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REVIEW ARTICLE

Is Jackfruit Holding the Key Answer to Male Infertility? Outcomes of a Preliminary Pre-clinical Study

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Abstract

Purpose: The antioxidant activity of jackfruit can be a potential treatment for male infertility. The objectives of the study were to evaluate the effect of jackfruit (*Artocarpus heterophyllus*) extract on testicular dysfunction and cytogenetic changes induced by Chlorpyrifos in male Wistar albino rats by using sperm analysis, histopathologic analysis of testis, biochemical analyses, and cytogenetic studies; and to determine if these were dose-dependent. **Methods:** Ethanolic extract of jackfruit (JFE) (450mg/kg, 600mg/kg) and Chlorpyrifos (CPF) (17.5mg/kg) were used. Thirty male rats grouped into control, toxic (CPF), jackfruit, treatment-1 and -2 received distilled water+corn oil, chlorpyrifos, JFE, CPF 17.5mg+JFE 450mg/kg, and CPF 17.5mg+JFE 600mg/kg, respectively. Network pharmacology was used to track, and identify the active ingredients and target pathways. **Results:** Treatment groups particularly treatment 1 displayed greater weight gain. JFE was associated with an increased testicular weight ($p<0.05$). Sperm motility significantly increased in the jackfruit, control, and treatment groups, particularly treatment 2. The increase in sperm count and motility were dose-dependent. There was a significant decrease in the DNA damage with JFE. There was an increase in the total protein in the treatment groups. Sperm motility, vitality, and total proteins are significantly reduced with CPF. There was a reduction in malondialdehyde (MDA) and an increase in catalase activity in the jackfruit treatment groups. Network analysis analysed 50 active compounds in JFE, and identified 12 potential targets. **Conclusion:** JFE has a positive impact on sperm count, motility, vitality, and chromatin integrity demonstrated by decreased DNA damage. Antioxidant assays, MDA, and catalase indicated strong antioxidant activity of JFE.

Keywords: *Artocarpus heterophyllus*, Chlorpyrifos, Network Pharmacology, Sperm Count, Sperm Motility, Sperm Vitality, Sperm Chromatin Integrity

1. Introduction

Male factor is the cause of infertility in 1/3rd of the infertile couples and the estimated prevalence is between 30% and 45%[1]. In India, primary infertility is on the increase[2,3]. There is a gradual decline in the sperm count of a normal Indian adult male to 20 million/mL from 60 million/mL in the last three decades[4]. Treatment of infertility is a very expensive process and takes about 2-10 years as per the patients' profiles[1]. There is a strong need for awareness to address the issues, and normalize them rather than associating them with any form of stigma or discrimination.

A high concentration of Reactive Oxygen Species (ROS) was detected in the semen in about 30 to 80 % of infertile men[5]. Increasing the antioxidant levels in the body to overcome the oxidative stress that is the primary cause of either spermatogenesis low sperm

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count or sperm motility is one of the treatment strategies for male infertility.

Jackfruit, a tropical fruit, contains a wide range of phytonutrients such as carotenoids, phenolic compounds and flavonoids along with vitamin C can act as antioxidants. It has also been explored in diabetic and hypertensive models for its antioxidant activity[6]. The use of jackfruit in infertility models has been minimal to almost nil. Its antioxidant activity could be a boon in the management of infertility and could emerge as a potential treatment option for male infertility.

Occupational exposure to pesticides has become common and is an increasingly alarming phenomenon. The health effects caused by this occupational exposure are massive and irreversible in some cases. Widespread use of organophosphorus compounds and high rates of food contamination could leave humans, animals, and birds exposed to high levels of pesticidal toxicity. Sperm quality in farmers exposed to pesticides showed significant variations of main semen characteristics in exposed subjects, such as a decrease in sperm concentration, reduced motility, and alterations in the sperm membrane[7].

Pesticide-induced infertility is known to increase ROS and the use of an antioxidant agent could potentially reduce the severity of this toxicity or reverse it. For the current study, an organophosphate pesticide chlorpyrifos was used for inducing testicular toxicity in the experimental rats[8].

With this backdrop, we evaluated the antioxidant activity of jackfruit by using an ethanolic extract of jackfruit in 2 different doses i.e., 450mg/kg and 600mg/kg in chlorpyrifos-induced infertility in male Wistar albino rats.

2. Materials and Methods

This study was carried out with the approval of the Institutional Animal Ethical Committee (IAEC/KMC/131/2019) before the start of the experiment, with an objective to evaluate the effect of *Artocarpus heterophyllus* extract on testicular dysfunction and cytogenetic changes induced by chlorpyrifos in male Wistar albino rats by using sperm analysis, histopathologic analysis of testis, biochemical analyses and cytogenetic studies; and to determine if these effects were dose-dependent.

2.1 Preparation of Jackfruit Extract (JFE)

Homegrown raw jackfruit (firm flesh variety) was used for the purpose of obtaining extract. The plant was authenticated at the Department of Botany, MGM College, Udupi. About 5 kg of the fruit was taken, deseeded such that only pulp and rind were made available. Then it was chopped into smaller pieces in a clean aseptic setting (preparation room) and dried using a hot air oven for a period of 24 hours at 50°C. The ethanolic extract was prepared using the Soxhlet method of extraction[9]. The extraction process was done at Manipal College of Pharmaceutical Sciences. The jackfruit extract (JFE) (*Artocarpus heterophyllus* extract) dissolved in corn seed oil just before dosing the animals.

