ISSN: 2456-2912
VET 2023; 8(4): 296-300

www.veterinarypaper.com
Received: 15-04-2023
Accepted: 17-05-2023

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Ameliorative effect of naringenin on 5-fluorouracil induced toxicity on body weights, organ weights and gross pathology

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Abstract
The aim of current experimental study was to evaluate migitated effect of NG against 5-FLurouracil-induced toxicity on organ weights at different intervals. For this investigation, male rats (N=48, Albino *Wistar*) were employed, divided randomly into four (N=12) groups. Groups 1 and 3 each contained a control (normal saline) and ameliorative agent NG @ 100 mg/kg b.wt/day given orally for 28 days respectively, group-2-5-FU @ 20 mg/kg b.wt was injected intraperitoneal (IP) for first 5 days, group 4 injected 5-FU + NG P/O for 28 days and rats were sacrificed on 14th and 28th day of experiment. Body weights were individually on the 0th, 7th, 14th and 28th day. Soon after, necropsy, gross lesions were recorded along with organ weights. A significantly reduced in body and organ weights were recorded, whereas, NG group increases body and organ weights reduced by 5-FU induced toxicity along with restored organ damage due to the anti-oxidant effect.

Keywords: 5-Flurouracil, Naringenin, Gross-lesions, Body weights

1. Introduction
Chemotherapy is frequently employed to treat a number of cancer forms [1]. However, the administration of chemotherapy drugs can lead to harmful side effects in a variety of organs and tissues [2]. 5-FU a second most commonly used chemotherapeutic drug, widely employed in human biology due to its exceptional anticancer effects against various solid cancers including skin and breast neoplasm [3]. It acts in S phase and it is intracellularly converted into various metabolites inhibiting thromidylate synthase thus deficit in thymidine, preventing DNA synthesis along with RNA, ultimately leading to cell death through various intracellular signalling pathways [4]. It was reported that overproduction of reactive oxygen species and inflammatory mediators are well known to have a significant role in 5-FU-induced toxic symptoms [5-7].
Nowadays usage of compounds with anti-oxidants and anti-apoptotic with anti-inflammation properties reduce adverse effects caused by 5-FU like NG. It has an anti-oxidant property by donating Hydrogen ions along with endogenous production of antioxidant agents like glutathione, thus, terminating formation of reactive oxygen species [8, 9]. Hence the present experimental study was to evaluate the ameliorative effect of NG at different intervals.

2. Material and Methods
Healthy male rats (Albino Wistar, N=48, 3 moon age with weight 180-200 g), were procured from Jeeva Life Science (ISO 9001:2015 certified company), Hyderabad, India. Rats were acclimatized for 10 days with a temperature of 25±2 °C, dark and daylight (12:12) ratio, Relative humidity (45-55%) with a standard pellet diet and *ad libitum* deionized water throughout the current experimental period for 28 days. The Experiment was carried out according to the guidelines and prior approval of the Institutional Animal Ethics Committee (No. 9/24/C.V.Sc., Hyd. IAEC-Rats/ 12.06.2021).

2.1 Experimental design
In our present study, the ameliorative effects of NG against 5-FU-induced toxicity in rats were studied. Healthy adult male rats (N=48) were separated into four groups (N=12) in one group.

1. **Group-1:** Control- normal saline orally for 28 days.
2. **Group-2:** 5-FU @ 20 mg/kg b.wt for first 5 days-IP.
3. **Group-3:** NG (100 mg/kg b.wt) orally for 28 days.
4. **Group-4:** 5-FU for five days + NG for 28 days.

2.2 Body weights
Electronic weight balances were used to record the body weights of all animals were on 0th, 7th, 14th and 28th day to know the body weight gains.

