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Original Research Article

Ex-vivo Assessment of Mango (*Mangifera indica*) and Cashew (*Anacardium occidentale*) Leaf Extracts on Male Fertility: Impact on Sperm Parameters

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ABSTRACT

Rapid population growth challenges developing nations. Mango (*Mangifera indica*) and Cashew (*Anacardium occidentale*) leaves, known for antimalarial effects, demonstrate spermicidal potential, suggesting natural fertility control options amid rising reproductive health concerns and limited access to conventional contraceptives. Hence, this study looked into the *in vitro* potential of the aqueous extracts of *M. indica* and *A. occidentale* leaves on sperm concentration, sperm motility, and sperm morphology. The extracts were prepared by dissolving 0.5 g of the dried leaf powder in 100 mL of distilled water. The aqueous extracts of the herbs (10 µL) were added directly to 10 µL of sperm suspension of the sperm collected from adult male albino rats. The spermicidal effects of the extracts were observed using a microscope and compared to those of normal control sperm at the same volume ratio. The results showed that *Mangifera indica* significantly reduced sperm concentration and sperm motility, with p-values of $p < 0.01$ and $p < 0.001$, respectively. *Anacardium occidenta*le leaves extracts significantly ($p < 0.01$) reduced sperm motility, with both extracts exerting varying effects on sperm morphology. Findings from this study revealed that *Mangifera indica* and *Anacardium occidentale* leaf extracts not only reduced sperm concentration and motility but also induced morphological abnormalities in spermatozoa (Head, neck, and tail sperm defects) when compared to the normal control sperm. This indicates that the studied leaves exhibit spermicidal effects, highlighting their potential use as natural agents for male fertility control.

Keywords: Birth control, Natural remedy, Infertility, Sperm parameter.

Introduction

There is an increase in population worldwide, with fertility rates at or below the replacement level following years of rapid demographic changes. According to the United nation estimates, the world's population will rise from its current 7 billion to 10 billion by the year 2100 with over half of that growth taking place in sub-Saharan Africa (SSA) mostly in high-fertility nations.[1] Population numbers in SSA increased steadily between 2013 and 2023 rising from 981 million in 2013 to 1241 million in 2023 according to World Bank 2024. In West African nations, the overall fertility rate is still high despite multiple interventions.[2] This emphasizes the necessity of creative approaches to curb fertility.

Fertility is the ability to establish a clinical pregnancy[3]. Birth control pills and condoms are two of the many ways to help control pregnancy.[4] Choosing an appropriate contraceptive method is often complicated by considerations such as cost, future reproductive plans, and potential side effects. [1]

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The mechanisms and adverse effects of available birth control options vary widely, with combined hormonal contraceptives such as pills, patches, and vaginal rings containing estrogen and progesterone, commonly associated with headaches, nausea, breast tenderness, and menstrual irregularities.[5,6] Progestin-only options like minipills can also induce headaches, depression, acne, reduced libido, and raise the risk of ectopic pregnancy.[7,8] Injectable progestin, such as Depo-Provera, administered every three months, is known to cause weight gain, mood swings, headaches, and decreased bone mineral density.[9] Barrier methods, including spermicides, diaphragms, and condoms, may trigger allergic reactions or skin irritation, particularly with latex-based products.[10] Emergency contraceptive pills, though useful after unprotected intercourse, are frequently accompanied by dizziness, nausea, exhaustion, and headaches.[11] Collectively, these varying effects highlight the need for more accessible, affordable, and well-tolerated fertility control options, especially in resource-limited settings.