JFE was made into 2 concentrations - 450mg/kg and 600mg/kg. Chlorpyrifos (Sigma-Aldrich Production GmbH, Switzerland) was used. The strength used was 17.5 mg/kg and it was given as a suspension in corn oil. Corn oil was procured from the Department of Pharmacology, Kasturba Medical College, Manipal (processed in the Manipal College of Pharmaceutical Sciences, Manipal, MAHE).

2.2 Study Animals

Campus-bred, male albino rats of Wistar strain weighing 180-250 gm and aged 10 – 12 weeks, were used for the study. They were acclimatized for a period of two weeks. The animals were housed under standard conditions, 12 hour:12-hour light-dark cycle, 50% humidity, and a temperature of 28°C. The animals were housed in groups of 3 in polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the study and supplied with standard laboratory food granules (VRK Nutritional Solutions, Pune, India) and water ad libitum.

Thirty male Wistar albino rats were used for the pesticide-induced model for testicular dysfunction and were randomly allotted into five groups of six rats each (Supplementary Figure 1) and grouped as control, toxic (CPF17.5mg/kg), Jackfruit (JFE 450mg/kg), treatment 1 (CPF (17.5mg/Kg)+JFE (450mg/Kg)) and treatment 2 (CPF (17.5mg/Kg)+JFE (6000mg/Kg)). Testicular damage was induced by oral administration of Chlorpyrifos with corn oil as a vehicle for a duration of 30 days. The CPF and JFE were administered through the oral route and using corn oil as a vehicle. All animals in different groups received their respective compounds/extract for 30 days.

At the end of the experiment macroscopic parameters such as body weight and testicular weight and microscopic parameters including sperm count[10], sperm motility[11], sperm viability[11] and chromatin integrity[12] were evaluated. A sample for sperm analysis was obtained from the epididymis. Samples were evaluated histopathologically. Sperm analysis and histopathological examination were carried out at the Department of Pathology, KMC, MAHE, Manipal, India; Biochemical tests including total protein, antioxidant assay including lipid peroxide, and catalase test were performed at the Department of Biochemistry, KMC, MAHE, Manipal, India.

2.2.1 Sperm Count

The epididymis collected was first minced manually and homogenized in 1mL of phosphate buffer saline (PBS) of pH 7.2 to obtain a suspension. The obtained suspension was used for semen analysis. The suspension was filtered through a nylon mesh (80µm). The sperm count was done using the filtrate as per the standard method with the Neubauer chamber. Briefly, an aliquot from the suspension was taken (up to 0.5 mark) in the leukocyte hemocytometer and diluted with PBS up to mark 11. The suspension was mixed well and then charged into the Neubauer counting chamber. Total sperm count in eight squares (except the central erythrocyte area) of 1 mm^2 each was determined and multiplied by 5x104 to express the number of spermatozoa per epididymis.

2.2.2 Sperm Motility

Manual method was used to assess sperm motility. In this assessment, an easy method is to count the number of stationary sperms in the sample, fix the sample and count the total number of sperm. The assessment of the percentage of progressively motile sperm, which is defined as the number of progressively motile sperm/ total number of sperm x100 was done.

2.2.3 Sperm Viability

Eosin staining was used for this procedure. About 10 µl of the aliquot was taken on a fresh and clean slide and then 0.5% Eosin stain was added to it to prepare a smear and was allowed to air-dry so that the sample is fixed. It was focused under 40X light microscope.200 spermatozoa were counted to segregate into dead (stained) and live (unstained) sperms. Viability percentage was calculated by taking a ratio of live sperms to the total number of sperms counted.

Chromatin Integrity was done using Aniline Blue (AB) staining. Smears of fresh sperm of each case was air dried and fixed in 3% buffered glutaraldehyde in 0.2 M phosphate buffer (pH = 7.2) for 30 min at room temperature. Each smear was treated with 5% aqueous AB stain in 4% acetic acid (pH = 3.5) for 5 min. At least, 200 spermatozoa were counted in each slide by light microscopy. Unstained or pale blue stained cells and dark blue cells were considered normal and abnormal spermatozoa, respectively. At least 200 sperm cells were evaluated in each slide and the percentage of abnormal spermatozoa was reported.

At the end of the study, all animals were euthanized with the standard technique of overdose of intraperitoneal ketamine.

IMPPAT 2.0 (Indian Medicinal Plants, Phytochemistry and Therapeutics) database[12,13] was used to search for the active compounds. The potential target and gene list thus obtained using various databases was imported into the Cytoscape to construct an active compound and target network[14].

Statistical analysis of samples was carried out using SPSS v23. One-way ANOVA was used followed by post hoc analysis using Tukey's test. The statistical significance was set at $p<0.05$ at a confidence interval of 95%. The data was expressed as MeanStandard deviation (S.D.)

3. Results

3.1 Body Weight

At baseline, there was no significant difference in the body weight of the Wistar albino rats in different groups. However, a statistically significant difference in the same was noted at the end of the study except in the control group. Overall, the weight gain in the treatment groups was greater, particularly with treatment 1 (Table 1).

3.2 Testicular Weight

There was an increase in the testicular weight of the rats fed on jackfruit extract compared to the normal control and was statistically significant ($p<0.05$). The testicular weight was less in the treatment groups compared to the toxic group, which was statistically significant (Figure 1).

