and tested group rats. Where $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ was consider as significant, more significant and extreme significant.

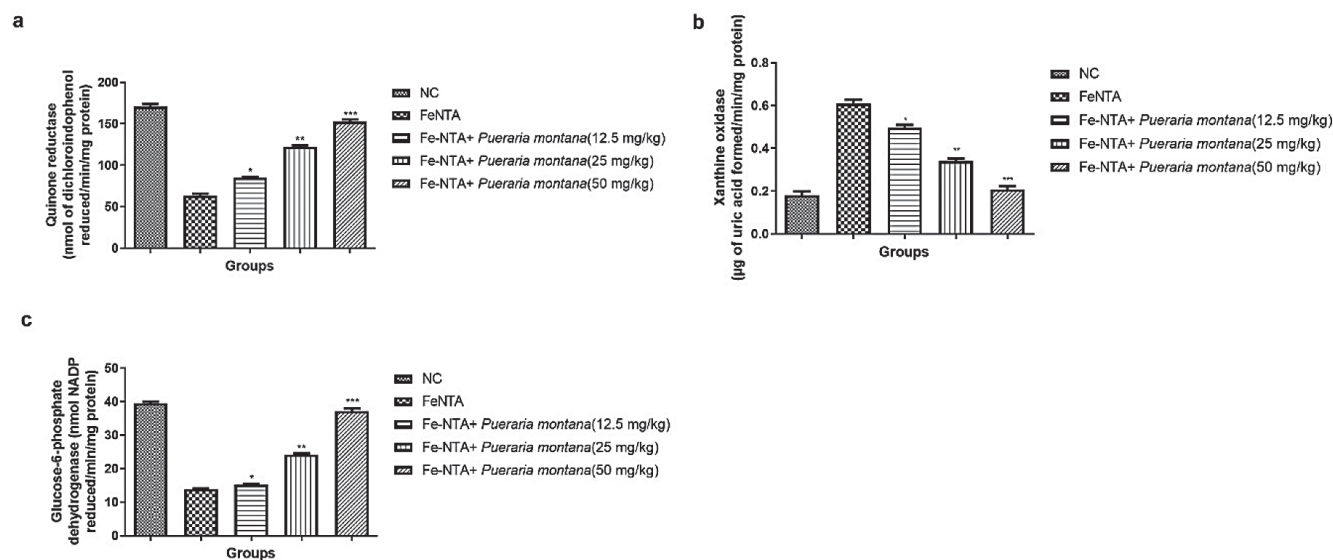


Fig. 6 Effect of *Pueraria montana* on the phase I and II enzymes of different group rats. a: quinine reductase, b: xanthine oxidase and c: glucose-6-phosphatase dehydrogenase. Dunnett's test was used for the statistical analysis between the disease control and tested group rats. Where $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ was consider as significant, more significant and extreme significant.

treated rats significantly ($p < 0.001$) altered the level of phase I and II enzymes.

3.6 Cytokines

During cancer disease, inflammatory responses play an important role. The similar momentum was observed in Fe-NTA group rats, this group rats exhibited the boosted level of TNF- α (Fig. 7a), IL-1 β (Fig. 7b), IL-6 (Fig. 7c) and

suppressed level of IL-10 (Fig. 7d) and suggesting the inflammatory reaction in this group rats. *Pueraria montana* treated rats exhibited the alteration in the level of cytokines, suggesting the suppression of the inflammatory reaction and anti-inflammatory effect of *Pueraria montana* extract.