More than 35,000 plant species are used medicinally in many cultures around the world.[12] Approximately 80% of the world's population depends on herbal remedies for primary healthcare, with the majority using herbal medicines.[13] Interest in using herbal medicines to treat various diseases and manage fertility is growing. Several plant products that exhibit spermicidal activity and alter hormone levels (including luteinizing hormone, testosterone, and follicle-stimulating hormone) have been shown to impair fertility in both males and females.[14, 15] These products may one day be turned into contraceptives. Few native plants have had their antifertility properties fully studied, despite many being known to induce pregnancy prevention. Considerable strides have been made in creating reversible, highly effective, and socially acceptable forms of birth control for women [16] Male contraceptive options and advancements are still scarce. It is necessary to create new male contraceptive methods in light of recent developments in the understanding of male reproductive physiology.[17]

According to reports, medicinal plants with antiplasmodial properties, such as cashew (*Anacardium occidentale*) and mango (*Mangifera*

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indica) leaves, can have adverse effects on the male reproductive system, including toxicity, damage to sperm parameters (Volume, concentration, motility, and morphology), and hormonal disruption.[18] This dual purpose raises the possibility of using these plants as natural birth control options. Identification of plant phytochemicals may be useful in understanding their mechanisms of action and the possible side effects of some constituents.[19]

Mangifera indica L leaves (Mango leaves) are prized for their therapeutic and nutritional qualities.[20] It is a member of the Anacardiaceae family. Anti-inflammatory, antimalarial, and antioxidant effects are among its documented pharmacological properties. [20] *Mangifera indica* leaves have been shown to have antimalarial properties in recent research. [21]

Anacardium occidentale, also known as cashew, is often referred to as such. This evergreen tree is native to Brazil and is a member of the Anacardiaceae family.[22] Extracts from these plant leaves are used to treat malaria[23] and noted to be rich in antioxidants[24]. Therefore, this study aims to assess the *in vitro* impact of aqueous extracts from cashew (*Anacardium occidentale*) and mango (*Mangifera indica*) leaves on sperm parameters to determine their potential effects as male contraceptives.

Materials and Methods

Sample Collection and Extract Preparation

Fresh leaves of *Mangifera indica* and *Anacardium occidentale* were obtained from the botanical garden of the Federal University of Technology, Akure (FUTA) (https://maps.app.goo.gl/ZptTKNKwavMPrxbcA) on the 16th June, 2024. They were first identified using morphological characteristics and reference materials at the Functional Food and Nutraceutical Laboratory of the Federal University of Technology, Akure.

Afterwards, the leaf specimens were authenticated at the Center for Research and Development (CERAD), FUTA, with voucher numbers FUTA 0406 and FUTA 0407, respectively. The collected samples were properly washed, air-dried for one week at room temperature, and then ground into a fine powder. The aqueous extracts were prepared individually following a method described by Shodehinde *et al* (2016), [25]. A fine powder (0.5 g) of either plant leaf was measured into 100 mL of distilled water, mixed, and stirred for 3 hours separately. The mixtures were filtered using filter papers (Whatman no.1) and centrifuged to obtain a clear supernatant for analysis.

Qualitative Phytochemical Screening

The presence of phenol was detected using the Ferric Chloride Test by adding 3-4 drops of ferric chloride solution to the aqueous extracts of *Mangifera indica* and *Anacardium occidentale*. The formation of a bluish-black colour indicates the presence of phenols.[26] The Test for Flavonoids was carried out by preparing 1.0 ml of *Mangifera indica* and *Anacardium occidentale* individual aqueous extracts from both plants with sodium hydroxide (NaOH) solution. The appearance of the yellow solution, which disappeared with the addition of hydrochloric acid, indicates the presence of flavonoids.[27] *Mangifera indica* and *Anacardium occidentale* individual aqueous extracts (0.5 ml) were added to 2ml of distilled water to test for the presence of Saponins. The mixture was shaken and observed for 10 minutes to assess its persistent foaming property. If the foam produced remains for ten minutes, it reveals the presence of saponins.[28] Three (3) drops of $FeCl_3$ were added to a mixture of 0.5 mL of *Mangifera* indica and *Anacardium occidentale* individual extracts, and 10 mL of distilled water, respectively, to detect the presence of tannins. A visible green precipitate indicated the presence of tannins.[29] Aqueous solution of NaOH was added to a mixture of 0.1 mg of *Mangifera indica* and *Anacardium occidentale* individual extract and 1 ml of distilled water to detect the presence of glycosides. The Formation of yellow colour indicates the presence of glycosides.[30] The leaf sample (0.5 g) was mixed with 2 ml of chloroform and 2 ml of sulphuric acid to form a layer. A reddish-brown colour was observed, indicating the presence of terpenoids.[31]

