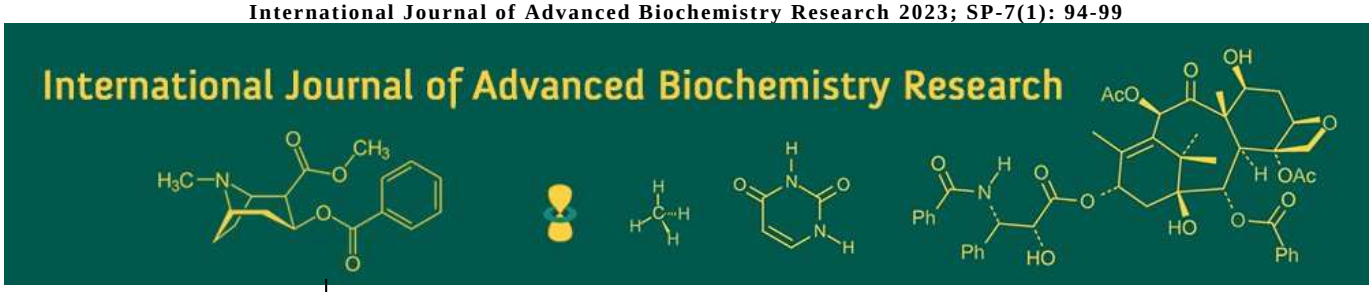

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The potential ameliorative role of naringenin against 5-Fluouracil induced nephrotoxicity toxicity

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Abstract
5-Fluouracil-induced nephrotoxicity causes alterations in renal markers and kidney morphology by increased oxidative stress, inflammation and apoptosis play an important role in the pathogenesis of kidnry injury. The present study was assessed to mitigative action of naringenin (NG) powder in 5-FU induced toxicity. We divide forty-eight animals into 4 groups (n=12) and sacrificed on 14th and 28th day and reported that NG treatment significantly decrease in blood urea nitrogen-BUN and creatinine value, lipid peroxidation and inflammatory markers - tumour necrosis factor (TNF-α), interleukin -1β (IL-1β), Nuclear factor Kappa beta (NF-κB) with increased concentrations of interleukin-10 (IL-10), albumin along with increase in endogenous antioxidant enzymes-superoxide dismutase (SOD) and glutathione peroxide (GSH). In addition to theses, histological alterations also clarified that 5-FU-induced distorted renal corpuscles and tubules with inflammatory cell infiltrations and severe congestion were decreased by NG treatment. Our results suggest that NG have ameliorative action against 5-FU-induced nephrotoxicity, however, NG on 28th day much better results than 14th day in all parameters.

Keywords: Nephrotoxicity, naringenin, creatinine, 5-fluouracil

Introduction
Cancer is a life-threatening disease posing challenges to the scientific community today, in spite of significant advancements in biological sciences (Rossi *et al.*, 2023) [1]. Several anticancer drugs are being used to inhibit proliferation of cancer cells (Alfarouk *et al.*, 2015) [2]. Under ideal conditions, anticancer treatments would destroy cancer cells without causing harm to healthy tissues. However, there is currently not even a single substance is totally safe without toxic effects (Rossi *et al.*, 2021) [3]. The fluoropyrimidine antimetabolite known as 5-FU was developed in 1957 by Heidelberg *et al.* and has been used clinically for the past 40 years, second most commonly used agent to treat a variety of solid tumours due to its broad antitumor activity and its synergism with other medications (Miura *et al.*, 2010) [4]. It exerts its effect through disrupts the metabolism of nucleosides in the malignant cell and incorporates them into DNA and RNA during S phase by inhibiting thymidylate synthase leading to cytotoxicity and cell death (Chibber, 2012) [5].
Apart from its benefits, 5-FU chemotherapy causes implications in a organs of patients like emesis, mucositis and organ toxicities-cardio, hepato and nephrotoxicity (Lamberti *et al.*, 2014) [6] Furthermore, previous studies observed that 5-FU is catabolized α-fluoro-β-alanine (FBAL), urea, ammonia and carbon dioxide resulting nephrotoxicity (Rashid *et al.*, 2014; Saif *et al.*, 2009) [8, 7]. In addition to it, 5-FU also responsible to increase oxidative stress, apoptosis and capable of damaging biomolecules, altered blood flow through disturbed renal tubules and decreasing the activity of antioxidant enzymes through stimulation of altered signaling pathways (Arab *et al.*, 2018, Badawoud *et al.*, 2017, Rashid *et al.*, 2014) [9, 10, 8]. Recent evidence shows overexpression of inflammatory mediators such as TNF-α, NF-κB and interleukins are markers in 5-FU-injected rats (Al-Asmari *et al.*, 2016) [11].
NG is an orally available natural phytochemical with anti-oxidant, anti-inflammatory (Regugadevi and prabu, 2009) [12] and anti-apoptotic property through activating intracellular anti-oxidant by inhibiting NF-κB pathway (Chen *et al.*, 2012) [13], reduce oxidative stress (Renugadevi and Prabu, 2009) [12] and it helps in an effort to further investigate beneficial potential of its use in clinical cancer treatments, we studied its effects against 5-FU-induced nephrotoxicity in rats.