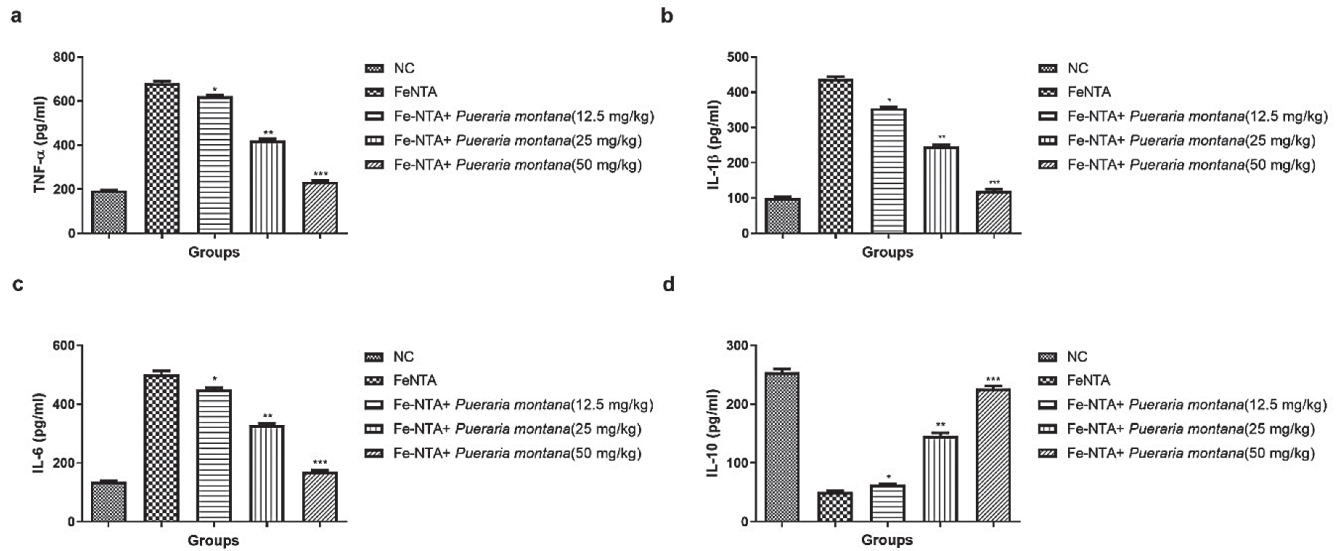


Fig. 7 Effect of *Pueraria montana* on the inflammatory cytokines of different group rats. a: TNF- α , b: IL-1 β , c: IL-6 and d: IL-10. Dunnett's test was used for the statistical analysis between the disease control and tested group rats. Where $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ was consider as significant, more significant and extreme significant.

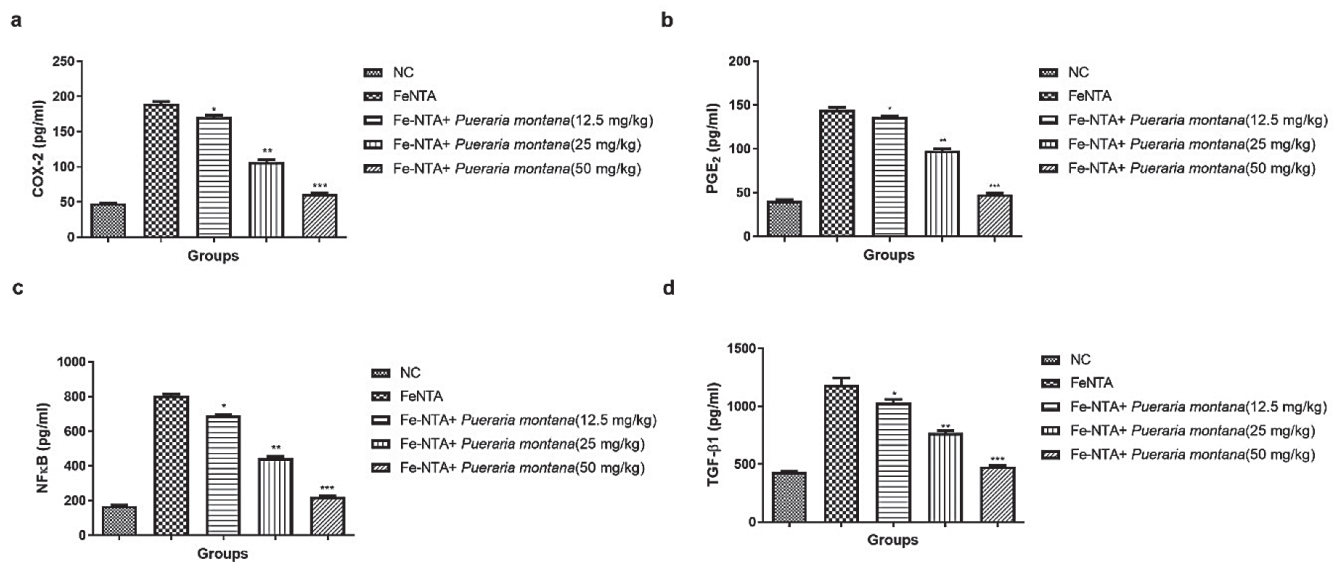


Fig. 8 Effect of *Pueraria montana* on the inflammatory parameters of different group rats. a: COX-2, b: PGE₂, c: NF- κ B and d: TGF- β 1. Dunnett's test was used for the statistical analysis between the disease control and tested group rats. Where $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ was consider as significant, more significant and extreme significant.