Animal Purchase

Six (6) adult male albino rats weighing 200 - 230 g were obtained from the Animal House, Federal University of Technology, Akure, Nigeria. They were given free access to water and a commercial diet *ad libitum* and handled according to the guidelines laid down by the Centre for Research and Development (CERAD), Federal University of Technology, Akure, Nigeria.

Sperm Analysis

The male albino rats were sacrificed under anaesthesia, and their epididymis was collected, cleared of fats, and the sperm weight was determined per gram caudal epididymis[32]. The volume determination was performed according to Archimedes' principle (Volumetric method). The epididymis was placed into a container and quantified using a measuring cylinder by water displacement. Sperm suspensions were created by placing the epididymis in a pre-warmed (37°C) cell-culture dish with 1 mL of Phosphate Buffered Saline[33]. The epididymis was minced with a sterile surgical blade and incubated at room temperature for 5 minutes to allow spermatozoa to swim out into the solution[34]. The leaf extracts (5 mg/mL) were added to the sperm in a 1:1 ratio; 10 µL of each extract was added individually to 10 µL of the sperm suspension. Changes in sperm characteristics were observed and compared with control semen, which was left at room temperature without the addition of the extract. The following parameters were evaluated:

Epididymal sperm concentration

The concentration counts of the sperm cells were done using a newly improved Neubauer's counting chamber (Haemocytometer) (Deep 1/10 mm. LABART, Germany). Ten microliters of the aliquot sample were aspirated with a Pasteur pipette and mounted in the counting chamber.[35] After 5 minutes, it was observed under a light microscope (3H-Tokyo, Japan). [36]

Sperm motility

Motility was determined by diluting the minced sample with 10 mL of 0.9% normal saline and allowing it to stand for 5 minutes. This solution (10 µL) was observed at a magnification of ×100 under the light microscope.[37]

Progressive Assessment

Sperm motility was assessed over a minimum of five square strips, with each square being observed for 10 seconds. After counting the non-motile sperm, only the sperm that showed flagellar activity were considered motile.[38] They were categorised based on the swiftness of their movement (Fast and slow progression).

Sperm Morphology

The morphology of the spermatozoa was assessed based on smears prepared from sperm suspension. The spermatozoa were diluted at a 1:20 ratio with 10% neutral buffered formalin and evaluated for specific abnormalities.[39] In steps, sperm suspension and 1% eosin-y 5% nigrosin were mixed in equal volumes and then smeared on clean glass slides and air-dried. The sperm cells were observed at ×400 magnification using an Olympus light microscope (3H-Tokyo, Japan) to determine morphological abnormalities.[39] Sperm cell morphology was classified according to the presence of specific abnormalities, such as head defects (round head, small or large size, double or detached head), neck and middle piece defects (distended, irregular, bent middle piece, abnormally thin middle piece), and tail defects (short, irregular, coiled, or multiple tails).[39]

Ethical Consideration

The Animal Ethics Committee of the Federal University of Technology, Akure, Nigeria's Center for Research and Development (CERAD) approved the use and handling of the experimental rat model with the ethical number FUTA/ETH/21/01.

Statistical Analysis

The data obtained were expressed as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) followed by Tukey's multiple

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comparisons test was performed using GraphPad Prism 8.0 for Windows. The level of significance was accepted at $P < 0.05$.

