NARINGENIN AMELIORATES 5-FLUOROURACIL-INDUCED CARDIOTOXICITY IN *WISTAR* RATS

Sravathi Vemula[1], Jeevanalatha Mylaram[2], Ravikumar Yadala[3], Gopalareddy Alla[4], Anilkumar Banothu[5*], Durga Veera Hanuman Donga[6], Nagarjuna Gandham[7]

Abstract

The present study evaluated the ameliorative effects of Naringenin (NG) against 5-Flourouracil (5-FU) induced cardiotoxicity in *Wistar* rats. A total of 48 male rats of uniform size were divided into 4 groups of 12 animals each. Control group 1 received normal saline (NS) per oral (P/O), group 2 served as 5-FU toxic was injected intraperitoneal (IP) (@ 20 mg/kg b.wt.) for first 5 days, group 3 received NG orally (100 mg/kg b. wt/day) for 28 days and group 4 was injected IP with 5-FU + NG P/O for 28 days and sacrificed on 14th and 28th day of experiment. NG treatment significantly reduces increased left ventricular wall thickness, cardiac markers-lactate dehydrogenase (LDH), creatine kinase myocardial band (CK-MB), cardiac troponin-I (cTn-I) and C-reactive protein (CRP)in serum in group 2 on 14th day and 28th day of the experiment. On microscopic examination, severe congestion of blood vessels along with degeneration of cardiac myocytes were noticed. At the same time, mild tissue alteration was observed in NG treatment group. On ultrastructurally, in SEM, a mild decrease in degenerative changes in tissue structure was observed in group4 rats. In conclusion, the findings underscore the substantial potential of NG in mitigating 5-FU-induced cardiotoxicity.

Key words: Naringenin, myocarditis, fluorocitrate, cardiotoxicity

[1]Sravathi-Ph.D Scholar, Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India E-Mail ID: sravanthiraghu38@gmail.com
[2]Jeevanalatha- Associate Professor & Head, Department of Veterinary Pathology, College of Veterinary Science, Mamnoor-Warangal, Telangana, India E-Mail ID: drjeenalatha@gmail.com
[3]Ravikumar- Assistant Professor & Head, Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India E-Mail ID: ravikumaryadala@gmail.com
[4]Gopalareddy-Controller of Examinations, PVNRTVU, Hyderabad, Telangana, India E-Mail ID: gopalareddy123@rediffmail.com
[5]Anil Kumar Banothu-Assistant professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India E-Mail ID: anilvetpharma@gmail.com
[6]Hanuman-Ph.D Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India E-Mail ID: handy.hanu@gmail.com
[7]Nagarjuna-MVSc Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India E-Mail ID: nagarjunagandham94@gmail.com

***Corresponding author:** Anilkumar Banothu Email: anilvetpharma@gmail.com

Introduction

Cancer has remained a major life-threatening disease until today, despite significant advancements in biological sciences (Alfarouk et al. 2015). Due to early identification and

advancement in treatment, the number of cancer survivors has grown in combination with the rising frequency of cancer over the previous decade(De Moor et al. 2013). Despite these advancements, cancer-related cardiac dysfunction (CRCD), which is a 10% prevalence and one of the most-dreaded and annoying chemotherapeutic side effects, can persist even after 20 years of treatment(Cardinale et al. 2010). According to the World Health Organization (WHO, 2020), 17.8 million people die every year due to cardiovascular disease. Its prevalence is increasing due to recent changes in lifestyle, pollution, dietary habits and increased use of chemotherapeutic drugs. In a similar vein, the International Agency for Research on Cancer predicted 19.3 million new cancer cases and over 10 million deaths from cancer in 2020 (Sung et al. 2021).

5-FU is an anti-cancer, anti-metabolite drug that has been used in early,s since the 1957's (Heidelberger, 1965). It is a Fluropyrimidene analogue which is anaromatic heterocyclic agent with a structural similarity to that of a pyrimidine molecule of Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA) with an analogue of uracil and fluorine atom at 5^{th} position in the place of hydrogen (Miura et al. 2010) which is 2^{nd} most widely used in colorectal, breast stomach, head, neck and pancreatic (Sorrentino et al. 2012) and skin cancer treatment (Malet-Martino, 2002).5-FU is metabolized to several active metabolites by DPD: 5-Fluro deoxyuridine monophosphate (5-FdUMP) and 5-Flurouridine thymidine monophosphate (5-FUTP)-these active metabolites disrupt the action of TS and RNA and DNA synthesis resulting in DNA and RNA damage (Longley et al. 2003).

The common chemotherapeutic side effects of 5-FU are emesis, diarrhoea, myelosuppression, mucositis and 3^{rd} most commonly used drug associated with cardiotoxicity (Sengul et al. 2021;Jyothi et al. 2010), pulmonary toxicity (Gedikli and Erbas, 2021; Maimonaparveen et al. 2021), hepatotoxicity (Pawankalyan et al. 2022; Vanisthasree et al. 2011), nephrotoxicity (Rashid et al. 2014) and hematotoxicity(Sravathi et al. 2022;Manvitha et al. 2019). Cardiotoxicity is the third most common toxicity of 5-FU, with an incidence of 1.2-7.6% (Kanduri et al. 2019). Clinical symptoms like myocardial infarction, dyspnea, left ventricular hypertrophy, cardiac arrest and death occur suddenly caused by 5-FU (Alter et al. 2006).

5-FU-induced cardiotoxicity includes myocardial infarction (MI), inflammatory reaction leading to fibrosis of interstitium, coronary artery spasm (Mosseri et al. 1993), and endothelial injury followed by thrombosis (Tsibiribi et al. 2006). Myocardial necrosis and ischemia, disruption of the tricarboxylic acid cycle (TCA) within cardiomyocytes and decreased ability of red blood cells (RBC) to transfer oxygen resulting in myocardial ischemia (Spasojević et al. 2008). 5-FU toxic effects also showed increased superoxide anion (O^{2-}) levels and a reduced antioxidant capacity due to oxidative stress, lipid peroxidation (LPO), apoptosis, DNA damage, and pro-inflammatory cytokine oxidation by increased NF-kB activity. (Sengul et al. 2021) (Fig**.**1).