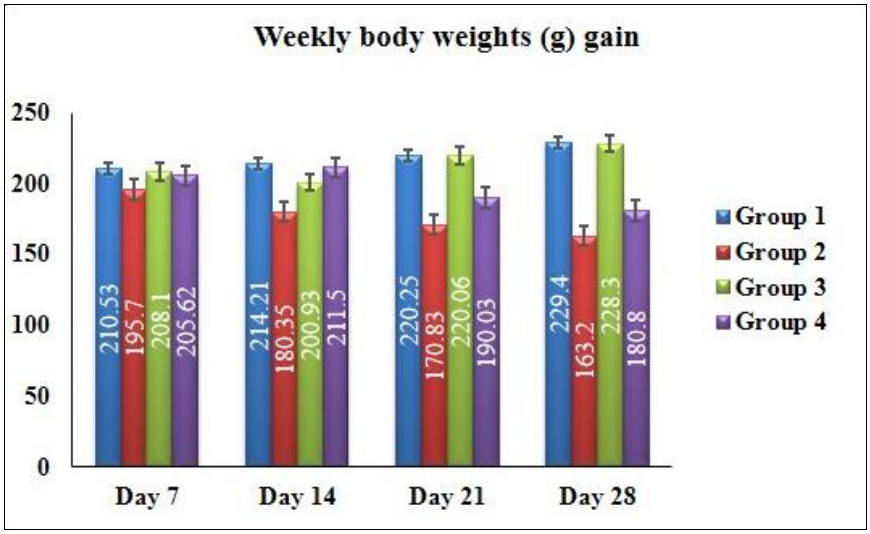
2.3 Organ weights
Organ weights of all animals were weighted using electronic balance on 14th and 28th day of experiment to access the ameliorative effect of NG on organ weights.

2.4 Gross pathology
Rats were sacrificed by using isoflurane (gaseous anaesthesia) on 14th and 28th day of experiment and gross lesions were recorded after detailed necropsy examination as per standard protocol given by Feinstein in lungs, liver and kidneys [10].

2.5 Statistical analysis
Data regarding weights were subjected to analysed using one way Analysis of variance (ANOVA) using statistical package for social sciences (SPSS) version 15.0. Duncan's multiple comparison tests were done for comparison among groups and significance level was set at $p<0.05$ [11].

3. Results and Discussion
In present study, clinical signs were observed in 5-FU treated rats (group 2) include dull and inactive and gathering themselves at one corner of the cage and mild to moderate diarrhoea was noticed. From the 3rd day onwards, appetite was greatly reduced, decreased water intake, decreased in body weights of rats, which might be due to atrophy of skeletal muscles and adipose tissue coupled with frequent diarrhea caused by interaction of 5-FU on intestinal epithelial cells. These similar findings were reported by Zhang *et al.* (2009) [17]; AI-Hamdany and AI-Hubaity (2014) [13]; Kadagi *et al.* (2014) [14] and Elghareeb *et al.* (2020) [7] in 5-FU induced toxicity [7, 12, 13]. Whereas in group 4 rats showed no clinical signs which might be due to protective effect of NG. The animals in group 1 and group 3 rats remained healthy throughout the experimental period.

3.1 Body weight (s)
In the present study, mean values were significantly ($p<0.05$) reduced in body weights of 5-FU treated rats, reason behind might be due to decrease in feed intake, atrophy of skeletal muscles and adipose tissue. There is also inhibition of RNA and DNA synthesis and stimulation of apoptosis by 5-FU in the highly proliferative organ like intestine leads to oral and intestinal mucositis leads to frequent diarrhoea along with painful conditions associated with inflammation and ulcerations of stomach and intestines further leads to difficulty in feed and water intake [14]. All above reasons are responsible for decrease in body weights of rats in 5-FU toxicity (Figure.1). These results are closely related to the findings of AI-Hamdany and AI-Hubaity (2014) [13]; Badawoud *et al.* (2017) [16] and Zheng *et al.* (2019) [13, 15-17].
Group 4 rats showed a significant increase in the mean values of body weights (Figure 1) might be due to protective effects of NG by scavenging free radicals generated and anti-inflammatory effects in 5-FU induced toxicity [8, 9].