3.7 Inflammatory mediators

We calculated the levels of several inflammatory mediators as well as the total number of inflammatory mediators such as COX-2 (Fig. 8a), PGE₂ (Fig. 8b), NF-κB (Fig. 8c) and TGF-β1 (Fig. 8d) in the all-group rats. Fe-NTA control group rats exhibited the boosted level of these inflammatory mediators and *Pueraria montana* treated rats significantly ($p < 0.001$) repressed the level of these inflammatory mediators. *Pueraria montana* (50 mg/kg) treated rats reached the level of these inflammatory parameter almost near to the normal level.

3.8 Histopathology

Normal rats histopathology did not show any alteration in the histopathology (data not shown). Fe-NTA induced rats exhibited the necrosis in the renal tissue, inflamed cells, inflammation in blood cells, cytoarchitecture deformation, tubular cost and increased the size of Bowman's capsule. *Pueraria montana* treated rats demonstrated the reduction of size of the Bowman's capsule and suppressed the inflammatory blood cells, inflamed cells (Fig. 9).

4 Discussion

Experimental study was carried out on various plants to scrutinize their efficacy against cancer. Searching the new chemotherapeutic and antitumor agent that are more effective along with less toxic³¹. Chemotherapeutic drugs have a preventative effect by slowing the spread and growth of invasive cancer by either stopping or reversing premalignant cell progression³². The plant and their phytochemical possess excellent antioxidant effects and has been reported to show the multiple biological effects such as antitumor and anti-inflammatory effects^{33, 34}. *Pueraria montana* contain the phenolic phytoconstituents like tannins, flavonoids and saponins in the extract and acts as the free radical scavengers due to potent antioxidant effects²⁰. In this study, we make attempt to scrutinize the chemotherapeutic effect of *Pueraria montana* against Fe-NTA induced renal cancer in rats.

ODC (rate limiting enzyme) is the 1st line enzyme in polyamine biosynthesis and it take part in induction and progression of tumors¹⁵. Previous research suggests that ODC use as an indicator for cell propagation and during the cancer disease, the level of ODC remarkably boosted due to expansion of carcinogenesis effect. For DNA duplication, ODC catalyzes ornithine decarboxylation to putrescine, which is then transformed to higher polyamines¹. Another