Many studies have been focused today on naturally occurring compounds with high phytochemical content, such as Curcumin, Rutin, Resveratrol (RES), Vincamine (Renushe et al. 2022),Tropoline derivatives and NG, which offer a pool of antioxidants and anti-apoptotic capabilities to increase their effectiveness in lowering free radicle-induced damage by highlighting bioactive constituents. NG is a flavonoid in citrus fruit, grapes, oranges and tomatoes and is very lipophilic, readily absorbed through the intestine than naringin(Harish et al. 2022; Igual et al. 2013). It has protective effects against cardiotoxicity, pulmonary (Gedikli and Erbas, 2021), nephrotoxicity (Gelen et al. 2017) and hepatotoxicity through an anti-oxidant, anticancer and immune-modulatory along with anti-inflammatory properties (Nie et al. 2012). No studies have been done on the impact of NG on 5-FU-induced cardiotoxicity except naringin,

which revealed microscopical alteration. Therefore, the present study aimed to explore possible cardioprotective mechanisms of NG by considering ultrastructural changes along with cardiac biomarkers against 5-FU-induced cardiotoxicity in rats at different weekly intervals.

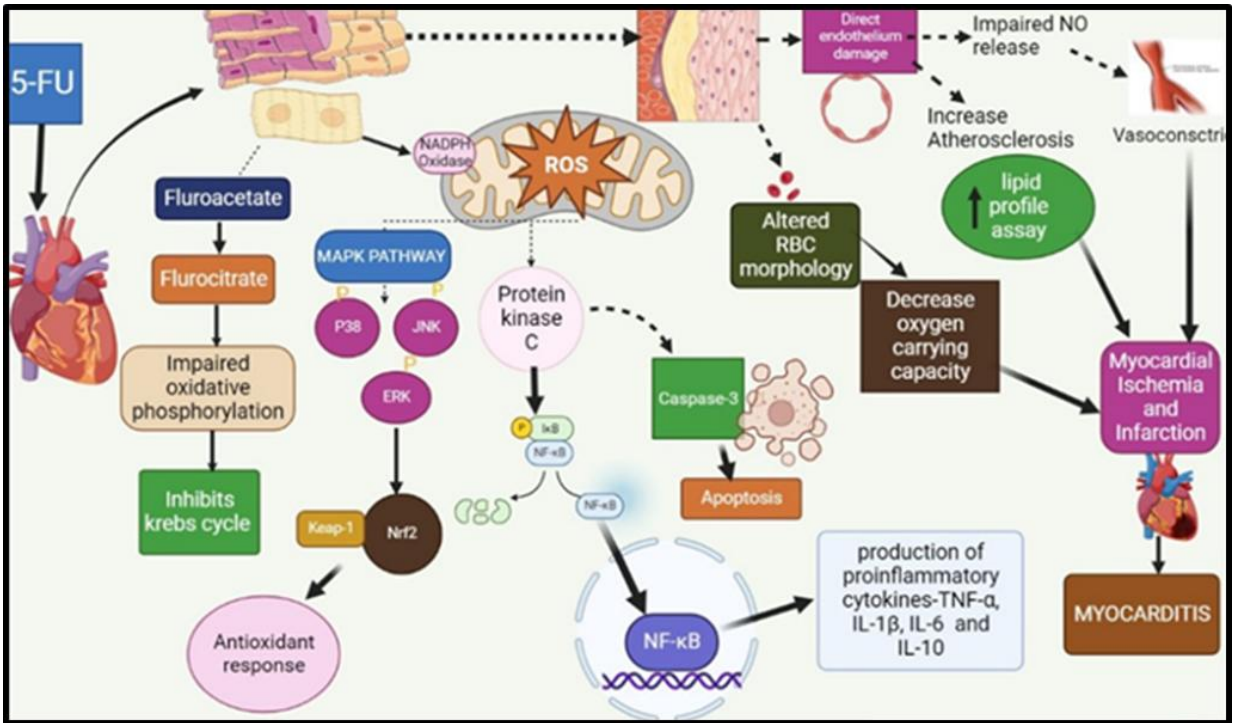

Fig. 1 Molecular mechanism of 5-FU induced cardiotoxicity. 5-FU converted flurocitrate by inhibitingthe Krebs cycle, altering RBC morphology to further myocardial ischemia, activatingthe NF-κB signaling pathway, and stimulating inflammation cause myocarditis. ERK-extracellular signal-regulated kinase, JNK-Jun N-terminal kinase, Nrf2-nuclear factor erythroid 2- related factor 2.

Materials and Methods

All chemicals are purchased from SRL Private Limited and Qualigens Private Limited in India (Mumbai). 5-FU was procured from Celon Laboratories Private Limited in Hyderabad, India. Sigma (SAC-St Louis, MO, USA) provided naringin (CAS No. 10236-47).

Experimental Animals

Male rats (48-Albino *Wistar*), aged 3 months and weighing 180–220 g, were purchased from Jeeva Life Science, a Hyderabad, India-based organisation 9001:2015 certification. Animals were acclimatised over 10 days. For the duration of the current experimental period, they were kept at a constant temperature of 25°C, a light-dark cycle of 12 hours (h), and a relative humidity of 45–55%. Additionally, all rats were provided a regular pellet diet (low-fat, nutritionally balanced food) and unlimited access to deionized water for 28 days.

Ethical statement

The experiment was completed in accordance with the directions and with the Institutional Animal Ethics Committee's prior consent (No. 9/24/C.V.Sc., Hyd. IAEC-Rats/ 12.06.2021).

Experimental design

In the present study, forty-eight healthy adult Wistar rats were separated into four groups, twelve animals in each group. 5-FU and NG dose was selected based on previously available

literature (Gelen et al. 2018). They were randomly allocated into the following groups. Group 1 rats, sham received normal saline per orally(P/O), group 2 rats received 5-FU alone at dose rate of 20 mg/kg b.wtfor an initial five days (20 mg/kg b.wt, IP, Group-3 received NG (100 mg/kg b. wt-P/O) and Group-4 rats were administered with 5-FU for five days along with NG for 28 days. After blood collection, each group with six rats were sacrificed on the 14th and 28th day, followed by cervical dislocation. Heart samples were harvested and went for various analysis. The study protocol was illustrated as a graphical representation in (Fig. 2).