Table 1. Change in body weight from baseline to the end of the study (original)

	Body weight(gms) (mean±SD)	
Group	**Day 1**	**DAY 30**
Control (CPC)	216.637.23	268.016.04
Chlorpyrifos(CPF)	231.623	263.016.43*
Jackfruit (JFK)	224.115.6	271.023.5*
CPF+JFE-450	221.618.34	359.049.9*
CPF+JFE-600	215.022.58	290.019.03*

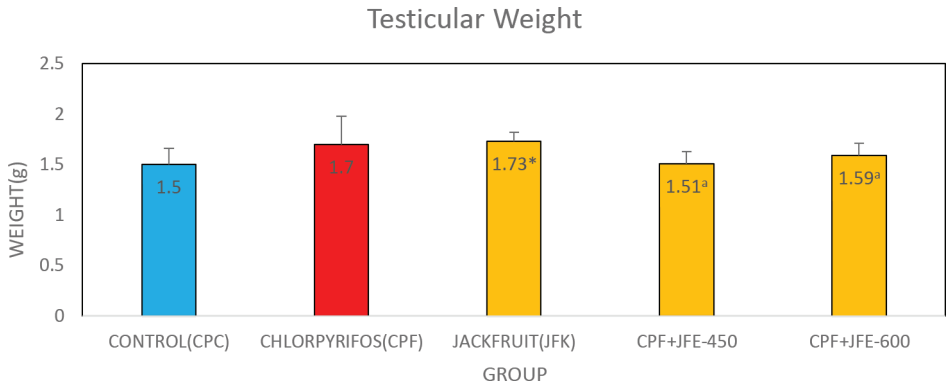
* statistically significant with p <0.05 for CPF vs treatment groups (JFK, JFE-450 and JFE-600)
JFE-450 denotes 450 mg/kg and JFE-600 denotes 600 mg/kg.
Comparison was done by one-way ANOVA followed by post hoc Tukey's analysis.

3.2 Sperm Count

Sperm count was maximum in the treatment groups, highest in the JFE-600 group, and least in the CPF group. The increase in the sperm count was dose-dependent as there was a significant increase in JFE-600 when compared to the JFE-450 group (Figure 2).

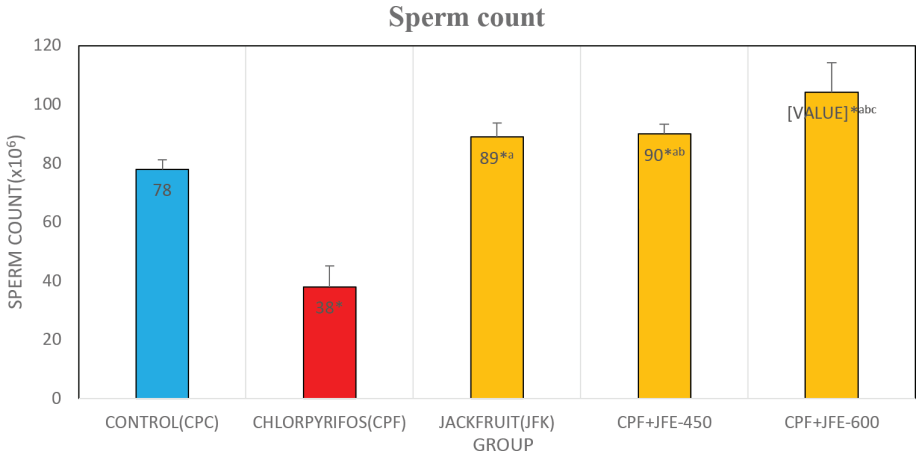
3.3 Sperm Motility

The sperm motility was significantly increased in the jackfruit control group as well as treatment groups, particularly the treatment 2 group, compared to the CPF group, which recorded the lowest. Among the treatment



* denotes p<0.05 for Control vs Jackfruit group
[a] statistically significant p<0.05 for Toxic (CPF) group vs Treatment groups (JFE-450 and JFE-600)
All values are expressed as MeanS.D. Comparison was done by one-way ANOVA followed by post hoc Tukey's analysis.

Figure 1. Mean change in the testicular weight (original).



All values are denoted
*denotes p<0.05 for Control vs All other groups (CPF, JFK, JFE-450, JFE-600)
a denotes p<0.05 for CPF(Toxic) vs JFK, JFE-450, JFE-600
b denotes p<0.05 for Control vs JFE-450, JFE-600
c denotes p<0.05 for JFE-450 vs JFE-600
All values are expressed as Mean± S.D. Comparison was done by one-way ANOVA followed by post hoc Tukey's analysis.

Figure 2. Comparison of Sperm count among the study groups (original).

groups, motility was highest in the JFE-600 group (Figure 3).

3.4 Sperm Viability

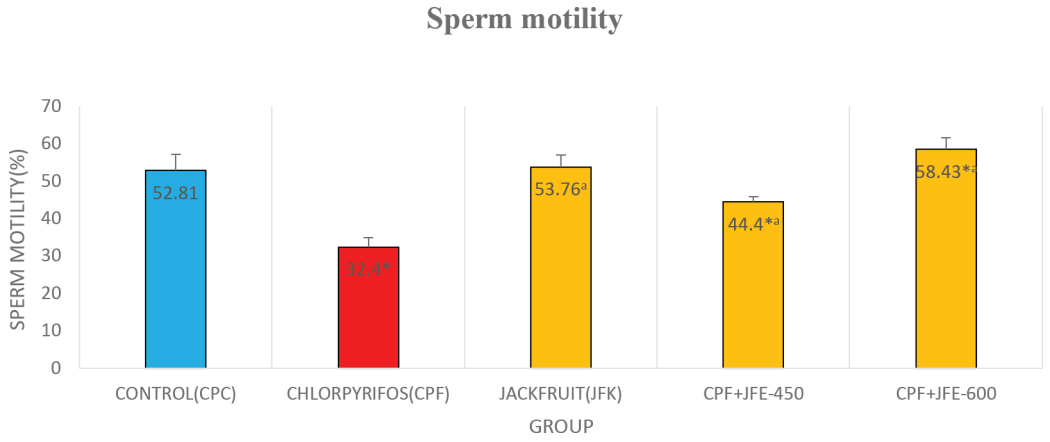
Sperm viability or vitality was significantly reduced with CPF and increased in all the other groups that received JFE (Figure 4). Supplementary Figure 2 depicts sperm motility under a high power field.