We hypothesised that NG would protect against 5-FU-induced nephrotoxicity *via* anti-oxidant and anti-inflammatory nuclear factor-kappa B (NF-κB) pathway.

Materials and Methods

Chemicals: All chemicals are procured from Qualigens Private limited, India (Mumbai) and SRL Private limited, India. 5-FU was procured from celon laboratories private limited, Hyderabad, India. Naringin (CAS No: 10236-47) was obtained from Sigma (SAC-St Louis, MO, USA).

Experimental animals and design. *Wistar* rats (n=48), weight (180-210 grams) at 3 months age included in this study were acquired from jeeva life sciences (ISO 9001:2015 certified company) With 10 days acclimatization prior to study, embeded in adequate moisture and controlled temperature at 70% Humidity, 12hours (h) light, 12 h dark, 25 °C temperature for experimental study with standard chow and water. The study was authorized by Animal Ethics Committee (No.9/24/C.V.Sc., Hyd. IAEC-Rats/ 12.06.2021). The rats were assigned to experimental four groups (N=12), Group I: control-received normal saline. Group II: 5-FU (20 mg/kg b. wt five intaperitoneal injections 1-5 days). Group III -NG 100 mg/kg b. wt orally. Group IV rats received both NG and 5-FU. The toxic dose for 5-FU-induced nephrotoxic was based previous literature (Arab *et al.,* 2018). Rats were anaesthetized on 14th and 28th day of sacrifice and blood samples were collected and removed the kidneys. Blood, followed by kidney tissue samples were collected for various analysis-oxidative stress parameters and inflammatory cytokines and histopathological examination was conducted.

Blood sample collection and determination of kidney function indices

Blood was collected into heparinized tubes and centrifuged for 10 minutes (min), used to evaluate BUN and creatinine levels. Quantitative estimation of the renal function markers-urea, BUN and creatinine was carried out in serum stored at 4 °C using commercial kits by RANDOX following manufacturer's instructions.

Oxidative stress parameters:

The kidney tissues were collected and transferred to eppendorf tubes and stored at -80 °C until further biochemical parameters were measured. Renal tissues were homogenized, centrifuged and super anted was collected to examined levels of MDA (Balasubramanian, *et al.,* 1988) [15], total protein (lowry *et al.,* 1951) [14] and antioxidant enzymes SOD (Madesh and Balasubramanian, 1998) [16], GSH (Ellman, 1959) [17] in the renal tissue homogenate.

Inflammatory cytokines

We assessed the levels of Renal tissue inflammatory cytokines (TNF-α, IL-6 and IL-10 were measured in renal tissue homogenates using ELISA kits; absorbance was recorded spectrophotometrically at 450 nm using the ELISA microplate reader. The results were expressed as mean ± standard deviation.

Histopathology

A 10% formalin solution was used to preserve the kidney for 48 h, rinsed under running water, then tissue were encased in paraffin blocks, sections were cut 5 µm thickness using a microtome device, dyed and observe under Leica DM microscope.

Statistical analysis

All data were presented as the mean standard error of mean (±SEM). Analysis of variance with Dunnett posthoc testing was carried out to determine differences in parameters between variables (Snedecor and Cochran, 1994) [18].

Results

Evaluation of oxidative stress parameters and inflammatory cytokine strom, there is no significant difference between group-1 (control) and group-3 (NG), used as comparison with other groups.

Serum biomarkers

There was a considerable increase in values of Creatinine and BUN and with decreased albumin in toxic group were observed. According to Fig. 1, there was a significant (P<0.05) decrease in these values of concentration due to anti-oxidant property in group-4 with increase in albumin concentration (Fig. 1).