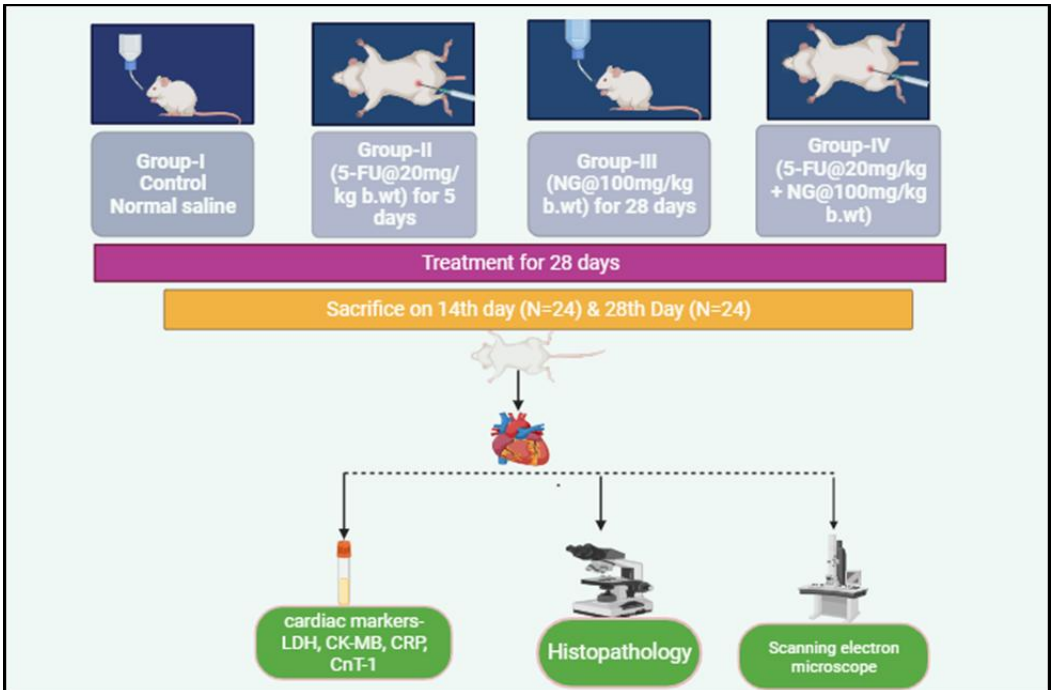

Fig.2 showsthe experimental design and research methodology.

Blood Sample collection

Blood was collected on 14th day and 28th day under light isoflurane anaesthesia. Blood (2 mL) was collected from the retro-orbital plexus through capillary,and serum was separated and placed -20°C until further analysis.Thermo Scientific's semiautomatic Multiskan GO Microplate (ELISA) Spectrophotometer and Erba Mannheim biochemical kits (Transasia Biomedicals Ltd., Solan, Himachal Pradesh, India) were used for the biochemical analysis.

The thickness of the left ventricle wall

Experimental animals were sacrificed, and the heart was excised and immersed in Tyrode's solution for 3 changes at room temperature to wash off the blood from the heart. The heart samples wereovernight fixed in 10% neutral buffered formalin (NBF). Later, the atria were separated from the ventricles. The right and left ventricles were then separated. The weight and height of the left ventricle were recorded. The left ventricle was incised into two halves. The left ventricle wall's thickness was measured using a Vernier Callipers with sensitivity of 0.01 (Shushma, 2002), and further hearts were collected and stored in NBF for histopathology. The remaining portion of tissue was stored at -80 ^{0}C for further analysis.

Cardiac enzymes analysis

Lactate dehydrogenase (LDH), cardiac troponin I (cTn-I), Creatine kinase myocardial band (CK-MB) and C-reactive protein (CRP) were determined by a sandwich ELISA technique by using ELISA kit, which were procured from Erba diagnostics, USA in serum.The units of cardiac enzyme activity were given as IU/L.

Histopathology of heart tissue

Fresh heart tissues were fixed in 10 per cent neutral buffer formalin (NBF) for HP and subjected to an ascending graded series of alcohol for dehydration, then cleared with xylene. This is followed by infiltration with embedding medium (Paraffin) with sectioned (5 μm) and stained by using haematoxylin and eosin (H and E). Observed under light microscopic examination is achieved as the standard procedure describes(Luna, 1968).

Effect of 5-FU on ultrastructural pathology

Immediately after sacrifice, thin slices of tissues wereprimarilyfixed with 2.5 per cent glutaraldehyde. Followed by secondary post-fixation done in osmium tetroxide. Section wasdehydrated in ascending graded acetone series (Qualigens fine chemicals, India) and embedded in Spurr's resin. For scanning electron microscopy (SEM), sections were incubated in a vacuum desiccator and mounted over aluminiumstubs with carbon conductivity tape. Sections were sputtering with a gold sputter coater (JFC-1600) for 180 seconds, as per the description given by Lakshman, 2019(Lakshman, 2019)**.**

Statistical analysis

Data regarding the study was run tostatistical analysis by using one-way Analysis of variance (ANOVA) using the statistical package for the social sciences (SPSS) version 15.0. Duncan's multiple comparison tests were done for the comparison among groups,and the significance level was set at $P<0.05$ (Snedecor and Cochran, 1994).

Results

Thickness of left ventricles

A significant ($P<0.05$) increase in the thickness of left ventricles (mm) were recorded in group 2 when compared to groups 1, 3 and group 4 rats on the 14^{th} and 28^{th} day of the experiment and a significant decrease in mean values were observed in group-4 due to ameliorative effect of NG in tissue restoration (Table.1).