3.4.1 Chromatin Integrity

There was a significant decrease in DNA damage in those groups that received JFE compared to the CPF group (Figure 5). Supplementary Figure 3 depicts chromatin integrity.

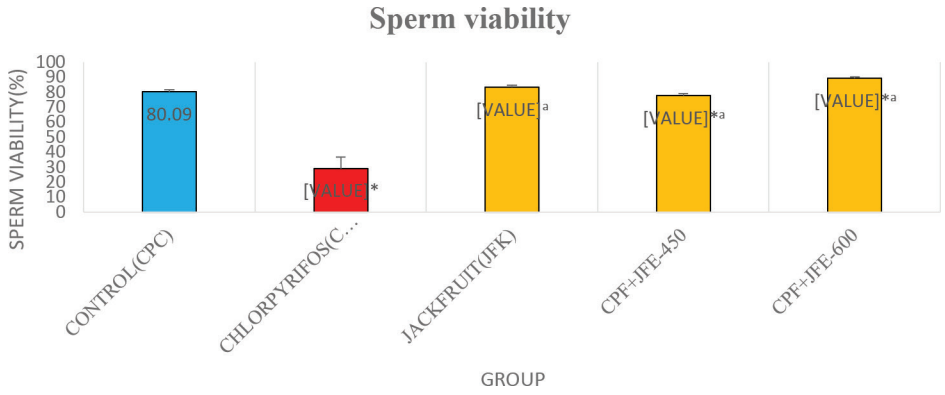
There was an increase in total protein in the treatment groups at par with the control group and a decrease in the toxic group. However, there was no significant difference between protein content in the control and treatment groups. There was a reduction in malondialdehyde (MDA) levels and an increase in Catalase activity in the jackfruit treatment groups (Supplementary Table 1).

Histopathological evaluation revealed normal tissue architecture in control, severe toxic changes, and no toxic changes, in CPF and JFE groups, respectively (Figure 6) and a reduction in toxic changes compared with CPF in JFE 450 and 600 groups (Figure 7). The degenerated tubules were



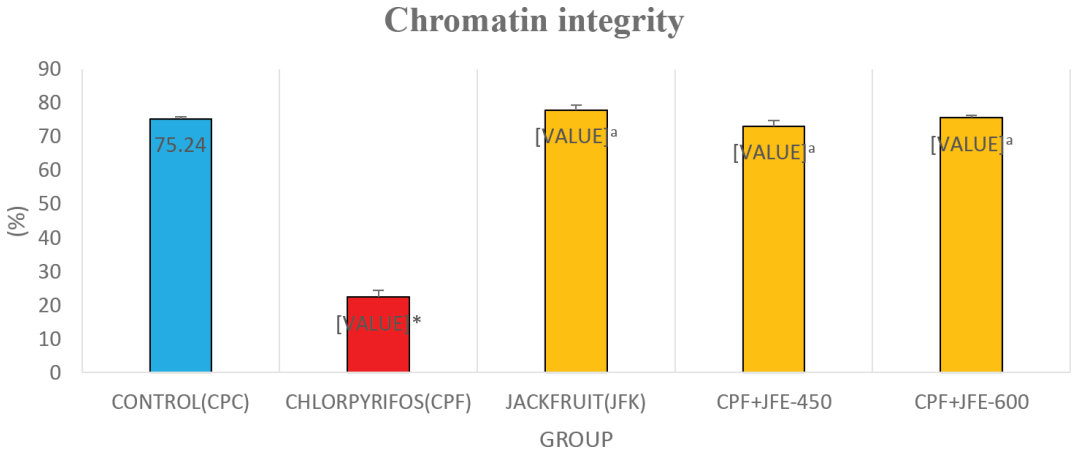
*denotes p<0.05 for Control vs other groups CPF, JFE-450,JFE-600
ª denotes p<0.05 for CPF vs JFK, JFE-450 and JFE-600
All values are expressed as Mean S. D. Comparison was done by one-way ANOVA followed by post hoc Tukey's analysis.

Figure 3. Sperm motility observed among the different groups (original).



*denotes p<0.05 for Control vs CPF, JFE-450 and JFE-600
ª denotes p<0.05 for CPF vs JFE, JFE-450 and JFE-600
All values are expressed as MeanS.D. Comparison was done by one-way ANOVA followed by post hoc Tukey's analysis.

Figure 4. Sperm viability among the different groups (original)



*denotes p<0.05 for Control vs CPF

[a] denotes p<0.05 for CPF vs JFK, JFE-450 and JFE-600

All values are expressed as MeanS.D. Comparison was done by one-way ANOVA followed by post hoc Tukey's analysis.

Figure 5. Chromatin integrity (original).

much less in the JFE 450 group compared to the CPF group.