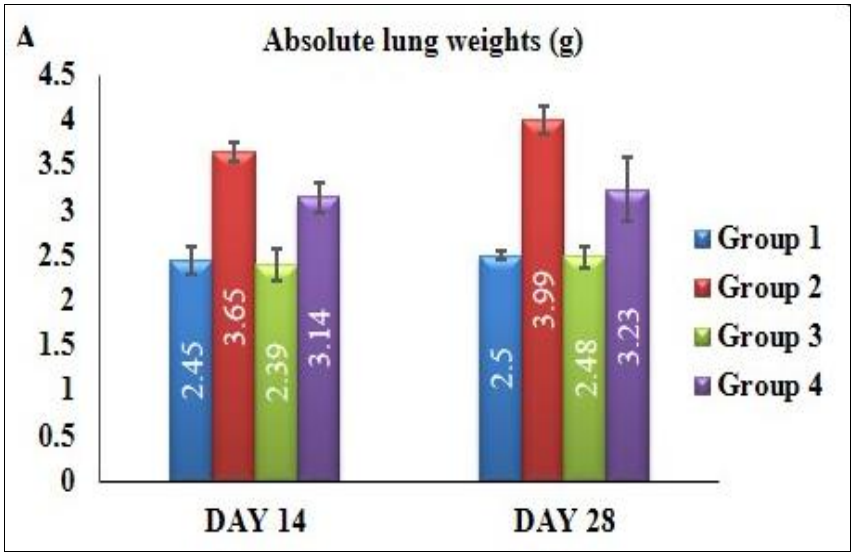
Values are Mean ± SE (n=6); One way ANOVA
Means with different superscripts in a column differ significantly at $p<0.05$ (*)