Oxidative stress

When compare to control group, there was a significant increase in MDA value, which indicate that renal tissue undergo lipid peroxidation. In addition to it, there was a lowered values of MDA is observed in group-4 rats due to anti-oxidant property (Fig. 2 A).

There was a significantly (P<0.05) lowered concentration of anti-oxidant enzymes-GSH and SOD levels were observed in group 2 rats. However, concentration of elevated levels of these enzymes were observed when treated with NG due to anti-oxidant property in group-4 rats (Fig. 2 B-C).

Inflammatory cytokines

Inflammatory cytokine levels (TNF-α, IL-1β and NF-κB) in renal tissue homogenate were found in the group-2 to be considerably (P 0.05) higher in concentration than the control group-1 rats (Fig. 3 A-C). Due to NG treatment, group 4 rats showed concentrations of lowered levels of these enzymes. Similarly concentration of IL-10 levels were increased in group-4 rats when compared with group-2 rats (Fig. 3 D).

Histopathology

The kidney sections of group 1and group 3 rats showed normal histological details of renal parenchyma on 14th and 28th day of experiment (Fig. 4 A, C). The kidney sections of group 2 on 14th day showed swollen to shrunken glomeruli and congestion of periglomerular blood vessles, mild tubular epitheliual degeneration, increase in Bowmen's capsule space, mild cystic dilatation, diffuse infiltration of MNCs (Fig.4 B). On 28th day of experiment of group 2 rats showed same lesions as 14th day but changes are severe with MNCs (interstitial nephritis) degeneration of tubular epithelium, cystic dilatations and periglomerular haemorrhages (Fig.4 E-H).

In group 4, the kidney sections revealed swollen and shrunken glomeruli, mild dilation of tubules, mild degeneration of tubular epithelium and infiltration of MNCs in the interstitial spaces. On 28th day, most of the kidney sections showed normal glomeruli and reconstructive appearance of tubules (Fig. 4 I).

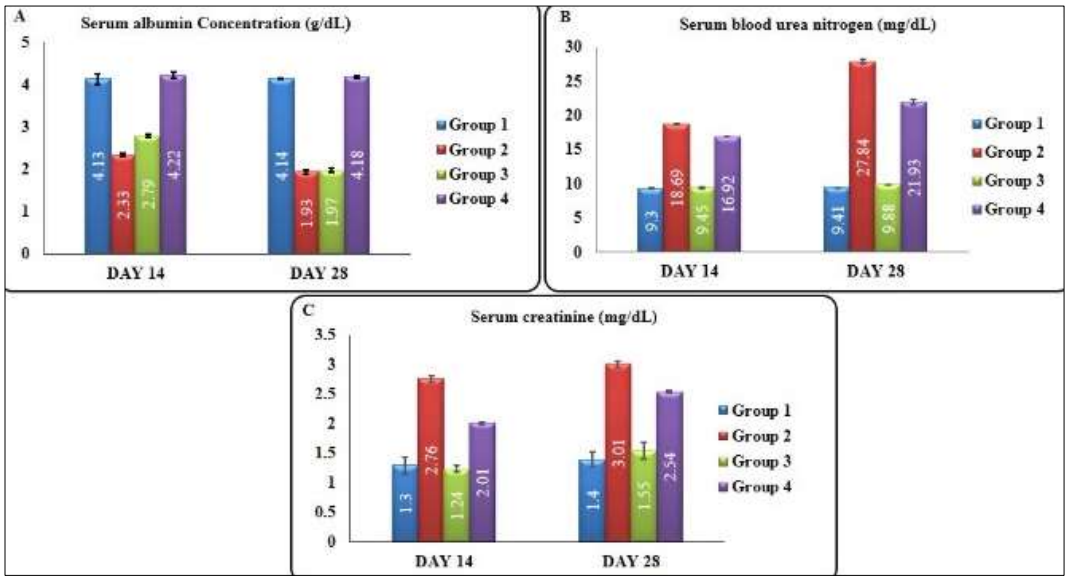

Fig 1: Effect of NG on serum kidney markers. A-albumin, B-BUN, C-creatinine.