Effect of NG on Cardiac Biomarkers

A significant ($P<0.05$) increase in mean values of cardiac enzymes (LDH, CK-MB, CRP and cTn-I) were recorded in 5-FU treated group on the 14^{th} and 28^{th} day of the experiment as shown in (Table 1). There was a significant ($P<0.05$) reduction in this enzyme mean values in group 4 rats when compared to group 2 on day 14^{th} and day 28^{th} was helped to lessen by decreasing tissue damage by 5-FU. There was no significant difference between groups 1 and 3 on the 14^{th} and 28^{th} day of the experiment (Table.1).

Groups	Group 1		Group 2		Group 3		Group 4	
days	14^{th}	28^{th}	14^{th}	28^{th}	14^{th}	28^{th}	14^{th}	28^{th}
Thickness	$2.44\pm.1^{c}$	$2.37\pm.03^{c}$	$4.52\pm.05^{a}$	$5.04\pm.06^{a}$	$2.46\pm.01^{c}$	$2.35\pm.02^{c}$	$2.70\pm.07^{b}$	$3.22\pm.03^{b}$
LDH	$447.18\pm.74^{c}$	502.6 ± 1.2^{c}	572.50 ± 1^{a}	637.6 ± 6.8^{a}	446.6 ± 1.07^{c}	501.2 ± 2.8^{c}	$513.9\pm.81^{b}$	590.2 ± 1.6^{b}
CK-MB	$140.92\pm.6^{c}$	$161.22\pm.15^{c}$	$294.80\pm.33^{a}$	$332.8\pm.04^{a}$	$141.5\pm.64^{c}$	161.8 ± 1.6^{c}	225.6 ± 3.70^{b}	$22.81\pm.11^{b}$
cTn-I	$10.94\pm.21^{c}$	$59.1\pm.40^{c}$	$28.11\pm.65^{a}$	$32.32\pm.71^{a}$	$11.04\pm.31^{c}$	$17.32\pm.31^{c}$	$17.41\pm.31^{b}$	$22.81\pm.11^{b}$
CRP	$53.71\pm.7^{c}$	$59.1\pm.40^{c}$	$90.45\pm.10^{a}$	112.0 ± 1.03^{a}	$53.30\pm.85^{c}$	$58.80\pm.50^{c}$	$75.0\pm.95^{b}$	$83.70\pm.65^{b}$
TBARS	$8.10\pm.01^{c}$	$8.75\pm.82^{c}$	$14.40\pm.13^{a}$	$16.43\pm.06^{a}$	$8.33\pm.13^{c}$	$8.74\pm.04^{c}$	$11.71\pm.35^{b}$	$13.24\pm.07^{b}$
GSH	$12.72\pm.13^{a}$	$11.2\pm.2^{a}$	$9.30\pm.1^{c}$	$7.30\pm.20^{c}$	$12.53\pm.16^{a}$	$11.17\pm.14^{a}$	$10.66\pm.10^{b}$	$10.66\pm.15^{b}$
SOD	$12.90\pm.40^{a}$	$10.03\pm.04^{a}$	$6.60\pm.22^{c}$	$5.22\pm.21^{c}$	$13.04\pm.20^{a}$	$8.81\pm.13^{a}$	$9.07\pm.11^{b}$	$8.90\pm.10^{b}$

Table. 1: Cardiac markers in serum

Values are Mean ± SE (n=6) on day 14th and 28th; One-way ANOVA.
Means with different superscripts in a column differ significantly at P<0.05 (*).

Ameliorative effect of NG on histopathological alterations induced by 5-FU

Heart sections of group 1 and group 3 rats showed a normal architecture with normal cardiac muscle fibres on 14th and 28th day of the experiment (Fig. 3A,E). The heart section of group 2 rats showed dilatation of cardiac muscle fibres, interfibrillar oedema, disruption, degeneration and splitting of cardiac muscle fibres and pericardial congestion with focal infiltration of MNCs between damaged cardiomyocytes (Fig. 3B,C,D). On the 28thday of the experiment, group 2 rats showed severe congestion and interfibrillar haemorrhages with MNCs infiltration, mild fibrosis, disruption, degeneration and necrosis of cardiac muscle fibres, accumulation of fat globules, and thrombosis formation (Fig. 3F-J). The heart sections of group 4 rats on the 14th day of the experiment showed mild degree of changes. On the 28th day, group 4 rats showed mild changes with the restoration of cardiac muscle fibres,indicating the ameliorative effect of NG (Fig. 3K,L). Table 2 scores histopathological alterations like congestion, odema, disruption of cardiac musclefibres, infiltration of inflammatory cells and haemorrhages from (-, +, ++ and +++).