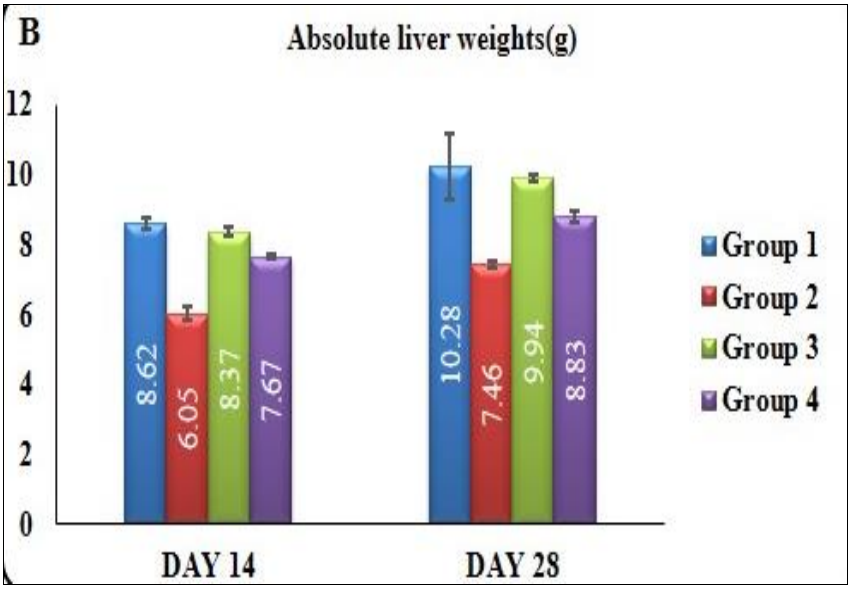
Fig 1: Weekly body weights

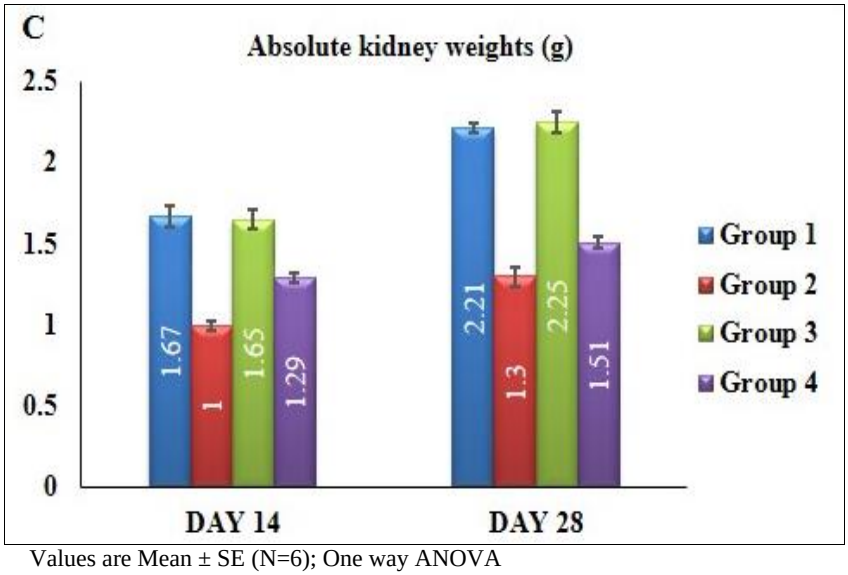
3.2 Organ weights
Significantly ($p<0.05$) elevated mean values of absolute lung weights were also recorded in 5-FU toxic group (Figure 2 A). Hence it is opined that increased lung weights could be due to increased ROS production and oedema formation due to destruction of surfactant-producing type II pneumocytes cells, which in turn results in increased surface tension, are in accordance with the observations of earlier studies [13, 18]. Significantly, lowered mean values of absolute lung weights were recorded in the group 4 rats when compared to group 2 rats on day 14th and 28th day which might be due to the antioxidant effect of NG will restored damaged lung tissue [19].
In the present study, a significant ($p<0.05$) decrease in mean values of absolute liver and kidney weights on 14th and 28th day and was noticed in group 2 rats when compared with groups 1 and 3 rats. The reduced mean values of liver weights might be due to hepatocyte degeneration, necrosis and hepatic enzyme leakage, which might have hampered the synthesis and utilization of enzymes. The decreased values of kidney

weights due to degeneration and necrosis of renal parenchyma, in agreement with the findings of Famurewa *and* it's coworkers [16, 20, 21].

Significantly higher mean values of absolute liver and kidney weights were recorded in group 4 rats when compared to group 2 rats on day 14th and 28th day which might be due to antioxidant effect of NG [22] (Figure 2 B-C).







Values are Mean ± SE (N=6); One way ANOVA
Means with different superscripts in a column differ significantly at $p<0.05$ (*)

Fig 2: Effect of NG on organ weights. A. lung weights B. Liver weights C. kidney weights

3.3 Gross pathology

The lungs of group 2 rats revealed gross lesions including mild congestion, haemorrhages and mild emphysematous areas of lungs on 14th day of experiment. On 28th day, lungs of group 2 rats showed severe haemorrhagic areas, oedema, congestion and emphysema of lungs which might be due to oxidative stress which was positively correlated with microscopic changes and oxidative stress parameters in the various study. The lungs of group 4 rats showed mild emphysema and focal petechial haemorrhages on lungs which might be due ameliorative effect of NG on pulmonary toxicity by reducing tissue oxidative damage [23] (Figure 3).

The liver and kidneys of group 2 rats in the present study showed mild to moderate congestion on 14th and 28th day of experiment (Figure 4. B, E). These observations are coinciding with the earlier study of AI-Hamdany and AI-Hubaity (2014) [13]. 5-FU induces liver and kidney damage due to overproduction of ROS and toxic intermediate metabolites- alpha fluoro beta alanine and ammonia in liver and kidneys [24]. In group 4 rats, there was a mild congestion of liver and kidneys on 14th and 28th day of experiment which might be due to due to protective effect of NG powder (Figure 4 D, F).

