Summary

To identify the risk variables contributing to FPV infection in Chennai, research was carried out. Using TaqMan RT-qPCR, 250 samples of feline faeces DNA were examined for FPV. A structured questionnaire was used to analyze risk variables such as temporal distribution, age, sex, breed, type of food, type of water, and interaction with stray cats. Chennai saw a 75.83% total case prevalence from January 2022 to May 2023. The biggest risk factors for FPV are the time following the rainy season, age under six months, imbalanced nutrition, contaminated drinking water, and interaction with feral cats.

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Reproductive Toxicity Induced by 5-Fluorouracil (5-FU) and the Potential Ameliorative Effects of Naringinin -28 Days Experimental Study in Wistar Rats

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Abstract

This study evaluates the potential ameliorative effects of Naringenin (NG) on testicular function in a 5-Fluorouracil (5-FU) induced testicular toxicity model. Male rats were divided into control, 5-FU treated, NG treated and NG + 5-FU combination groups with 6 animals each. Hormonal assays revealed a significant decrease in 5-FU treated group.Furthermore, pro-inflammatory cytokines (TNF-α, IL-1ß, IL-6) exhibited a marked increase in the 5-FU group, which was significantly attenuated by decrease pro-inflammatory levels in NG + 5-FU combination group. Anti-inflammatory cytokine (IL-10) levels significantly decreased in 5-FU group but were notably restored with NG treatment along with hormone levels by mitigating chemotherapy-induced testicular damage.

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5-Fluorouracil (5-FU) has remained a prominent chemotherapy medication extensively employed for the treatment of solid cancers affecting solid tumours like gastrointestinal system, pancreas, ovaries, esophagus, colorectal and breast (Vodenkova *et al.,* 2020). Following administration, a significant portion of 5-FU undergoes metabolism into inactive dihydrofluorouracil, primarily catalyzed by the rate-limiting enzyme dihydropyrimidine dehydrogenase (DPD) in the liver (Diasio and Harris, 1989). The remaining administered 5-FU is predominantly converted into active metabolites, including fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP). FdUMP forms a complex with TS, inhibits the normal function of thymidylate synthetase (TS) and hindering the synthesis of deoxythymidine monophosphate and causes imbalances in other deoxynucleotides and disruption interferes with DNA synthesis and repair processes along with breaking of DNA strands(Noordhuis *et al.,* 2004). However, it's worth noting that 5-FU also negatively affects the metabolism and viability of normal cells, which accounts for its developmental and reproductive toxicities (Naren *et al.,* 2022). Nevertheless, it remains uncertain the clear mechanism of 5-FU reproductive toxicity, which holds significance in assessing fertility. The impact of cancer treatment drugs on reproductive toxicity is a critical aspect to contemplate, given its potential consequences for the well-being of future generations.NG-4,5,7-trihydroxyflavanone, a naturally occurring dietary flavonoid abundant in citrus fruits, grapefruits, tomatoes, cocoa and various herbal products, possesses valuable therapeutic properties(Alboghobeish *et al.,* 2019). Previous resear ch has established that NG exhibits antioxidant anti-inflammatory, antidiabetic, cardioprotective, anticancer, antistress, and neuroprotective properties (Hernández-Aquino and Muriel, 2018). It plays a crucial role in supporting reproductive health. It contributes to germ cell development, promotes spermatogenesis and enhances semen quality by regulating sperm volume and motility (Wang *et al.,* 2021). So, in this experiment we assessing genotoxicity using chemotherapy drugs holds with ameliorative agent to significant scientific significance for 28 days in *Wistar* rats.

Materials and Methods

Twenty four adult male, weighing approximately 250-300 grams (gm), were obtained from Jeeva Life Sciences, Hyderabad, India-based organization-ISO 9001:2015 certification. The experimental protocol received approval from the Institutional Animal Ethics Committee (IAEC) under approval no (3/25/C.V.Sc,Hyd. IAEC). Total of 24 rats were procured and assigned randomly into 4 groups (n=6) and experimental design is given as : Group-I: Sham, Group-II treated with 5-FU at dose rate of 20 mg/kg-IP for initial five days, Group-III: orally treated with Naringenin 100 mg/kg b wt for 28 days, Group-IV: Combination group5-FU (first 5 consecutive days 20 mg/kg b. wt) + (Naringenin 100 mg/kg b wt).In all groups, rats were sacrificed at end of the experimental period (28 days) and light isoflurane anaesthesia were given was blood collection from the retro-orbital plexusthrough capillary tubes, allowed to clot for 4 hours, centrifuged and serum was separated and placed -20°C until hormone analysis.For Immunoexpression, binding primary antibody to antigen of interest and detecting antibody bounded to the antigen of interest for immunostaining is primary principle and ELISA kits were used to estimate inflammatory cytokines.