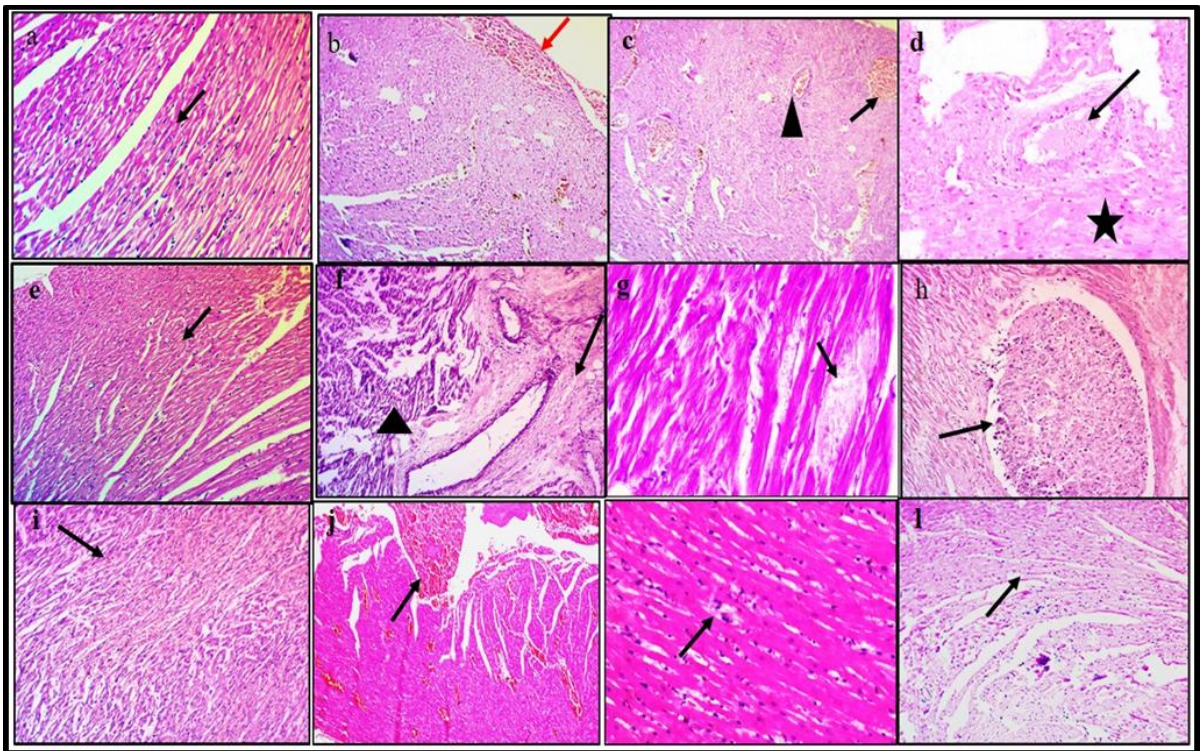

Fig. 3 Microscopic picture of a and e showing normal cardiac muscle fibres, b- pericardial congestion, c-inflammatory, and congestion of blood vessels. c-d. Degeneration and disruption of muscle fibres, necrosis. f-j. Sections showed severe degenerative changes. k-l. Mild disruption of muscle fibres. H&E 100x. K-200x

Parameters		Group-1	Group-2	Group-3	Group-4
HP	Congestion	-	+++	-	++
	Haemorrhages	-	++	-	+
	Pericardial haemorrhages and disruption of cardiac fibre	-	++	-	+
	Degenerations and necrosis of myocardial fibre	-	+++	-	++
	Infiltration of inflammatory cells	-	++++	-	+++
	Fat cell accumulation and thrombus formation	-	++	-	+

Table. 2: Table showing scoring of HP in heart tissue lesions

Scanning electron microscopy

SEM of heart sections of group 1 and 3 rats revealed normal appearance structure of cardiomyocytes with cardiac muscle fibres on the 14th and 28th day of the experiment (Fig. 4A,B,E). The cut surface of group 2 heart section on 14th day reveals mild dilatation of cardiac muscle fibres with mild fibrous tissue proliferation, presence of erythrocytes in lumen with distortion of cardiomyocytes,extravasation of erythrocytes in between cardiac muscle fibres with changed morphology due to the effect of 5-FU on RBC morphology (Fig. 4C-D). On 28th day of the experiment group 2 rats showed infiltration of inflammatory cells on surface area, dilatation and disruption of cardiac muscle fibres with mild fibrosis (Fig. 4F,G,H). In group 4 rats, the heart section showed mild disruption of cardiac muscle fibres with mild fibrosis (Fig. 4I).

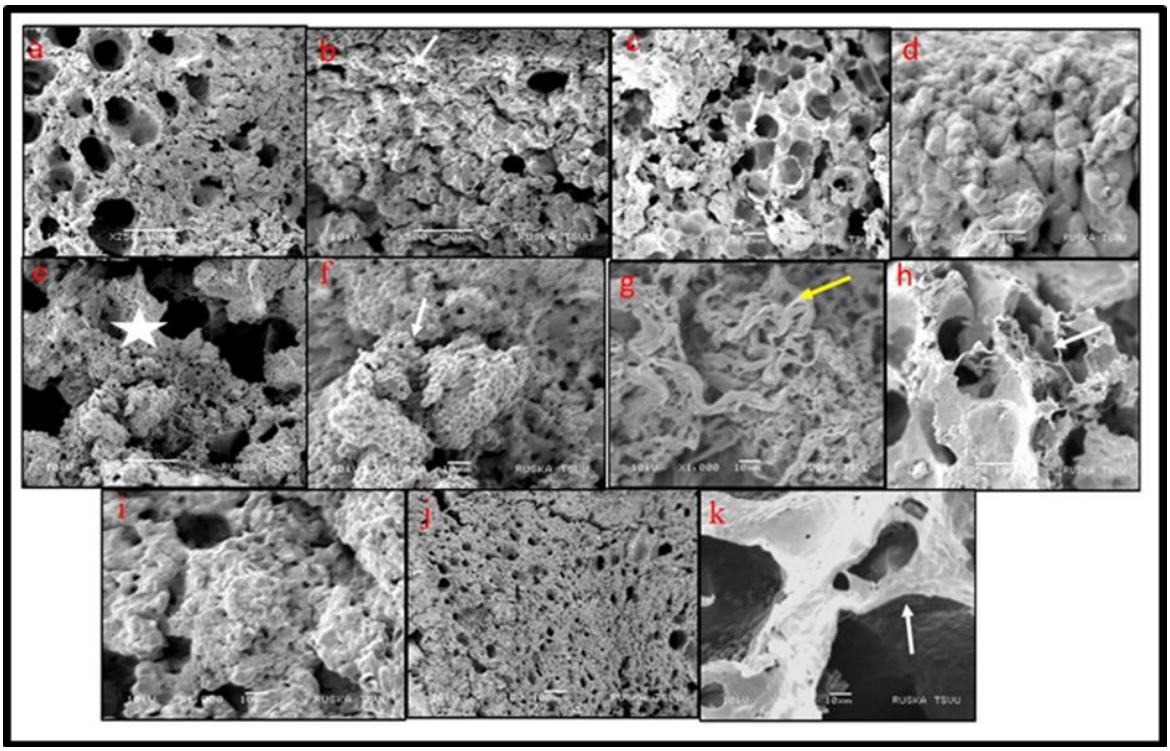

Fig. 4a,b,e) normal appearance of cardiomyocytes, c,d) Mild dilatation and distortion of cardiac muscle fibres, mild fibrous tissue proliferation, presence of erthrocytes, g,h) Inflammatory cells, dilatation, disruption of cardiac muscle fibres with mild fibrosis, i) mild disruption of cardiac muscle.