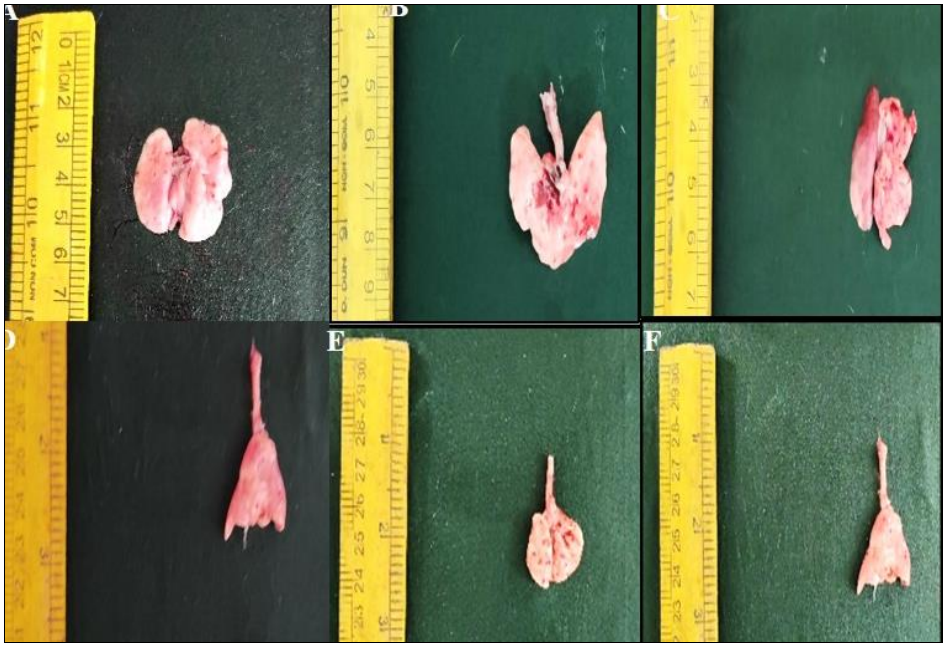


Fig 3: Gross lesion of lung. A, D. normal appearance of lung. B-C. Moderate congestion, emphysematous with petechial haemorrhages in 5-FU group on 14th day. D. Mild congestion of lungs on 14th day E. severe congestion of lungs with petechial haemorrhages. F. Mild congestion of lungs by restoration of damaged tissue in the combination group.

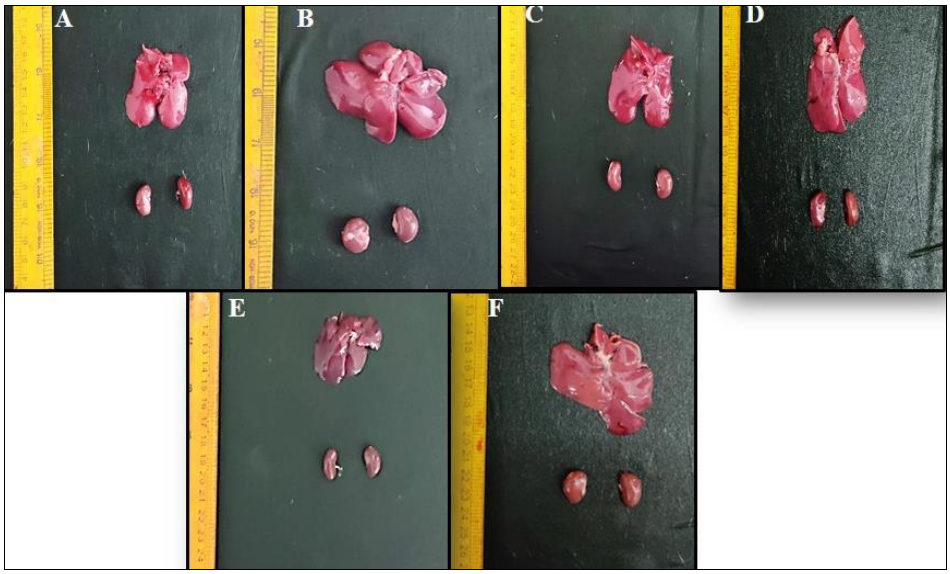


Fig 4: Gross lesion of liver and kidney. A, C. Normal appearance of liver and kidney. B. congestion of liver and kidney on 14th day. D. mild congestion of kidney and liver. E. severe congestion of liver and kidney. F. mild congestion of liver and kidney due to NG on 28th day.

4. Conclusion

5-FU causes a decrease in body weights, organ weight along with gross pathological lesions due to increasing oxidative stress. Whereas NG will ameliorative in decrease in tissue damage due to anti-oxidant and anti-apoptotic properties.

5. Acknowledgement

We want to thank to PV Narsimha Rao Telangana Veterinary University for smooth conduct of the clinical research by providing research facilities.

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