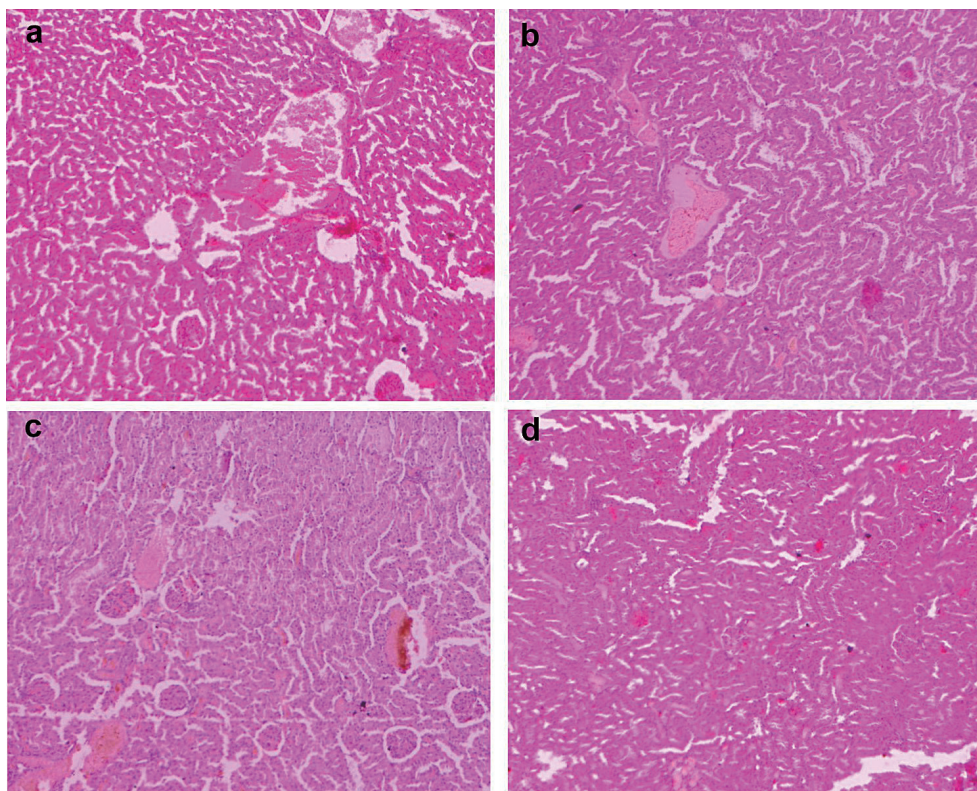


Fig. 9 Effect of *Pueraria montana* on the histopathology of different group of rats. Where **a**: showed Fe-NTA induced renal cancer, **b**: showed FE-NTA treated with *Pueraria montana* (12.5 mg/kg), **c**: showed FE-NTA treated with *Pueraria montana* (25 mg/kg) and **d**: showed FE-NTA treated with *Pueraria montana* (50 mg/kg), respectively.

tumor marker, 3[H]thymidine also used as the indicator for identification of cancer disease^{15, 35}. ODC and ODC and 3[H]thymidine is vigorously used for estimation of tumor progression and hyperproliferative effect¹. During the renal cancer, the level of ODC and 3[H]thymidine boosted and similar result was observed in the Fe-NTA group rats. *Pueraria montana* treatment dramatically reduced ODC and 3[H]thymidine levels, implying anti-hyperproliferative activity against renal cancer.

The boosted level of uric acid is considered as the indicator in the injury of joints and difference tissues. The significant enhance level of uric acid leads to induces the permanent injury in the tissue and finally induces the acute inflammation³⁶. XO is a crucial enzyme that catalyze the hydroxylation of hypoxanthine to xanthine and further xanthine to uric acid which is excreted from the kidneys. The excess production of XO, unable to excretion of uric acid from the kidney and ultimately causes the hyperuricemia³⁷. The XO inhibition suppress the vascular oxidative stress and also reduce the level of uric acid. Mostly, the researcher targeting the XO for the treatment of renal stone and nephropathy associated with the hyperuricemia^{37, 38}. During the renal injury, suppressed the glomerular filtration rate, which further start the excretion of creatine from the glomerulus and tubular^{11, 24}. Pandey *et al.* exhibited that boosted level of uric acid also suppressed the glomerular filtration rate³⁹. During the renal injury, start the degradation of purine nucleotides and start the generation of urea, which is the sign of metabolism of nucleotide¹. The concentration of creatine and urea is estimate for the acute renal failure, kidney blood flow, accumulation of blood flow and suppression of renal excretion³⁹. The modification of the renal parameters suppresses the glomerular filtration rate and similar result was observed in the Fe-NTA induced renal cancer rats and *Pueraria montana* remarkably altered the level of renal parameters and suggest the renal protective potential.

Oxidative stress and the inflammatory response are well-known to be exacerbated by cancer. Anwar *et al.*, suggest that oxidative stress and inflammatory reaction boosted during the DEN induced renal cancer disease¹. During the carcinogen's influence on the required metabolism, the carcinogenic effect is transferred via Phase I and II enzymes. DEN metabolized via phase I enzyme and catalyzes the free radical reaction, while phase II enzyme detoxifies the various carcinogen³⁵. During the cancer, increased the level of free radical likes O_2 and H_2O_2 , which suppress the endogenous antioxidant enzymes such as GPx, SOD, CAT^{10, 11}. During the Fe-NTA induced renal cancer, suppress the level of endogenous antioxidant enzymes. Previous study suggest that the ROS generation may induces the subcellular and cellular injury via peroxidation of lipid membrane which denaturing the cellular proteins and breaking the DNA strands and finally induces