Discussion

The current experiment results reveal that 5-FU has potentially toxic effects on the heart and ameliorative effect of NG on cardiotoxicity. In the present study, we noticed an increase inthe thickness of the left ventricular wall in toxic group 2 rats, indicating hypertrophy of ventricles due to left ventricular dysfunction, which might be due to oxidative stress, interstitial oedema, fibrosis and cellular infiltration of inflammatory cells (Namrathaet al, 2021; Shushma, 2002)In group 4, the mean values were decreased which could be due to the ameliorative effect of NG on oxidative stress by inducing restoration of cardiac function by myocardial tissue preservation (Gelen et al. 2021).

Cardiotoxicity is assessed by using parameters of diagnostic markers found in the myocardium (LDH, CK-MB, CRP and cTnI**)**(Kumaret al. 2019; Cardinale et al. 2010). These biomarkers increase cardiotoxicity with cardiac injury, heart failure, myocarditis with myocardium ischemia. A rapid drop in blood flow occurs during acute myocardial injury, and enzymes are released, which are utilised as testing indices in hypoxic-ischemic myocardial damage (Yadala et al. 2020). 5-FU produces cell membrane breakdown, leading to intracellular protein release like LDH, CK-MB, cardiac troponins and CRP, which are assayed to determine the existence and amount of 5-FU-induced myocardial damage(Sengul et al. 2021).

LDH increases in 5-FU-induced toxicity due to necrotic damage of the myocardium and cardiac dysfunction (Lamberti et al. 2012). cTn-I is a contraction-regulating protein complex specific to the myocardium tissues and is considered a gold standard biomarker for MI, necrosis, LVH and drug-induced cardiotoxicity(Khudhair and Numan, 2014). CK-MB releases in case of HF and reflects plasma membrane integrity and permeabilityand CRP is a marker of inflammation and a significant predictor of cardiovascular risk(Mohamed and Safwat, 2016).

In the present experimental study, mean values of cardiac serum parameters (LDH, CK-MB, CRP and cTn-I) were significantly ($P<0.05$) increased in 5-FU treated groups on 14^{th} and 28^{th} day might be due to direct toxic effects, leakiness of plasma membrane, excessive production of free radicals, haemodynamic stress, myocardial necrotic damage due to ROS production, decreased of blood supply to the heart could be responsible for hypoxic injury to the myocardium resulting in cell damage, rupture of cell membrane and leakage of the enzymes into the blood stream. Similar findings were recorded by Khudhair and Numan by 5-FU induced cardiotoxicity (Khudhair and Numan, 2014; Refaie et al. 2022) and heavy metal toxicity (Anilkumar et al. 2014).

In the present experiment, heart sections of group 2 rats on the 14^{th} day showed disruption and degeneration of cardiomyocytes, dilatation of cardiac muscle fibres, pericardial congestion with focal pericardial infiltration of MNCs, congestion of blood vessels, interfibrillar oedema. On 28^{th} day group 2 rats showed severe congestion of blood vessels, degeneration and necrosis of muscle fibres, mild fibrosis and focal areas of interfibrillar haemorrhages and severe infiltration of inflammatory cells along with thrombosis formation, which might be responsible for atherosclerosis formation. These changes might be due to oxidative stress, direct myocardial injury and ischemia of muscles. The structural and functional changes of the heart in group 2 rats are positively complement each other with respect to histopathological and biochemical results (elevated values of cTn-I, LDH, CK-MB and CRP)(Sengul et al. 2021; Anilkumar et al. 2014).

In the present study, the heart sections of group 4 rats on day 28th showed similar lesions to that of group 2 rats with mild infiltration of inflammatory cells, mild degeneration with restoration of cardiac tissue. These changes might be due to ameliorating activity of NG over oxidative stress (Gelen et al. 2017; Anilkumar et al. 2010).

On SEM, we observed the cut surface of group 2 rats on 14th day of the experiment of the heart section showed mild dilatation of cardiac muscle fibres with mild fibrous tissue proliferation, presence of RBCs in the lumen with distortion of cardiomyocytes and extravasation of RBCs in between cardiac muscle fibres with changed morphology of erythrocyte with inflammatory cells on the surface area. On 28th of the experiment, group 2 rats showed dilatation and disruption of cardiac muscle fibres with mild fibrosis mild infiltration of inflammatory cells. The changes in group 2 rats could be due to 5-FU induced oxidative damage. Change in the morphology of erythrocytes might be due to shift in the metabolism of phosphate compounds in RBCs, which leads to deoxygenation of Hb and causesa severe drop in ATP levels which leads to echinocytes along with oxidative stress (Spasojevic et al. 2008; Chiluka et al. 2015).

In group 4 rats, the heart section showed mild disruption of cardiac muscle fibres and mild infiltration of inflammatory cells which could be due to the ameliorative effect over 5-FU induced toxicity.We inferred that NG has anti-inflammatory, anti-apoptotic, and anti-oxidant properties based on the results.

Conclusion

This present experimental study and statistical analysis indicate 5-FU (20 mg/kg b.wt) exposure elevated levels in left ventricular wall thickness, cardiac enzymes by free radical-induced damage, inflammatory cytokine levels and expression of inflammatory markers. Furthermore, histological damage and ultrastructural changes were induced in the cardiac tissues following the 5-FU exposure. Naringenin powderhas mitigated cardiac toxicity may be due to potent anti-inflammatory and anti-oxidant activity by activation of NF-κB/Nrf2 signaling cascade pathway. Furthermore, future studies were required to understand the detailed mechanisms of ameliorative effect by using molecular techniques.

Conflict of interest statement

The authors declare with no conflict of interest.

Acknowledgements

We would express our gratitude to P.V. Narsimha Rao Telangana Veterinary University.

Funding

The authors received no extramural funding for experimental study

REFERENCES

Alfarouk, Khalid O, Christian-Martin Stock, Sophie Taylor, Megan Walsh, Abdel Khalig Muddathir, Daniel Verduzco, Adil HH Bashir, Osama Y Mohammed, Gamal O Elhassan, and Salvador. 2015. Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp. *Journal Cancer cell international Harguinde*y, 15: 1-13.