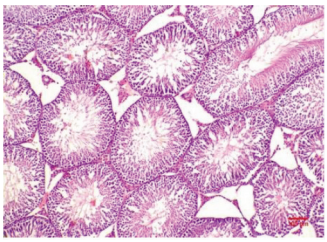
Of the 51 active compounds collected from the IMPPAT 2.0 database, one compound (Propanol) had canonical SMILES of < 5, hence 50 active compounds were run through the Swiss target prediction database and ~100 targets were obtained for each active compound. With the help of Cytoscape, 12 potential targets were analyzed that identified 42 nodes and 48 edges for male infertility. Our network analysis suggested that the mechanism of action of *A. heterophyllus* to treat male infertility might be related to the Androgenic Receptor (AR), estrogen receptor gene (ESR1), B-cell leukemia/lymphoma 2 (BCL2) protein, mouse double minute 2 (MDM2), chemical carcinogenesis receptor activation, phosphoinositide 3-kinase (P13K-AKT) signaling pathway and pathways in cancer.

4. Discussion

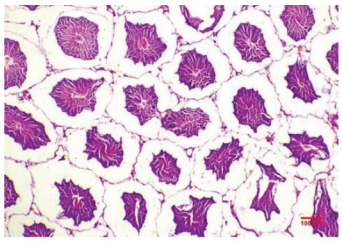
Nature has provided solutions to our health problems, and exploration of which will be of tremendous benefit to humans. One such plant whose health benefits remain unexplored is *Artocarpus*, belonging to the Moraceae family, grown abundantly in the Western Ghats of India, Asia, Africa, and certain regions of South America. Non-toxicity in animal studies established its safety[15], strengthening its promotion in human health. Various parts of the plant have been used as food additives in ice-creams, jams, jellies, and marmalade[16-18]. It is tested for its clinical utility as an anticarcinogenic, antimicrobial, and antifungal agent; it is believed to possess anti-inflammatory, wound healing, laxative, and hypoglycemic properties[19-22]. It also elevates the low-density lipoprotein: high-density lipoprotein ratio and is considered to have a beneficial impact on high blood pressure, heart disease, strokes, and bone loss, and improves muscle and nerve function[23]. It's also a rich source of vitamins B12, and C, and minerals. However, its properties remain under-explored due to complexities in processing, high perishability, and variation in the physical and biochemical properties making it less attractive as a commercial crop, despite its huge medicinal potential. Ours is an attempt to tap its potential use in male infertility, in which we evaluated the possible ameliorative effect of *Artocarpus* (Jackfruit) on chlorpyrifos-induced infertility.

In the diabetic models, the hypoglycemic effect of jackfruit was seen through inhibitory action on α-amylase enzyme and reduction in ROS level[24,25]. In immunomodulatory and anti-inflammatory studies, *Artocarpus* showed increased proinflammatory and anti-inflammatory cytokine activity[26-29].

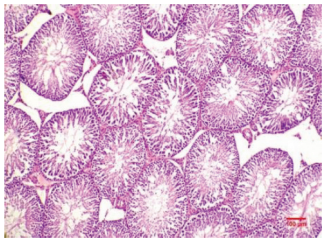
The concept of using an antioxidant to overcome oxidative stress and adopted as a treatment option in a number of diseases or disorders[30], including infertility,



Control: sections showing seminiferous tubules consisting of several layers of cells i.e., sertoli cells, spermatogonia, spermatocytes, spermatids and all stages of spermatogenic cells closely arranged in rows. Image demonstrates normal spermatogenesis and spermiogenesis with high proportion of spermatozoa.



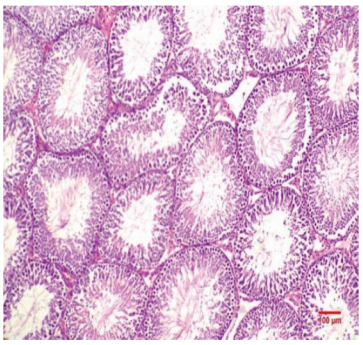
CPF: Sections showing atrophy and degeneration of tubules in the periphery with damaged epithelium, incomplete and irregular spermatogenesis; depletion of germ cell layers in tubules, with necrosis in few tubules. Image also depicts vacuolar degeneration of tubule, retained spermatids loss of tissue architecture



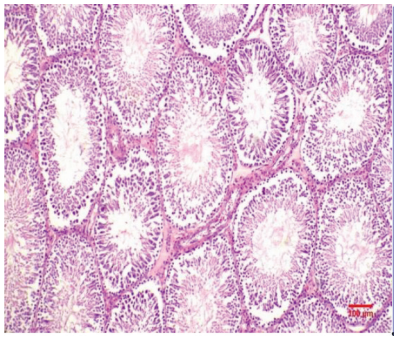
JFE: There was no degeneration, atrophy, or necrosis of the tubules. The germ cells were arranged in an organized manner in the tubules. Spermatogenesis and spermiogenesis process was normal with high proportion of spermatozoa.

Figure 6. HPE features of testicular tissue of animals of control, toxic and JFE groups (original).

wherein a reduction in ROS levels has been linked to improving male and female infertility[3]. Female infertility using antioxidants is studied considerably, but male infertility has not received the deserved attention.



JFE-450: There were no necrosed tubules, showed few degenerated tubules, which were very less compared to the CPF group. There were retained spermatids in few tubules. There was a good proportion of spermatozoa.



JFE-600: There were no necrosed tubules. Very few tubules showed degenerated germ cells and mild germ cell loss. There was a good proportion of spermatozoa.

Figure 7. HPE features of testicular tissue of animals of treatment JFE-450 and JFE-600 (original).

We conducted this study using the antioxidant property of jackfruit to explore its therapeutic application in male infertility.