Results and Discussion

Reproductive toxicity is a crucial point to consider as its important potential influence on the health of offspring. This consideration becomes particularly pertinent, given the substantial number of young individuals battling cancer and undergoing treatments that include drugs such as 5-FU (Delessard *et al.,* 2020). The exact mechanism of 5-FU toxic on reproductive system is not known. The cytotoxic effects induced by 5-FU can be attributed to various mechanisms. Based on previous literature, studies demonstrated that 5-FU is converted into active metabolites and inhibits TS enzyme and inhibits synthesis of DNA and RNA synthesis and interfere with repair processes along with breaking of DNA

and RNA strands (Waxman *et al.,* 1990). In our current study, serum hormone testosterone (ng/ml), Follicle stimulating hormone (FSH-mIU/mL) and Luteinzing hormone (LH-mIU/mL) levels in all the groups were estimated and toxic 5-FU showed significant (P<0.05) reduction in these levels when compared to control and ameliorative group-3 rats. It might be due to inhibitory effect of 5-FU on synthesis of deoxynucleotide which are required for DNA synthesis, results in decrease synthesis of cells like sertoli and leydig cells and further decrease in hormonal production(Takizawa and Horii, 2002). 6-Mercaptopurine (6-MP) is classified as an antimetabolite drug, as 5-FU. This medication triggers the production of ROS, initiates the activation of Caspase 3 and facilitates the formation of apoptosomes (Lynch *et al.,*2016). These processes collectively result in the depletion of Leydig cells with decrease testosterone levels in our study. In previous findings, results suggest that increase in pulsatile secretions of gonadotropins hormones at pituitary axis, which further increase in FSH and LH levels (Takizawa and Hori, 2002). This might be the same reason in our study and same findings were given in previous literature (D'souza and Narayana, 2001).Furthermore, group-4 combination rats showed significant (P<0.05) improvement in these values compared to group-2 toxic rats indicating mitigation action of NG. Whereas, control and NG showed similar mean values and results of serum testosterone of all the groups on day 28 were depicted in the fig. 4 (Mostafa *et al.,* 2016) (Table I).

Spermatogenesis is a crucial post-meiotic developmental process, which is highly vulnerable to testicular inflammation, oxidative stress and apoptosis. The mean concentration of pro-inflammatory cytokine TNF-α, IL-1ß and IL-6 (pg/mg protein) in the testicular tissue disclosed a highly significant (P<0.05) increase in 5-FU treatment group when compared to sham. Moreover, cytotoxic chemotherapy can activate the nuclear factor-kappa B (NF-κB) family, which mediates inflammation and leads to testicular damage. It's worth noting that oxidative stress can also activate NF-κB signaling pathway. The excessive generation of free radicals, inhibition of ROS removal through the suppression of antioxidant systems, induction of DNA damage and initiation of hormonal changes following chemotherapy all contribute to gonadal and sperm alterations (Fahmy*et al.,*2020). Whereas, treatment with NG + 5-FU showed a significant drop in the levels of TNF-α, IL-1ß and IL-6 were observed as compared to group-2 toxic rats. Furthermore, the mean values of NG and sham showed no significant variation displays safety of this agent-NG. In addition to pro-inflammatory cytokines we also observed anti-inflammatory cytokine levels (IL-10) in testicular tissues and we found to be decreased with the significance of (P<0.05) in 5-FU toxic group. Besides, IL-10 levels were significantly increase at P<0.05 in combination group due to pharmacological intrusion of NG (Sengul *et al.,* 2023) (Table I).

Conclusion

In conclusion, the combination of NG and 5-FU treatment shows promise in mitigating complications, particularly inflammation, by harnessing their anti-inflammatory action with gonadropic properties. However, further extensive research is imperative to elucidate the full extent of NG's

Table. I : Effect of NG on Hormonal assay and inflammatory cytokines

Groups days=28	Group 1	Group 2	Group 3	Group 4
Testosterone level (ng/mL)	1.56±2.3^{a}	0.92±2.45^{c}	1.55±0.23^{a}	0.45±0.02^{b}
FSH (mIU/mL)	2.74±1.2^{a}	1.18±2.57^{c}	2.73±0.12^{a}	1.45±0.2^{b}
LH (mIU/mL)	3.56±2.4^{a}	1.84±1.24^{c}	3.54±0.71^{a}	2.34±0.23^{b}
TNF-α	8.34±2.6^{c}	12.89±1.64^{a}	8.32±0.23^{c}	10.23±0.16^{b}
IL-1β	36.43±1.8^{c}	56.74±1.48^{a}	34.23±0.14^{c}	44.21±0.21^{b}
IL-6	23.41±0.19^{c}	43.75±0.24^{a}	23.37±0.24^{c}	28.71±0.21^{b}
IL-10	89.23±0.15^{a}	43.75±0.49^{c}	88.34±0.13^{a}	62.36±0.52^{b}

benefits in reducing oxidative stress and its associated pathogenicity across varying time intervals.

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