Results and Discussion

Sperm parameters are essential in determining sperm quality.[40] Sperm quality, which is a major criterion for male fertility, is necessary to ascertain alternative birth control agents. The effect of *Mangifera indica* and *Anacardium occidentale* leaf aqueous extracts on sperm parameters *in vitro* was investigated during the screening for the presence of specific phytochemicals. The presence of phenol, flavonoid, tannin, glycoside, saponins, and terpenoid was screened in the selected leaf extracts. Phytochemicals present in the leaf extracts are represented in Table I. Results from the phytochemical screening (Table I) carried out on the extracts revealed the detectable presence of phenol, flavonoids, saponins, tannins, and terpenoids in *Mangifera indica* leaf extract and phenol, tannins, and saponins in *Anacardium occidentale* leaf extracts. Glycosides were not detected in either extract, with the *Anacardium occidentale* leaf extract also showing a non-detectable presence of flavonoids and terpenoids. Studies have shown that phytochemicals present in plants can have either positive or negative biological impacts on the plants. [19]

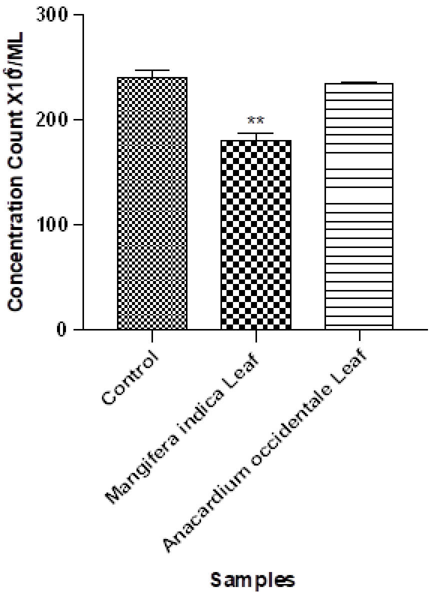

Figure 1: *In-vitro* effect of *Mangifera indica* and *Anacardium occidentale* aqueous leaf extracts on sperm concentration. Results are expressed in (Mean ± SD). ** indicates $p < 0.01$ versus control

The sperm analysis of extracts from Mangifera indica and *Anacardium occidentale* revealed negative effects on sperm parameters, including concentration, count, motility, progressive motility assessment, and morphology, as presented in Figures 1-8. *Mangifera indica* and *Anacardium occidentale* leaf extracts each produced a statistically significant reduction in sperm motile count and progressive assessments with p-values of $p < 0.001$ and $p < 0.01$ respectively as represented in Figures 2-4 with *Mangifera indica* leaf significantly ($p < 0.01$) reducing the concentration count (Figure 1) and exerting morphological abnormalities to the sperm (Figures 5-8). *Anacardium occidentale* leaf extract had its major effect on the morphological head defect as shown in Figure 6.

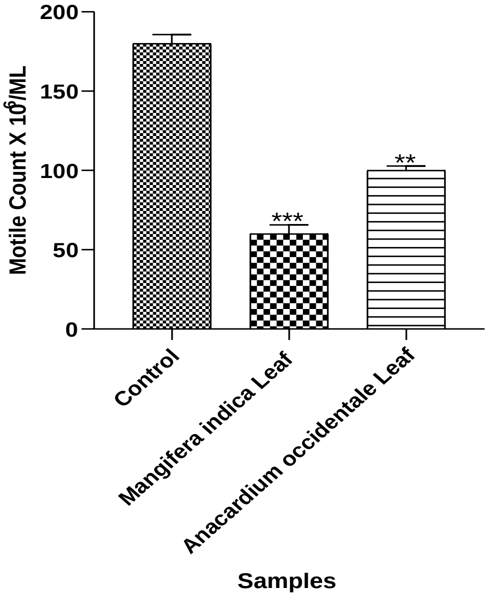

Figure 2: *In-vitro* effect of *Mangifera indica* and *Anacardium occidentale* aqueous leaf extracts on sperm motile count. Results are expressed in (Mean ± SD). ** indicates $p < 0.01$; *** indicates $p < 0.001$ versus control.