the dysfunction the cellular functions³⁵. After generation the free radicals, the first line antioxidant starts to scavenge the free radicals. SOD convert the superoxide (O_2) radicals into hydrogen peroxide (H_2O_2) and CAT scavenge the H_2O_2 ^{40, 41}. GSH is a tripeptide and play a crucial role in maintaining the cellular redox status. GSH mostly present in the mammalian tissue and non-protein sulfur (90%). GSH control the physiochemical activity of peptides and different cellular proteins, which further detoxify the free radicals^{7, 11, 42}. There is a relationship between the restoration of these enzymes and anticancer chemoprotective properties, according to several research^{3, 43}. The phase enzyme initiates the carcinogens metabolism to full form and exhibit the maximum carcinogen effect⁴. During the carcinogen, GST (phase II enzyme) detoxify the carcinogens metabolites by thiol conjugation. GR supports the glutathione S-Transferases (GST) to balance the GSH pool. QR suppress the various substrates via catalyzing the suppression the carcinogenicity or toxicity induced through free radicals⁴⁴. In this study, *Pueraria montana* treated rats demonstrated the alteration in the level of endogenous antioxidant enzymes and suggesting the antioxidant effect via suppression of oxidative stress.

Inflammatory cytokines have a key role in the spread of disease, particularly cancer, due to their proliferative and mutagenesis capabilities⁴⁵. TNF- α play a crucial role in transformation and later deposition of mutations²⁴. TNF- α boost the production of ROS via oxidative stress responsive gene which further enhanced the inflammatory reaction⁴¹. FeNTA-induced rats produced more cytokines, while *Pueraria montana* treatment lowered the levels of inflammatory cytokines, indicating an anti-inflammatory effect.

It is widely known that oxidative stress increases the inflammatory response⁴¹. Continuous free radical production is crucial for the generation of cytokines and the inflammatory response. Oxidative stress also shows the effect on the NF- κ B, along with control of several genes involved in cell proliferation, transformation, and angiogenesis⁴⁰. NF- κ B has an important role in the progression of different cancers, including kidney carcinoma. Due to enhance the level of NF- κ B during the cancer disease, it also involved in the various process such as apoptosis, cellular proliferation, angiogenic and expansion of tumor cells^{7, 11, 46}. NF- κ B involved in the regulation of various agent such as growth factors, vasoactive agents, cytokines and enhance level of NF- κ B regulated the inflammatory and immune related proteins. Recent year, the researcher targeting the NF- κ B for the treatment of cancer disease^{7, 11, 40, 42}. In this study, the level of NF- κ B boosted due to expansion of cancer disease and *Pueraria montana* treatment suppressed the level of NF- κ B suggesting the cancer protective effect. Other inflammatory parameter such as COX-2, play a significant role in regulation of NF- κ B^{11, 41}. Its also involve in the development of the relationship between the renal in-

inflammation and tumor expansion²³⁾. The reduction of the NF- κ B is considered as the significant target to suppress the expansion of carcinogenesis. COX-2 is believed to play a critical function in the gene transcription of NF- κ B, according to a variety of sources^{23, 35)}. The level of COX-2 is boosted during the renal cancer due to increased level of NF- κ B. PGE₂ plays a crucial role in cell proliferation, vascular permeability and inflammatory reaction. During the renal cancer, the level of PGE₂ is boosted, which suggests the inflammatory reaction in the disease^{35, 45)}. FeNTA induces rats exhibited the increased level of NF- κ B, PGE₂ and COX-2 and *Pueraria montana* treatment considerably suppressed the level of these inflammatory parameters and suggesting the anti-inflammatory effect.

5 Conclusion

Taking together, *Pueraria montana* extract exhibited the renal protective effect via suppression of renal nodules, renal weight and enhanced the body weight. *Pueraria montana* extract suppressed the renal tumor marker, which was estimated in terms of ODC and ³[H]thymidine incorporation. *Pueraria montana* extract suppressed the oxidative stress via altering the level of endogenous antioxidant, phase I and phase II enzymes. *Pueraria montana* extract significantly suppressed the inflammatory reaction via reducing the level of inflammatory cytokines and inflammatory mediators such as NF- κ B, COX-2, PGE₂.