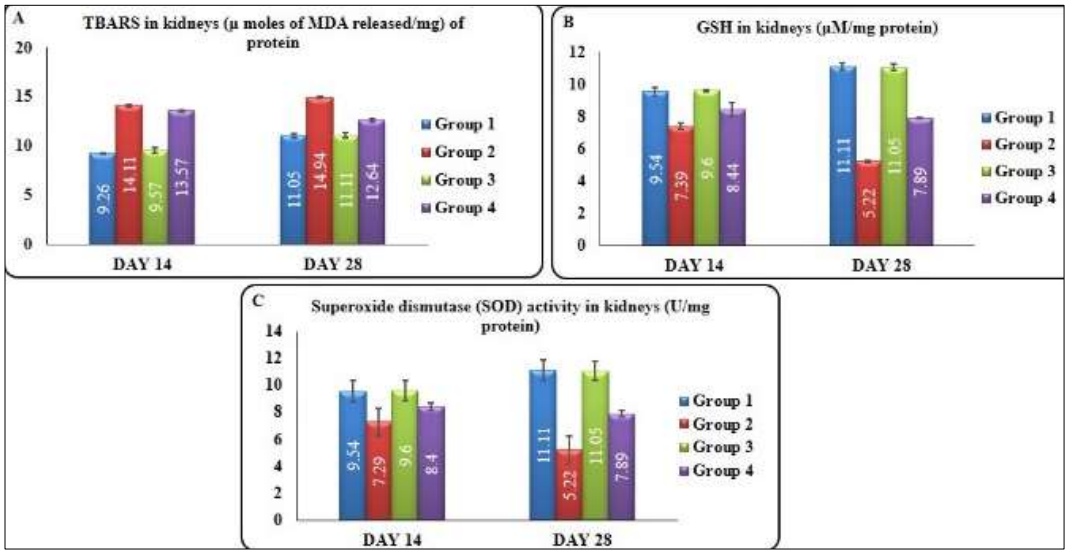

Fig 2: Effect of NG on oxidative stress indices on 14th and 28th day interval

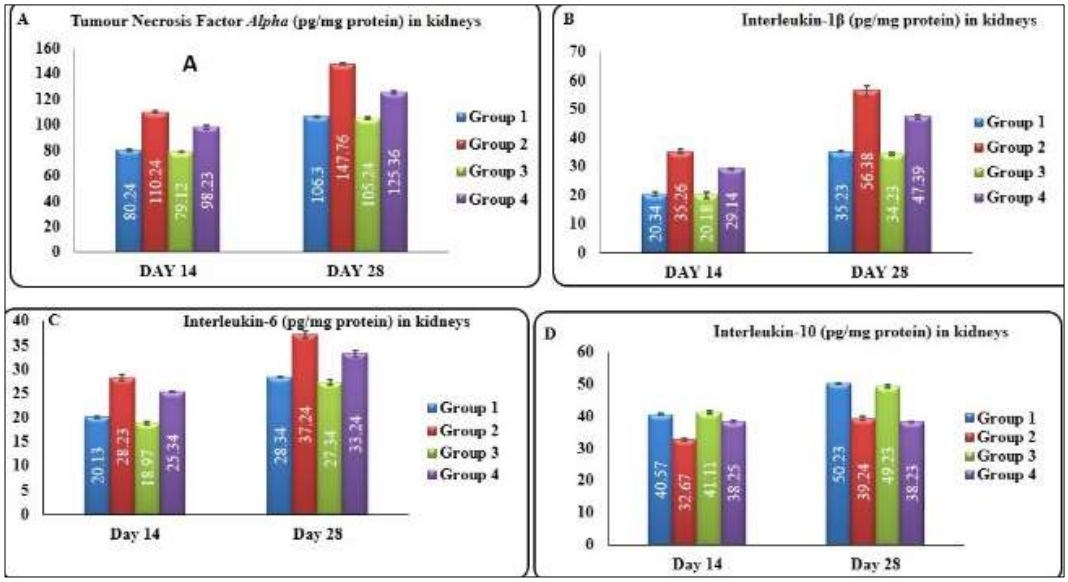

Fig 3: Ameliorative effect of NG on inflammatory cytokine strom at different intervals