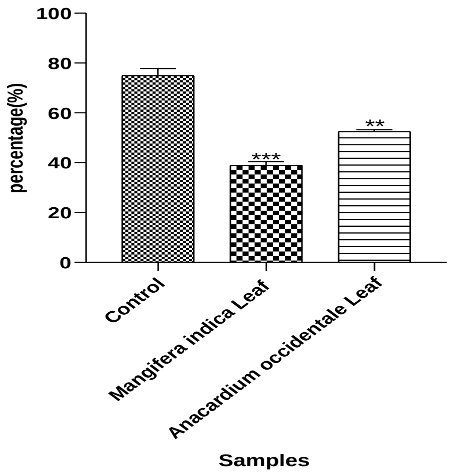

Figure 3: In-vitro effect of Mangifera indica and Anacardium occidentale aqueous leaf extracts on sperm progressive assessment (Fast). Results are expressed in (Mean ± SD). ** indicates p < 0.01; *** indicates p < 0.001 versus control.

The concentration count measures the number of sperm per millilitre of semen.[41] A significant ($p < 0.01$) reduction was noted in the concentration count of sperm treated with 5 mg/ml *Mangifera indica* leaf extract when compared to the control, as shown in Figure 1. This can be attributed to the strong presence of terpenoids, as represented by the (+) sign in Table I. Elevated levels of certain terpenoids may be harmful, potentially lowering sperm concentration.[42] Kumar *et al*. (2021) also revealed that *Mangifera indica* leaves contain a significant amount of terpenoids.[43] Motile Count signifies actively moving sperm.[44] A reduction in the motility of sperm disrupts its progressive ability to reach an egg compared to a normal sperm. *Mangifera indica* and *Anacardium occidentale* leaf aqueous extracts significantly reduced the motile count of sperm when compared to the control sperm, with p-values of $p < 0.001$ and $p < 0.01$, respectively. (Figure 2).

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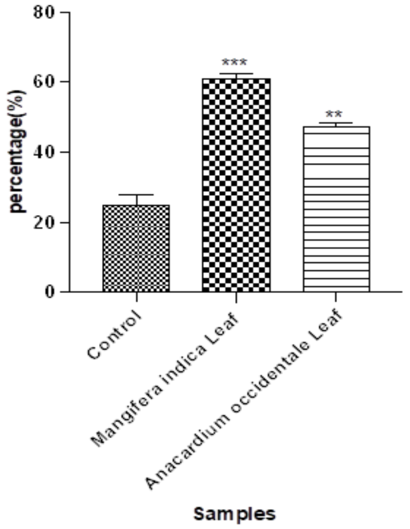

Figure 4: *In-vitro* effect of *Mangifera indica* and *Anacardium occidentale* aqueous leaf extracts on sperm progressive assessment (Slow). Results are expressed in (Mean ± SD). ** indicates $p < 0.01$; *** indicates $p < 0.001$ versus control.

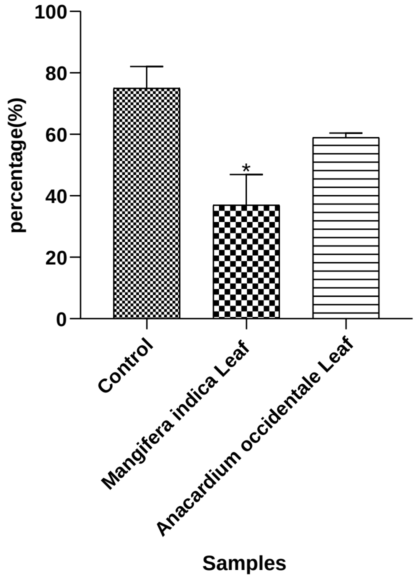

Figure 5: *In-vitro* effect of *Mangifera indica* and *Anacardium occidentale* aqueous leaf extracts on sperm morphology (Normal). Results are expressed in (Mean ± SD). *$p < 0.05$ versus control.