Alter P, M Herzum, M Soufi and JR Schaefer. 2006.Cardiotoxicity of 5-Fuorouracil. *Cardiovascular & Hematological Agents in Medicinal Chemistry*, 4: 1-5.

Anilkumar B, Reddy AG, Ravikumar P, Reddy YR, Haritha C. 2014. Oxidative Stress-induced Cardiotoxicity Due to Lead, Cadmium and Their Co-exposure in Male Wistar Rats and Protective Role of N-acetyl L-cysteine (NAC). *Inventi Rapid: Molecular Pharmacology*.2(4):1-6.

Anilkumar B, Gopala Reddy A, Anand Kumar A, Ambica G, Haritha C. 2013. Toxicopathological interaction of lead and cadmium and amelioration with N-Acetyl-L-Cysteine. *Veterinary World,*6(10)823-827.

Anilkumar B, Gopala Reddy A, Kalakumar B, Jyothi K, Gopi KS. 2010. Effect of Bt cotton plants on oxidative stress in sheep. *Indian Journal of Animal Sciences,*80(4):313.

Arundhathi S, Anand Kumar A, Ravi Kumar Y, Anil Kumar B. 2015. Haematological and histopathological alterations due to combined toxicity of Isoniazid and Rifampicin; amelioration with Withania somnifera and Vitamin-E in Wistar rats. *International Journal of Pharma and Bio Sciences*, 6:222-229.

Cardinale D, Colombo A, Lamantia G, Colombo N, Civelli M, De Giacomi G, Rubino M, Veglia F, Fiorentini C and Cipolla CM. 2010. Anthracycline-induced cardiomyopathy: clinical relevance and response to pharmacologic therapy. Journal of the American College of Cardiology, 55: 213-20.

Chelpuri Y, Pabbathi S, Alla GR, Yadala RK, Kamishetti M, Banothu AK, Boinepally R, Bharani KK, Khurana A. 2022. Tropolone derivative hinokitiol ameliorates cerulein-induced acute pancreatitis in mice. *International Immunopharmacology,* Aug 1;109:108915.

Chiluka H, Alla GR, Yerradoddi RR, Banothu AK. 2015. Pharmacodynamic interaction of fenugreek with insulin and glimepiride in streptozotocin-induced oxidative stress in Sprague Dawley rats. *Journal of Advanced Veterinary and Animal Research*, 2(3):353-356.

De Moor, Janet S, Angela B Mariotto, Carla Parry, Catherine M Alfano, Lynne Padgett, Erin E Kent, Laura Forsythe, Steve Scoppa, Mark Hachey and Julia H. 2013. Cancer survivors in the United States: prevalence across the survivorship trajectory and implications for car. *J Cancer Epidemiology, Biomarkers and Prevention,* 22: 561-70.

Gedikli S and Erbas E. 2021. Protective Effects of Naringin on Lung Toxicity Induced by 5-Fluorouracil in Rat. *Kocatepe Veterinary Journal,* 14: 16-25.

Gelen V, E Sengul, S Gedikli, G Atila, H Uslu and Mustafa. 2017. The protective effect of rutin and quercetin on 5-FU-induced hepatotoxicity in rats. *Asian Pacific Journal of Tropical Biomedicine,* 7: 647-653.

Gelen V, E Sengul, S yildrim and Gozde. 2018. The protective effects of naringin against 5-fluorouracil-induced hepatotoxicity and nephrotoxicity in rats. *Iranian journal of basic medical sciences,* 21: 404-410.

Gelen V, E Sengul, S yildrim, E senturk, S Tekin and Abdulsamed. 2021. The protective effects of hesperidin and curcumin on 5-Fluorouracil-induced nephrotoxicity in mice. *Environmental Science and Pollution Research,* 28: 47046-47055.

G Sushma. 2002. "Ameliorating Effect of Terminalia arjuna Against 5-Fluorouacil Induced Cardiotoxicity in *Wistar* Rats.*Acta Scientific Veterinary Sciences,* 29: 235-242.

HarishB, Shivakumar P, KumarBK, Ramya B, BegumN, Rani MU and Kumar BA. 2022. Amelioration of experimental nephrotoxicity due to 5-Flourouracil by Resveratrol in comparison to Vitamin-E.*The Journal of Phytopharmacology,* 11(4):243-246.

Heidelberger, C. 1995. Progress in nucleic acid research, and molecular biology. 1965. 'Fluorinated pyrimidines', 4: 1-50.

Igual ME, Garcia-Martinez, MM Camacho and Nuria. 2013. Jam processing and storage effects on β-carotene and flavonoids content in grapefruit. *Journal of Functional Food,* 5: 736-44.

Jyothi K, Gopala Reddy A, Ramana Reddy Y and AnilkumarB. 2010. Protective role of n-acetyl cysteine against cyclophosphamide-induced cardiotoxicity. *Indian Journal of Animal Sciences*, 80(9), p.871.

Khudhair A R and Numan IT. 2014. Possible cardio-protective effects of telmisartan againist 5-fluorouracil-induced cadiotoxicity in *Wistar* rats. *International Journal of Pharmacy and Pharmaceutical Science*, 6: 397-400.

Kanduri J, More LA, Godishala A and Asnani A. 2019. Fluoropyrimidine-associated cardiotoxicity. *Cardiology Clinics*, 37: 399-405.

Kumar YR, Madhuri D, Reddy AG, Anadkumar A, Kumar BA, Chandravathi T. 2019.Cardiotoxicity Induced by Doxorubicin in Wistar Rats and its Amelioration with Ascorbic Acid and Spirulina.*International Journal of Livestock Research*, 9(7): 49-56.

Lakshman M. 2019. Application of conventional electron microscopy in aquatic animal disease diagnosis: A review. *Journal of Entomology Zoological Studies*, 7: 470-475.

Lamberti M, Porto S, Marra M, Zappavigna S, Grimaldi A, Feola D, Pesce D, Naviglio S, Spina A, Sannolo N and Caraglia M. 2012. 5-Fluorouracil induces apoptosis in rat cardiocytes through intracellular oxidative stress. *Journal of Experimental and Clinical Cancer Research*,31:1-8.