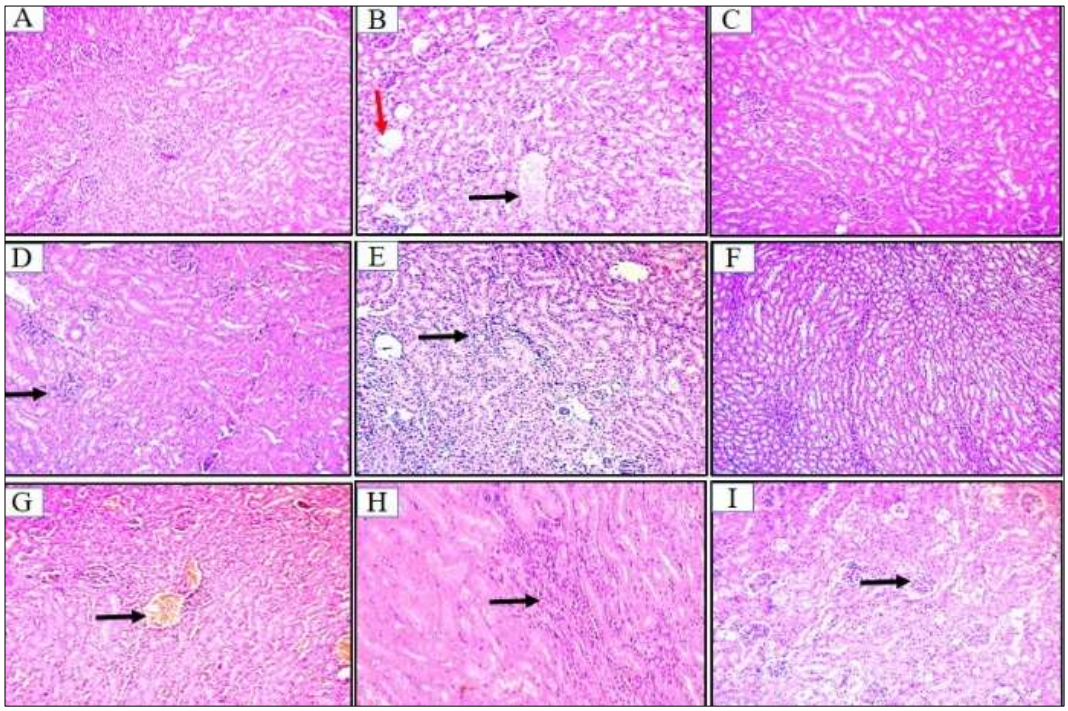

Fig 4: Microscopic pictures of kidney tissue.

Discussion

5-FU, an anticancer drug, is the third most commonly associated with organ toxicity due to ROS production (Sara *et al.*, 2018) [19]. A promising strategy for reducing the nephrotoxic side effects of chemotherapy based on the 5-FU alternative ameliorative drug NG may be demonstrated to have significant success with add-on usage with it. In the current experimental study we observed the ramifications of research, which gives an idea of the ability of NG to mitigate 5-FU-induced nephrotoxicity in this section. NG exerts its nephroprotective properties and aids in maintaining the redox balance within renal cells by neutralizing ROS production, preventing oxidative stress and preserving cellular integrity. The anti-inflammatory properties of NG also prevent the synthesis of pro-inflammatory cytokines, which lessens the inflammatory response and subsequent tissue damage in the kidneys.

5-FU, an antimetabolite, has two metabolic pathways (Maring *et al.*, 2002) [20].

1. Primarily in anabolic metabolism, it inhibits thymidylate synthase, which is a deficiency of thymidine, in addition, it further causes cytotoxic cell death by interfering with the synthesis of DNA and causing cell death (Miura *et al.*, 2021) [4]
2. Secondly, as a catabolic process, it converted into Flurocitrate and inhibits krebs cycles, and decreases ATP production, further causing cell death. It also affects iron homeostasis, which is responsible for ROS production *via* fenton reaction (Yousef and Aboelwafa, 2017) [21].
3. Also convereted into alpha fluro beta alanine, ammonia and co_2 in liver, reaches the kidney which is the main reason to cause nephrotoxicity by 5-FU (Li *et al.*, 2022) [22].

The disturbance in levels of BUN and creatinine in elevated and lowered in albumin concentration in present study are suggestive of renal dysfunction, which implies glomeruli, which were positively correlated in the present study through histopathological changes. These similar findings were observed by Rashid *et al.* 2014 [8] and reported that intraglomerular blood flow alteration, further disturbance in GFR, results in hemodynamic imbalance between vasoconstrictive and endothelial permeability interactions, causing increases in creatinine and urea, due to 5-FU nephrotoxicity associated with direct drug metabolites FBAL might induce tubular and glomerular damage followed by activation of pro-inflammation, oxidative stress, apoptosis and retard renal ultra renal filtration might be cause for elevated BUN and creatinine levels and lowered albumin value by Rashid *et al.* (2014) [8]; Badawoud *et al.* (2017) [10]; Arab *et al.* (2018) [9] and Famurewa *et al.* (2019) [23].