The percentage progressive assessment of the sperm treated with the extract, shown in Figures 3 and 4, further complements the Motile count. It revealed that the sperm treated with the extracts had significantly reduced the percentage of sperm with fast progression (Figure 3), which brought about an increase in the percentage of sperm with slow progression (Figure 4).

The effect of *Mangifera indica* and *Anacardium occidentale* leaf aqueous extracts on sperm motility can be linked to the presence of phenol. Studies have shown that phenol can negatively impact sperm motility. [45] *Mangifera indica* and *Anacardium occidentale* leaf extracts contain a detectable amount of phenol, which has been known to bring about negative effects on sperm motility. Findings from an *in vitro study* conducted by Saha *et al*. (2010) revealed that saponins are a potent spermicidal agent, exerting their effect even at low concentrations.[46] Saponins can potentially affect sperm by their ability to disrupt sperm membranes.[47] This disruption can impair sperm motility and viability, thereby contributing to reduced fertility. *Mangifera indica* and *Anacardium occidentale* leaf extracts exerted their spermicidal effect on sperm motility, which can also be due to the presence of saponins in the extracts, as seen in Table I.

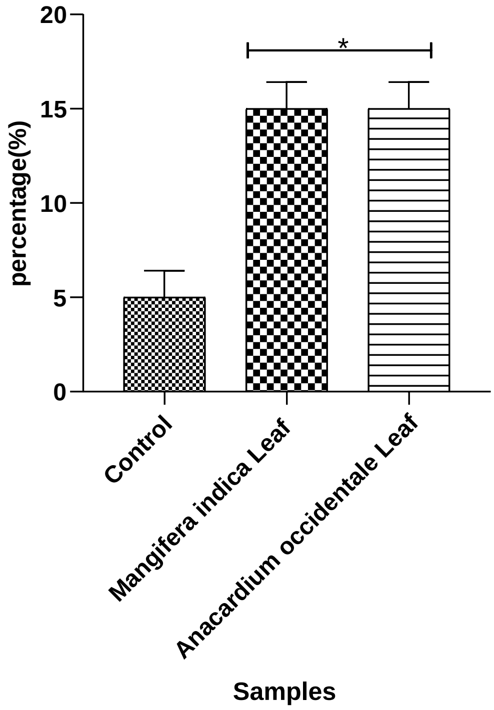

Figure 6: *In-vitro* effect of *Mangifera indica* and *Anacardium occidentale* aqueous leaf extracts on sperm morphology (Head Defect). Results are expressed in (Mean ± SD). *$p < 0.05$ versus control.

The morphology of sperm indicates the percentage of sperm with a normal shape, which entails an oval head and a long tail responsible for its penetrating and swimming efficiency.[48] *Mangifera indica* aqueous leaf extract significantly ($p<0.05$) reduced the percentage of sperm with normal morphology, as presented in Figure 5, thereby increasing the percentage of defective sperm. *Mangifera indica* leaf extract exerted its highest effect on the tail (Figure 8), bringing about a major significant ($p<0.01$) increase in the percentage of sperm with tail defect, while also causing a significant increase in defect on the head (Figure 6) and Neck (Figure 7) of sperm with p-values $p <0.05$ and $p<0.01$ respectively.