References

- 1) Anwar, F.; Al-Abbasi, F.A.; Bhatt, P.C.; Ahmad, A.; Sethi, N.; Kumar, V. Umbelliferone β -D-galactopyranoside inhibits chemically induced renal carcinogenesis via alteration of oxidative stress, hyperproliferation and inflammation: Possible role of NF- κ B. *Toxicol. Res. (Camb)* **4**, 308-1323 (2015).
- 2) Singh, D.; Yadav, E.; Kumar, V.; Verma, A. *Madhuca longifolia* embedded silver nanoparticles attenuate diethylnitrosamine (DEN)-induced renal cancer via regulating oxidative stress. *Curr. Drug Deliv.* **18**, 634-644 (2020).
- 3) Siddiqi, A.; Saidullah, B.; Sultana, S. Anti-carcinogenic effect of hesperidin against renal cell carcinoma by targeting COX-2/PGE2 pathway in Wistar rats. *Environ. Toxicol.* **33**, 1069-1077 (2018).
- 4) Shaheen, N.E.M. Oxidative stress of diethylnitrosamine on the functions of kidney in male rats and effective role of rutin and / or selenium. *J. Appl. Sci. Res.* **9**, 6684-6691 (2014).
- 5) Prasad, L.; Khan, T.H.; Jahangir, T.; Sultana, S. Abrogation of DEN/Fe-NTA induced carcinogenic response, oxidative damage and subsequent cell proliferation response by *Terminalia chebula* in kidney of Wistar rats. *Pharmazie* **62**, 790-797 (2007).
- 6) Bansal, A.K.; Bansal, M.; Soni, G.; Bhatnagar, D. Protective role of Vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. *Chem. Biol. Interact.* **156**, 101-111 (2005).
- 7) Rehman, M.U.; Tahir, M.; Khan, A.Q.; Khan, R.; Lateef, A. *et al.* Chrysin suppresses renal carcinogenesis via amelioration of hyperproliferation, oxidative stress and inflammation: Plausible role of NF- κ B. *Toxicol. Lett.* **216**, 146-158 (2013).
- 8) Pracheta, P.; Sharma, V.; Singh, L.; Paliwal, R.; Sharma, S. *et al.* Chemopreventive effect of hydroethanolic extract of *Euphorbia neriifolia* leaves against DENA-induced renal carcinogenesis in mice. *Asian Pacific J. Cancer Prev.* **12**, 677-683 (2011).
- 9) Iqbal, M.; Shah, M.D.; Vun-Sang, S.; Okazaki, Y.; Okada, S. The therapeutic potential of curcumin in alleviating N-diethylnitrosamine and iron nitrilotriacetate induced renal cell tumours in mice via inhibition of oxidative stress: Implications for cancer chemoprevention. *Biomed. Pharmacother.* **139**, 111636 (2021).
- 10) Rezaie, A.; Fazlara, A.; Karamolah, M.H.; Shahriari, A.; Zadeh, H.N.; Pashmforosh, M. Effects of *Echinacea purpurea* on hepatic and renal toxicity induced by diethylnitrosamine in rats. *Jundishapur J. Nat. Pharm. Prod.* **8**(2), 60-64 (2013).
- 11) Singh, D.; Chaudhary, D.; Kumar, V.; Verma, A. Amelioration of diethylnitrosamine (DEN) induced renal oxidative stress and inflammation by *Carissa carandas* embedded silver nanoparticles in rodents. *Toxicol. Rep.* **8**, 636-645 (2021).
- 12) Poornima, K.; Gopalakrishnan, V.K. Anticancer activity of *Tabernaemontana coronaria* against carcinogen induced clear cell renal cell carcinoma. *Chinese J. Biol.* **2014**, 1-8 (2014).
- 13) Umemura, T.; Sai, K.; Takagi, A.; Hasegawa, R.; Kurokawa, Y. Oxidative DNA damage, lipid peroxidation and nephrotoxicity induced in the rat kidney after ferric nitrilotriacetate administration. *Cancer Lett.* **54**, 95-100 (1990).
- 14) Tsao, B.; Curthoys, N.P. The absolute asymmetry of orientation of gamma-glutamyltranspeptidase and aminopeptidase on the external surface of the rat renal brush border membrane. *J. Biol. Chem.* **255**, 7708-7711 (1980).
- 15) Khan, N.; Sultana, S. Chemomodulatory effect of *Ficus racemosa* extract against chemically induced renal carcinogenesis and oxidative damage response in Wistar rats. *Life Sci.* **77**, 1194-1210 (2005).
- 16) De Freitas, J.M.; Meneghini, R. Iron and its sensitive balance in the cell. *Mutat. Res. Fund. Mol. Mech. Mutagen.* **475**, 153-159 (2001).