Reactive oxygen species (ROS) and oxidative stress have previously been mentioned as contributing factors to the multiorgan toxicity caused by 5-FU (Diba *et al.*, 2021) [24] through interfering with the mitochondrial electron transport chain and impairing kidney redox status (Adikwu, 2019) [25]. This finding was confirmed by decreasing kidney antioxidant status-GSH and SOD along with increase in MDA levels in our study. Thus, reduce the ability to scavenge free radicals and cause oxidative stress (Gelen *et al.*, 2017) [26]. Lipid peroxide (MDA), a reliable marker for assessment of oxidative stress is significantly increased, SOD and GSH are the cellular antioxidant enzymes, essential in reducing tissue damage brought on by free radicals, which result in cellular damage and necrosis of kidneys, which are decreased which are responsible for 5-FU nephrotoxicity (Gulcin and Beydemir, 2013) [27] This results coincides with previous studies (Rashid *et al.*, 2014) [8]; Aikemu *et al.* (2016) [28]; Arab *et al.* (2018) [9] and Sengul *et al.* (2021) [29]. Whereas, NG ameliorates increasing oxidative stress through scavenging free radicals with consequent suppression of hydroxyl radical generation produced by 5-FU and ameliorative effect is on 28^{th} is more compare to 14^{th} day indicating the chronic protective effect of NG, these similar findings are also in agreement with previous studies (Pari and Amudha, 2011; Turgut *et al.* 2016) [30, 31].

The current findings revealed that 5-FU instigated robotics inflammatory cytokines by increasing in inflammatory cytokines-TNF-α, IL-1β, NF-κB and decreasing in anti-inflammatory cytokines which was evidenced in 5-FU toxic

group. Previous reports have evidenced that particularly NF-κB are involved in 5-FU induced kidney injury (Rashid *et al.*, 2014) [8] translocates to the nucleus and transcription of inflammatory genes-TNF-α and IL-1β. The current research showed that NG efficiently inhibited the proinflammatory and NF-κB pathways, demonstrating its extensive anti-inflammatory IL-10 levels in group-4 suggesting that NG has anti-inflammatory effect (Mahmoud *et al.*, 2013) [32]. These results are in harmony with the previous studies through Nrf2 pathway (Chen *et al.*, 2012) [13].

In addition to alteration to these above changes in serum biomarkers, oxidative stress and inflammation, histopathological changes observed are severs degenerated renal, swollen to shrunken glomeruli, mild tubular epithelium degeneration, focal areas of mild intertubular haemorrhages and increase in Bowmen's capsule space, mild cystic dilatation and diffuse infiltration of MNCs on 14th day of experiment. On 28th day, the sections revealed shrunken glomeruli with vacuolar degeneration with vacuoles, severe periglomerular congestion, interstitial nephritis, degeneration of tubular epithelium and intertubular haemorrhages. The structural and functional changes of kidneys in 5-FU group are positively complementing each other with respect to biochemical results (elevated mean values of BUN and serum creatinine) and oxidative stress parameters of present study and it might be due to excessive production of free radicals along with toxic metabolite production (FBAL, urea and ammonia) (Arab *et al.*, 2018 and Gelen *et al.*, 2021) [9, 33].

In group 4 rats, the kidney sections showed mild shrunken glomeruli and mild tubular changes were observed. This might be due to protective effect of NG antioxidant effect on 5-FU nephrotoxicity (Turgut *et al.*, 2016) [31].

Conclusion

Our research study gave an idea of comparing 5-FU treatment with NG to 5-FU treatment alone has consistently shown enhanced renal function through glomerular filtration rate (GFR), lower serum creatinine and blood urea nitrogen levels and maintain the overall structure of the kidneys along with increasing antioxidant status and decreasing in the inflammatory marked through NF-κB pathway more on chronic study (28 days) than 14 days. These results indicate that naringenin is essential for preventing nephrotoxicity against 5-FU. Hence we conclude that NG action is much better protective action in chronic studies.

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