Table 1: Phytochemicals present in *Mangifera indica* and *Anacardium occidentale* leaves

Phytochemicals	***Mangifera indica* Leaf**	***Anacardium occidentale* Leaf**
Phenol	+	+
Flavonoid	+	-
Tannin	+	+
Glycoside	-	-
Saponin	+	+
Terpenoid	+	-

(-) represents absent, (+) represents present

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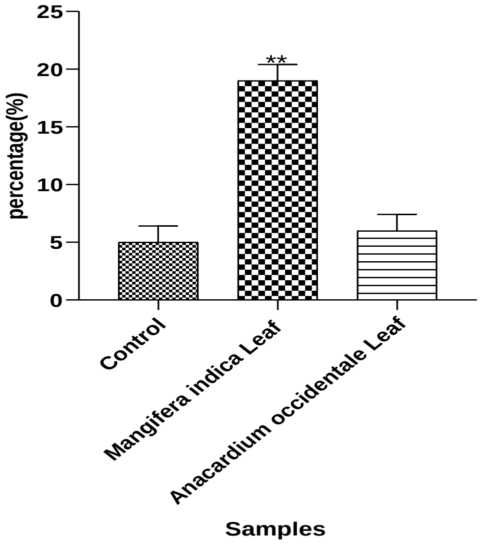

Figure 7: *In-vitro* effect of *Mangifera indica* and *Anacardium occidentale* aqueous leaf extracts on sperm morphology (Neck Defect). Results are expressed in (Mean ± SD). ** indicates p < 0.01 versus control.

Sperm treated with *Anacardium occidentale* had a significant (p<0.05) increase in the percentage of sperm with head defects, as represented in Figure 6, when compared to the control. The high and low effects of these extracts on morphology can be attributed to the level of tannin they contain.

It has been demonstrated that tannins, a class of water-soluble plant polyphenols, increase oxidative stress, which can harm sperm cells and lower their quality, motility, and viability.[49] This oxidative stress can bring about abnormalities in sperm morphology. The tannin level of *Mangifera indica* leaf extracts which is represented by (+) in Table I indicates a strong presence in the extract, this can be the possible cause of its high impact on sperm morphology, *Anacardium occidentale* leaf which increased only the morphological head defect significantly can be attributed to the weaker presence of tannins as represented by (+) on table I.

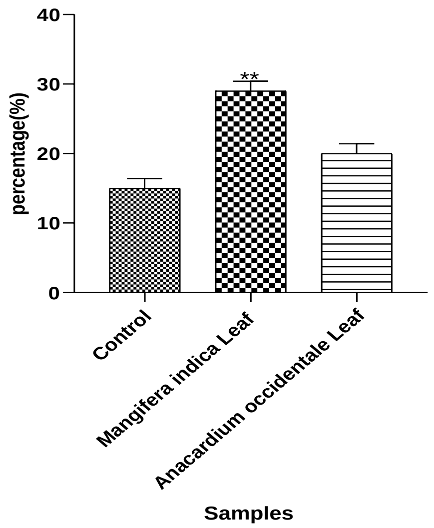

Figure 8: *In-vitro* effect of *Mangifera indica* and *Anacardium occidentale* aqueous leaf extracts on sperm morphology (Tail Defect). Results are expressed in (Mean ± SD). ** indicates p < 0.01 versus control.

Flavonoids have been shown to enhance sperm quality by improving motility and morphology.[50] However, the positive effect of flavonoids in *Mangifera indica* leaf extract (Table I) was not significant because the concentration of flavonoids was too low to exert an effect based on the qualitative assay (+). Findings from this study revealed that leaf extracts of *Mangifera indica* and *Anacardium occidentale* had significant spermicidal effects, primarily affecting various sperm parameters, as shown in Figures 1-8. The overall effect of *Mangifera indica* and *Anacardium occidentale* aqueous leaf extracts on Sperm parameters indicates a potential negative impact on male fertility, which can be attributed to the phytochemicals present in the extracts.

Conclusion

This research confirms reports that medicinal plants with antiplasmodial properties may have the potential to negatively impact sperm parameters due to the phytochemicals associated with this effect. The negative impact of *Mangifera indica* and *Anacardium occidentale* aqueous leaf extracts on sperm parameters makes them suitable as potential alternatives for birth control contraceptives. However, *in vivo* studies should be conducted to confirm the effects of these extracts on male fertility and to determine safe concentrations and doses.

Conflicts of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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