Chlorpyrifos, an organophosphorus compound and a pesticide with the anti-androgenic property. We administered chlorpyrifos 17.5 mg/kg to male Wistar albino rats, which resulted in minimal weight gain (~20-30 g) though with a slight increase in testicular weight compared to the control; this observation was in line with previous reports[31]. There was a major drop in the sperm count as well as sperm motility and chromatin integrity. Degeneration of seminiferous tubules along with severe damage to the spermatogenesis cycle such as retained spermatids and depletion of the germ layers was noticeable in HPE, which is a characteristic feature of CPF toxicity[32-34]. In our study, histopathology displayed

the microscopic level of damage and protection offered by JEE was proved. There was a significant tissue degeneration in the toxic group whereas minimal or nil degeneration in the treatment groups. Mortality, resultant of toxic effect of CPF can be expected and we report one death in the CPF treated group.

Oxidative stress is the major cause for the CPF associated toxicity[35]. Reactive oxygen species are required in extremely low and controlled levels for sperm proliferation, differentiation and function, which is similar in humans as well. High seminal ROS levels, reported in ~40% of infertile men, is a major limiting factor in bringing about positive outcome in an IVF cycle[36]. It is very interesting to note that a sperm is also capable of producing very small amounts of ROS and after the process of spermiation, the cytoplasm released consists of antioxidants that neutralise the ROS produced. Lack of cytoplasm also results in lack of antioxidants and increase in the free radical concentration[37]. In our study, investigational antioxidant assays including MDA and catalase indicated a drop in antioxidant levels in the CPF group owing to the oxidative stress due to the CPF.

Extracts of jackfruit possess antioxidant compounds[38], which was explored in an animal study designed for neurogenerative disorders where the peroxyl and hydroxyl radicals were scavenged by the phytochemicals present in ripe fruit extract[39]. This study explores the antioxidant activity of jackfruit in testicular dysfunction as a therapeutic option, which has remained unexplored. In our study, there was a very significant weight gain in the jackfruit groups probably due to its high fat content[29,40], and no significant changes were observed in the sperm count similar to the previous studies[39]. We noted a dose response relationship, with higher dose having better efficacy as demonstrated by improvement in sperm parameters such as sperm count, motility as well as chromatin integrity despite being administered a toxic agent like chlorpyrifos. Higher dose of JFE (600mg/kg) showed almost absolute reversal of testicular toxicity induced by CPF at the end of the study. Histopathology also showed a very minimal testicular degeneration and damage among the treatment groups compared to the toxic group. Our results demonstrate that effects of JFE of 450mg/kg are comparable to that of control group, and higher doses proved better efficacious, it would be a better option to use dose of 600mg/kg in the treatment group.

There are very few studies on the effect of *Artocarpus* on male fertility. Ratnasooriya, *et al.*,[41] have reported that *A. heterophyllus* seeds inhibits sexual competence but not fertility of male rats, which were independent of general toxicity profile and was attributed to induced sedation. It has shown no difference in ejaculating capability and fertility.

Chlorpyrifos was hypothesized to cause hypoproteinaemia but our study demonstrated that there is no significant difference in the protein content between the groups. Chlorpyrifos was thought to reduce protein content but it seems to act through some other pathway.

Additionally, we observed an increase in the body weight with JFE possibly due to the high carbohydrate and fat content of the fruit, while CPF did not significantly decreased the body weight of rats. The testicular weight was seen to be higher in the toxic group compared to the treatment groups which was an unexpected outcome. The exact cause for this is not known and probably due to the damage caused by CPF resulting in edema or inflammation. Sperm count and sperm motility significantly improved in the treatment groups despite being administered the toxic agent. Also, these parameters were even better than the control group. Sperm vitality and chromatin integrity was seen to be greatly compromised in the toxic group. These parameters had similar outcomes in normal and jackfruit control groups.

At the end of the study, we noticed reversal of changes, but we did not follow it up for longer time to note the nature of the reversal if it was temporary or permanent.

Antioxidant levels were greatly increased in the groups where jackfruit was used and CPF group showed low antioxidant activity. Total protein content was higher in the treatment groups when compared to toxic group but not much difference in comparison to the control group.

Analysis of active ingredient of jack fruit was supportive of utility in male infertility, demarking the pathways indicating the possible mechanisms of action. Our network analysis suggests that the mechanism of action of *A. heterophyllus* to treat male infertility might be related to AR, ESR1, BCL2, MDM2, chemical

carcinogenesis receptor activation, P13K-AKT signaling pathway and pathways in cancer.

JFE has a positive impact on various sperm parameters such as sperm count, motility, vitality, chromatin integrity demonstrated by decreased DNA damage. Antioxidant assays, MDA and Catalase clearly indicated the strong antioxidant activity of the jackfruit extract the toxic group shows very poor antioxidant levels.

Our results with jackfruit extract are promising, which can be further explored in male fertility. If successful, it will be a ray of hope for many with male infertility without any surgically correctable cause, and reducing the cost and waiting time in *in-vitro* fertilization.

5. Funding

Supported by the Department of Pharmacology, Kasturba Medical College, MAHE, Manipal. Chemicals were procured from the department fund. Laboratory investigations were done at the Department of Biochemistry and sperm analysis and histopathological analysis was done at the Department of Pathology, KMC, MAHE, which were done free of cost.