- 17) Hirayama, T.; Tsuboi, H.; Niwa, M.; Miki, A.; Kadota, S. *et al.* A universal fluorogenic switch for Fe(II) ion based on N-oxide chemistry permits the visualization of intracellular redox equilibrium shift towards labile iron in hypoxic tumor cells. *Chem. Sci.* **8**, 4858-4866 (2017).
- 18) Li, J.N.; Kang, S.H.; Yang, D.M.; He, Q.; Li, S.; Tao, S.L. Effects of substituting dietary alfalfa meal with kudzu vine (*Pueraria lobata*) meal on serum biochemical indexes, apparent nutrient digestibility and growth performance in Boer crossbred goats. *Acta Prataculturae Sin.* **30**(8), 146-153 (2021).
- 19) Koyama, T.; Yoshinaga, M.; Maeda, K.; Yamauchi, A. Room temperature reductions in relation to growth traits of kudzu vine (*Pueraria lobata*): Experimental quantification. *Ecol. Eng.* **70**, 217-226 (2014).
- 20) Chang, B.Y.; Lee, D.S.; Lee, J.K.; Kim, Y.C.; Cho, H.K.; Kim, S.Y. Protective activity of kudzu (*Pueraria thunbergiana*) vine on chemically-induced hepatotoxicity: *In vitro* and *in vivo* studies. *BMC Complement. Altern. Med.* **16**, 39 (2016).
- 21) Waldron, G.E.; Larson, B.M.H. Kudzu vine, *Pueraria montana*, adventive in Southern Ontario. *Can. Field-Naturalist* **126**, 31-33 (2012).
- 22) Kirakosyan, A.; Kaufman, P.B.; Chang, S.C.; Warber, S.; Bolling, S.; Vardapetyan, H. Regulation of isoflavone production in hydroponically grown *Pueraria montana* (kudzu) by cork pieces, XAD-4, and methyl jasmonate. *Plant Cell Rep.* **25**, 1387-1391 (2006).
- 23) Pandey, P.; Bhatt, P.C.; Rahman, M.; Patel, D.K.; Anwar, F. *et al.* Preclinical renal chemo-protective potential of *Prunus amygdalus* Batsch seed coat via alteration of multiple molecular pathways. *Arch. Physiol. Biochem.* **124**, 88-96 (2018).
- 24) Verma, A.; Ahmed, B.; Anwar, F.; Rahman, M.; Patel, D.K. *et al.* Novel glycoside from *Wedelia calendulacea* inhibits diethyl nitrosamine-induced renal cancer via downregulating the COX-2 and PEG2 through nuclear factor- κ B pathway. *Inflammopharmacology* **25**, 159-175 (2017).
- 25) Berton, T.R.; Conti, C.J.; Mitchell, D.L.; Aldaz, C.M.; Lubet, R.A.; Fischer, S.M. The effect of skin vitamin E acetate on ultraviolet-induced mouse skin carcinogenesis. *Mol. Carcinog.* **23**, 175-184 (1998).
- 26) Askari, F.; Azadbakht, M.; Gholami, S.; Akbari, F.; Shaki, F. *et al.* Protective effects of *Phyllanthus emblica*, *Acorus calamus*, and *Chelidonium majus* in an animal model of cataract. *J. Maz. Univ. Med. Sci.* **31** (200), 1-10 (2021).
- 27) Sinha, A.K. Colorimetric assay of catalase. *Anal. Biochem.* **47**, 389-394 (1972).
- 28) Sedlak, J.; Lindsay, R.H. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* **25**(C), 192-205 (1968).
- 29) Carlberg, I.; Mannervik, B. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *J. Biol. Chem.* **250**, 5475-5480 (1975).
- 30) Lawrence, R.A.; Burk, R.F. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem. Biophys. Res. Commun.* **71**, 952-958 (1976).
- 31) Sehrawat, A.; Khan, T.H.; Prasad, L.; Sultana, S. *Butea monosperma* and chemomodulation: Protective role against thioacetamide-mediated hepatic alterations in Wistar rats. *Phytomedicine* **13**, 157-163 (2006).
- 32) Malyszko, J.; Tesarova, P.; Capasso, G.; Capasso, A. The link between kidney disease and cancer: complications and treatment. *Lancet* **396**, 277-287 (2020).
- 33) Pan, K.; Ullah, I.; Mussarat, S. *et al.* A systematic review on ethnomedicines of anti-cancer plants. *Phytother Res.* **31**, 202-264 (2017).
- 34) Roy, A.; Ahuja, S.; Bharadvaja, N. A review on medicinal plants against cancer. *J. Plant Sci. Agric. Res.* **2**, 8 (2017).
- 35) Verma, A.; Bhatt, P.C.; kaithwas, G.; Sethi, N.; Rashid, M. *et al.* Chemomodulatory effect *Melastoma malabathricum* Linn against chemically induced renal carcinogenesis rats via attenuation of inflammation, oxidative stress, and early markers of tumor expansion. *Inflammopharmacology* **24**, 233-251 (2016).
- 36) El-Desouky, M.A.; Mahmoud, M.H.; Riad, B.Y.; Taha, Y.M. Nephroprotective effect of green tea, rosmarinic acid and rosemary on N-diethylnitrosamine initiated and ferric nitrilotriacetate promoted acute renal toxicity in Wistar rats. *Interdiscip. Toxicol.* **12**, 98-110 (2020).
- 37) Kostić, D.A.; Dimitrijević, D.S.; Stojanović, G.S.; Palić, I.R.; Dordević, A.S.; Ickovski, J.D. Xanthine oxidase: Isolation, assays of activity, and inhibition. *J. Chem.* **2015**, 294858 (2015).
- 38) Kapoor, N.; Saxena, S. Xanthine oxidase inhibitory and antioxidant potential of Indian Muscodor species. *3 Biotech* **6**(2), 248 (2016).
- 39) Pandey, P.; Bhatt, P.C.; Rahman, M.; Patel, D.K.; Anwar, F. *et al.* Preclinical renal chemo-protective potential of *Prunus amygdalus* Batsch seed coat via alteration of multiple molecular pathways. *Arch. Physiol. Biochem.* **124**, 88-96 (2018).
- 40) Siddiqi, A.; Hasan, S.K.; Nafees, S.; Rashid, S.; Saidullah, B.; Sultana, S. Chemopreventive efficacy of hesperidin against chemically induced nephrotoxicity and renal carcinogenesis via amelioration of oxidative stress and modulation of multiple molecular pathways. *Exp. Mol. Pathol.* **99**, 641-653 (2015).
- 41) Rashid, S.; Ali, N.; Nafees, S.; Hasan, S.K.; Sultana, S. Amelioration of renal carcinogenesis by bee propolis: A chemo preventive approach. *Toxicol. Int.* **20**, 227-234 (2013).