Longley DB, Harkin DP and Johnston PG. 2003. 5-Fluorouracil: mechanisms of action and clinical strategies. *Nature reviews cancer*, 3: 330-338.

Luna, G HT. 1968. Manual of histological and special staining techniques.: The Blakistone Division McGraw-Hill Book Company. *Inc. New York, Toronto London*, 1: 9-34.

Maimonaparveen S, Madhuri D, Lakshman M, Anilkumar B. Anti-inflammatory Potential of Whole Pomegranate Fruit Juice (POM) against Bleomycin Induced Lung Injury in Rats.*Journal of Animal Research*,11(6): 989-993.

Manvitha V, Reddy AG, Kumar BA, Jeevanalatha M, Priyanka G. 2019. Protective Role of Ashwagandha and Selenium against Chlorpyrifos (CPF) Induced Haemato-Biochemical and Hepatic Alterations in Wistar Rats. *Int. J. Curr. Microbiol. App. Sci*, 8(11):941-9.

Malet-Martino M, Jolimaitre P,and Martino R. 2002. The prodrugs of 5-fluorouracil. *Current Medicinal Chemistry-Anti-Cancer Agents*, 2: 267-310.

Miura K, Kinouchi M, Ishida K, Fujibuchi W, Naitoh T, Ogawa H, Ando T, Yazaki N, Watanabe K, Haneda S and Shibata C. 2010. 5-Fu metabolism in cancer and orally-administrable 5-Fu drugs. *Cancers*, 2: 1717-30.

Mohamed ET and Safwat GM. 2016. Evaluation of cardioprotective activity of Lepidium sativum seed powder in albino rats treated with 5-fluorouracil. *Beni-Suef university journal of basic and applied sciences*, 5: 208-215.

Mosseri M, Fingert HJ, Varticovski L, Chokshi S, Isner JM. 1993. In vitro evidence that myocardial ischemia resulting from 5-Fluorouracil chemotherapy is due to protein kinase C-mediated vasoconstriction of vascular smooth muscle. *Cancer research*, 53: 3028-3033.

Namratha ML, Lakshman M, Jeevanalatha M, Kumar BA.2021. Assessment of Vitamin C Protective Activity in Glyphosate-Induced Hepatotoxicity in Rats. *Pakistan veterinary journal*, Jul 1;41(3).

Nie YC, Wu H, Li PB, Luo YL, Long K, Xie LM, Shen JG and Su WW. 2012. Anti-inflammatory effects of naringin in chronic pulmonary neutrophilic inflammation in cigarette smoke-exposed rats. *Journal of medicinal food*, 15: 894-900.

Pawankalyan S, Kalakumar B, Shivakumar P, Madhuri D, Anilkumar B, Ravikumar Y. 2022. Hepatoprotective Effect of Pomegranate Juice Extract in Methotrexate Induced Hepatic Dysfunction in Rats. *Journal of Animal Research*, 12(4):473-81.

Rashid S, Ali N, Nafees S, Hasan SK and Sultana S. 2014. Mitigation of 5-Fluorouracil induced renal toxicity by chrysin via targeting oxidative stress and apoptosis in wistar rats. *Food and Chemical Toxicology*, 66: 185-93.

Refaie MM, Shehata S, Bayoumi AM, El-Tahawy NF and Abdelzaher WY. 2022. The IL-6/STAT signaling pathway and PPARα are involved in mediating the dose-dependent cardioprotective effects of fenofibrate in 5-Fluorouracil-induced cardiotoxicity. *Cardiovascular Drugs and Therapy*, 36: 817-827.

RenusheAP, Banothu AK, Bharani KK, Mekala L, KumarJM, Neeradi D, HanumanDDV, Gadige A and Khurana A. 2022. Vincamine, an active constituent of Vinca rosea ameliorates experimentally induced acute lung injury in Swiss albino mice through modulation of Nrf-2/NF-κB signaling cascade. *International Immunopharmacology*, 108, p.108773.

Sengul E, gelen V and Gedikli S. Cardioprotective activities of quercetin and rutin in sprague dawley rats treated with 5-Fluorouracil. 2021. *Journal of Animal and Plant Sciences*, 1: 31(2).

Snedecor G W and Cochran WG. 1994. Statistical methods. 8th ednEast West Press Pvt. *Ltd., New Delhi, India, 313*.

Sorrentino MF, Kim J, Foderaro AE, Truesdell AG.2012. 5-Fluorouracil induced cardiotoxicity: review of the literature. *Cardiology journal,* 19:453-457.

Spasojevic I, Jelic S, Zakrzewska J, Bacic G. 2008. Decreased oxygen transfer capacity of erythrocytes as a cause of 5-Fluorouracil related ischemia. *Molecules*, 14:53-67.

Sravathi, V, M Jeevanalatha, Y Ravikumar, A T Gopalareddy, B Swathi, Chandravathiand M. Lakshman. 2022. '5-Flurouracil induced hematological alterations in wistar rats and its amelioration with naringenin. *The journal of pharma innovation,*4: 12.

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. a cancer journal for clinicians, 71: 209-249.

Tsibiribi P, Bui-Xuan C, Bui-Xuan B, Lombard-Bohas C, Duperret S, Belkhiria M, Tabib A, Maujean G, Descotes J and Timour Q. 2006. Cardiac lesions induced by 5-Fluorouracil in the rabbit. *Human & experimental toxicology*, 25: 305-309.

Vanisthasree K, Reddy AG, Kalakumar B, Haritha C, Anilkumar B. 2011. Hepatotoxicity studies in the progeny of pregnant dams treated with methimazole, monocrotophos and lead acetate. *Toxicology International*, 18(1):67.

Yadala R, Madhuri D, Lakshman M, Gopalareddy A, Kalakumar B, Anilkumar B, Sawale GK, Sandhyarani K, Ramesh J. 2020. Cadmium and Chlorpyrifos induced histopathological alterations in the heart of male rats. *The Pharma Innovation Journal.* 2020;9(9):18-20.