6. References

1. Deshpande PS, Gupta AS. Causes and prevalence of factors causing infertility in a public health facility. J Hum Reprod Sci. 2019; 12(4):287-93. https://doi.org/10.4103/jhrs.HRS_140_18
2. Katole A, Saoji AV. Prevalence of primary infertility and its associated risk factors in urban population of central India: A community-based cross-sectional study. Indian J Community Med. 2019; 44(4):337-41. https://doi.org/10.4103/ijcm.IJCM_7_19
3. Ganguly S, Unisa S. Trends of infertility and childlessness in India: findings from NFHS data. Facts Views Vis Obgyn. 2010; 2(2):131-8.
4. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. J Hum Reprod Sci. 2015; 8(4):191-6. https://doi.org/10.4103/0974-1208.170370
5. Alahmar AT. The effects of oral antioxidants on the semen of men with idiopathic oligoasthenoteratozoospermia. Clin Exp Reprod Med. 2018; 45(2):57-66. https://doi.org/10.5653/cerm.2018.45.2.57
6. Omar HS, El-Beshbishy HA, Moussa Z, Taha KF, Singab AN. Antioxidant activity of *Artocarpus heterophyllus* Lam. (Jack Fruit) leaf extracts: remarkable attenuations of hyperglycemia and hyperlipidemia in streptozotocin-diabetic rats. Scientific World Journal. 2011; 11:788-800. https://doi.org/10.1100/tsw.2011.71
7. Miranda-Contreras L, Gómez-Pérez R, Rojas G, Cruz I, Berrueta L, Salmen S, Colmenares M, *et al.* Occupational exposure to organophosphate and carbamate pesticides affects sperm chromatin integrity and reproductive hormone levels among Venezuelan farm workers. J Occup Health. 2013; 55(3):195-203. https://doi.org/10.1539/joh.12-0144-FS
8. Zhang X, Cui W, Wang K, Chen R, Chen M, Lan K, *et al.* Chlorpyrifos inhibits sperm maturation and induces a decrease in mouse male fertility. Environ Res. 2020; 188:109785. https://doi.org/10.1016/j.envres.2020.109785
9. Tramontin DP, Cadena-Carrera SE, Bella-Cruz A, Cé Bella Cruz C, Bolzan A, Quadri MB. Biological activity and chemical profile of Brazilian jackfruit seed extracts obtained by supercritical CO_2 and low pressure techniques. The Journal of Superficial Fluids. 2019; 152: 104551. https://doi.org/10.1016/j.supflu.2019.104551
10. Bae WJ, Ha US, Kim KS, Kim SJ, Cho HJ, Hong SH, *et al.* Effects of KH-204 on the expression of heat shock protein 70 and germ cell apoptosis in infertility rat models. BMC Complementary and Alternative Medicine. 2014; 14(1):1-9. https://doi.org/10.1186/1472-6882-14-367
11. Lotti F, Maggi M. Sexual dysfunction and male infertility. Nat Rev Urol. 2018; 15(5):287-307. https://doi.org/10.1038/nrurol.2018.20
12. Sai L, Li X, Liu Y, Guo Q, Xie L, Yu G, *et al.* Effects of chlorpyrifos on reproductive toxicology of male rats. Environment and Toxicology. 2014; 29(9):1083–8. https://doi.org/10.1002/tox.21838
13. Vivek-Ananth RP, Mohanraj K, Sahoo AK, Samal A. IMPPAT 2.0: An enhanced and expanded phytochemical atlas of Indian medicinal plants. ACS Omega. 2023; 8(9):8827-45. https://doi.org/10.1021/acsomega.3c00156
14. Li S, Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application. Chin J Nat Med. 2013; 11(2):110-20. https://doi.org/10.1016/S1875-5364(13)60037-0
15. Sairam S, Urooj A. Safety evaluation of *Artocarpus altilis* as pharmaceutical agent in wistar rats. J Toxicol. 2014; 2014:980404. https://doi.org/10.1155/2014/980404
16. Mushumbusi DG. Production and characterization of jackfruit jam. [Master of Science in Food Science Thesis] Morogoro, Tanzania: Sokoine University of Agriculture; 2015. Available from: https://www.suaire.sua.ac.tz/handle/123456789/1203
17. Roy SK, Joshi GD. Minor fruits-tropical. In: Salunkhe DK, Kadam SS, editors. Handbook of Fruit Science and Technology. NY. USA: Marcel Dekker Inc, 1995. p. 570–97.
18. Nakasone HY, Paull RE. Tropical Fruits. Oxon, UK: CAB International; 1998. Available from: https://www.cabdirect.