- 42) Ahmad Lone, I.; Sarwar Alam, M.; Athar, M. Amelioration of diethylnitrosamine (DEN)/ferric nitrotriacetate (Fe-NTA) mediated renal carcinogenesis by dietary silymarin-- A mechanistic approach. *Free Radic. Biol. Med.* **87**, S68-S69 (2015).
- 43) Sharmila, R.; Sindhu, G. Modulation of angiogenesis, Proliferative response and apoptosis by β -sitosterol in rat model of renal carcinogenesis. *Indian J. Clin. Biochem.* **32**, 142-152 (2017).
- 44) Talalay, P. Chemoprotection against cancer by induction of Phase 2 enzymes. *BioFactors* **12**, 5-11 (2000).
- 45) Anwar, F.; Al-Abbasi, F.A.; Bhatt, P.C.; Ahmad, A.; Sethi, N.; Kumar, V. Umbelliferone β -D-galactopyranoside inhibits chemically induced renal carcinogenesis via alteration of oxidative stress, hyperproliferation and inflammation: Possible role of NF- κ B. *Toxicol. Res. (Camb)* **4**, 1308-1323 (2015).
- 46) Paliwal, R.; Sharma, V.; Pracheta; Sharma, S.; Yadav, S.; Sharma, S. Anti-nephrotoxic effect of administration of *Moringa oleifera* Lam in amelioration of DMBA-induced renal carcinogenesis in Swiss albino mice. *Biol. Med.* **3**(2), 27-35 (2011).

CC BY-SA 4.0 (Attribution-ShareAlike 4.0 International). This license allows users to share and adapt an article, even commercially, as long as appropriate credit is given and the distribution of derivative works is under the same license as the original. That is, this license lets others copy, distribute, modify and reproduce the Article, provided the original source and Authors are credited under the same license as the original.