org/cabdirect/abstract/19980307057 last accessed on Dec 10, 2022
19. Shanmugapriya K, Saravana PS, Payal H, Peer Mohammed S, Binnie W. Antioxidant activity, total phenolic and flavonoid contents of *Artocarpus heterophyllus* and *Manilkara zapota* seeds and its reduction potential. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(5):256–60.
20. Ranasinghe RASN, Maduwanthi SDT, Marapana RAUJ. Nutritional and health benefits of jackfruit (*Artocarpus heterophyllus* Lam.): A review. Int J Food Sci. 2019; 2019:4327183. https://doi.org/10.1155/2019/4327183
21. Yeh CJ, Chen CC, Leu YL, Lin MW, Chiu MM, Wang SH. The effects of artocarpin on wound healing: *in vitro* and *in vivo* studies. Sci Rep. 2017; 7(1):15599. https://doi.org/10.1038/s41598-017-15876-7
22. Gupta A, Marquess AR, Pandey AK, Bishayee A. Jackfruit (*Artocarpus heterophyllus* Lam.) in health and disease: a critical review. Crit Rev Food Sci Nutr. 2022; 1-35. https://doi.org/10.1080/10408398.2022.2031094
23. Swami SB, Thakor NJ, Haldankar PM, Kalse SB. Jackfruit and its many functional components as related to human health: a review. Comprehensive Reviews in Food Science and Food Safety. 2012; 11(6):565–76. https://doi.org/10.1111/j.1541-4337.2012.00210.x
24. Gupta N, Jain UK, Pathak AK. Wound healing properties of *Artocarpus heterophyllus* Lam. Anc Sci Life. 2009; 28(4):36-7.
25. Yao X, Wu D, Dong N, Ouyang P, Pu J, Hu Q, *et al*. Moracin C - A Phenolic Compound Isolated from *Artocarpus heterophyllus*, suppresses lipopolysaccharide-activated inflammatory responses in murine raw264.7 macrophages. Int J Mol Sci. 2016; 17(8):1199. https://doi.org/10.3390/ijms17081199
26. de Souza LP, Ramos EL, Santana SS, Silva MV, Santiago FM, Mineo TW, *et al*. Lectins from *Synadenium carinatum* (ScLL) and *Artocarpus heterophyllus* (ArtinM) are able to induce beneficial immunomodulatory effects in a murine model for treatment of *Toxoplasma gondii* infection. Frontiers in Cellular and Infection Microbiology. 2016; 6:164. https://doi.org/10.3389/fcimb.2016.00164
27. Wiater A, Paduch R, Trojnar S, Choma A, Pleszczyńska M, Adamczyk P, *et al*. The effect of water-soluble polysaccharide from jackfruit (*Artocarpus heterophyllus* Lam.) on human colon carcinoma cells cultured *in vitro*. Plants. 2020; 9(1):103. https://doi.org/10.3390/plants9010103
28. Prakash O, Kumar R, Mishra A, Gupta R. *Artocarpus heterophyllus* (Jackfruit): an overview. Pharmacognosy Reviews. 2009; 3(36):353-58. Available from: https://www.phcogrev.com/sites/default/files/PhcogRev-3-6-353.pdf last accessed on Dec 10, 2022.
29. Li J, Pang G, Ren F, Fang B. Chlorpyrifos-induced reproductive toxicity in rats could be partly relieved under high-fat diet. Chemosphere. 2019; 229:94-102. https://doi.org/10.1016/j.chemosphere.2019.05.020
30. Ajiboye BO, Ojo OA, Adeyonu O, Imiere OD, Fadaka AO, Osukoya AO. Ameliorative activity of ethanol extract of *Artocarpus heterophyllus* stem bark on pancreatic β-cell dysfunction in alloxan-induced diabetic rats. J Evid Based Complementary Altern Med. 2017; 22(4):538-43. https://doi.org/10.1177/2156587216685510
31. Kheradmandi R, Jorsaraei SGA, Feizi F, Moghadamnia AA, Neamati N. Protective effect of n-acetyl cysteine on chlorpyrifos-induced testicular toxicity in mice. Int J Fertil Steril. 2019; 13(1):51-6. https://doi.org/10.22074/ijfs.2019.5494
32. Recio-Vega R, Ocampo-Gómez G, Borja-Aburto VH, Moran-Martínez J, Cebrian-Garcia ME. Organophosphorus pesticide exposure decreases sperm quality: association between sperm parameters and urinary pesticide levels. J Appl Toxicol. 2008; 28(5):674-80. https://doi.org/10.1002/jat.1321
33. Singh N, Lawana V, Luo J, Phong P, Abdalla A, Palanisamy B, *et al*. Organophosphate pesticide chlorpyrifos impairs STAT1 signaling to induce dopaminergic neurotoxicity: Implications for mitochondria mediated oxidative stress signaling events. Neurobiol Dis. 2018; 117:82–113. https://doi.org/10.1016/j.nbd.2018.05.019
34. Bisht S, Faiq M, Tolahunase M, Dada R. Oxidative stress and male infertility. Nat Rev Urol. 2017; 14(8):470-85. https://doi.org/10.1038/nrurol.2017.69
35. Aitken RJ. Reactive oxygen species as mediators of sperm capacitation and pathological damage. Mol Reprod Dev. 2017; 84(10):1039-52. https://doi.org/10.1002/mrd.22871
36. Majzoub A, Arafa M, Mahdi M, Agarwal A, Al Said S, Al-Emadi I, *et al*. Oxidation-reduction potential and sperm DNA fragmentation, and their associations with sperm morphological anomalies amongst fertile and infertile men. Arab J Urol. 2018; 16(1):87-95. https://doi.org/10.1016/j.aju.2017.11.014
37. de Lamirande E, Jiang H, Zini A, Kodama H, Gagnon C. Reactive oxygen species and sperm physiology. Rev Reprod. 1997; 2(1):48-54. https://doi.org/10.1530/ror.0.0020048
38. Daud MNH, Fatanah DN, Abdullah N, Ahmad R. Evaluation of antioxidant potential of *Artocarpus heterophyllus* L. J33 variety fruit waste from different extraction methods and identification of phenolic constituents by LCMS. Food Chem. 2017; 232:621-32. https://doi.org/10.1016/j.foodchem.2017.04.018
39. Stenqvist A, Oleszczuk K, Leijonhufvud I, Giwercman A. Impact of antioxidant treatment on DNA fragmentation

index: A double-blind placebo-controlled randomized trial. Andrology. 2018; 6(6):811-6. https://doi.org/10.1111/andr.12547

40. Fernando MR, Wickramasinghe N, Thabrew MI, Ariyananda PL, Karunanayake EH. Effect of *Artocarpus heterophyllus* and *Asteracanthus longifolia* on glucose tolerance in normal human subjects and in maturity-onset diabetic patients. J Ethnopharmacol. 1991; 31(3):277-82. https://doi.org/10.1016/0378-8741(91)90012-3
41. Ratnasooriya WD, Jayakody JR. *Artocarpus heterophyllus* seeds inhibits sexual competence but not fertility of male rats. Indian J Exp Biol. 2002; 40(3):304-8.