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Effect of *Mimosa pudica* in Sulphanamide-Induced Male Infertility in Wistar Rats

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Abstract

Introduction: Herbs have been a source of medicinal compounds for centuries. Herbal therapy has been reported to treat fertility problems, liver disease, central nervous system disorders, digestive and metabolic disorders. Researchers are currently focusing on the medicinal properties of potentially bioactive plant products with excellent therapeutic properties. *Mimosa pudica* is a creeping annual or perennial herb. The aim of this study is to determine the effects of *Mimosa pudica* in sulphanamide-induced male infertility in wistar rats. Methods: Forty-two wistar rats were used for the study and distributed into seven different groups with different pattern of treatments: Group 1: Rats taking water only (negative control). Group 2: Rat and Sulphonamides (positive control). Group 3: sulphanamide (3000mg) and silymarin (210mg). Group 4: Sulphanamide (3000mg) and aqueous extract of *Mimosa pudica* (100mg). Group 5: sulphanamide (3000mg) and aqueous extract of *Mimosa pudica* (200mg). Group 6: sulphanamide (3000mg) and aqueous extract of *Mimosa pudica* (300mg). Group 7:100mg/kg of the aqueous extract of *Mimosa pudica*. Result: Rats that were induced with sulphoanamide & Silymarin and sulphoanamide & Plant (300mg) has the highest mean level of testosterone (20.0±1.4) compared to other groups while rats that were induced with sulphoanamides only has the lowest mean level of testosterone (3.7±1.6) compared to other groups ($p<0.001$). Rats that were induced with sulphoanamides only has the highest mean level of Prolactin (25.0±2.4) compared to other groups while those that were in the control group has the lowest mean level of Prolactin (7.8±2.6) compared to other groups ($p<0.001$). Rats that were induced with plant extract (100mg) only has the lowest mean±SD of LH (6.7±0.5) compared to other groups, those in the control groups, rats induced with sulphoanamides & plant extract (200mg), and sulphoanamides & plant extract (300mg) has the same mean level of LH (6.0±0.7), while the rats induced with only sulphoanamides the lowest mean level of LH (3.8±1.2) compared to other groups ($p<0.001$). Also, rats induced with sulphoanamides & plant extract (300mg) has the highest mean level of FSH (12.2±1.2) compared to other groups, rats induced with sulphoanamide & silymarin and those induced with sulphoanamides & plant extract (100mg) has the same mean level of FSH (11.0±0.9), while the rats induced with only sulphoanamides the lowest mean level of FSH (0.9±0.1) compared to other groups ($p<0.001$).

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Keywords

Mimosa pudica, Sulphanamide, Male, Infertility, Wistar, Rat.

Introduction

Mimosa pudica (from latin: *pudica* "shy, bashful or shrinking"; also called sensitive plant, sleepy plant, action plant, touch-me-not, shame plant) is a creeping annual or perennial flowering plant of the pea/legume family *fabaceae*. It is often grown for its curiosity value: the compound leaves fold inward and droop when touched or shaken, defending themselves from harm, and re-open a few minutes later (Azmi, 2011). The species is native to the Caribbean and South and Central America, but is now a pantropical weed, and can now be found in the southern united states, South Asia, East Asia, Micronesia, Australia, South Africa, and West Africa as well. It is not shade-tolerant and is primarily found on soils with low nutrient concentrations (Parasuraman *et al.,* 2019). *Mimosa pudica* is well known for its rapid plant movement like a number of other plant species, it undergoes changes in leaf orientation termed "sleep" or nyctinastic movement. The foliage closes during darkness and reopens in light (Abramson & Chicas-Mosier, 2016).

Infertility is defined as the inability of a couple to conceive even after one year of unprotected, frequent sexual intercourse (Winters & Walsh, 2014). It affects about 15% of all couples in the United States and at least 180 million worldwide. Male infertility is defined as the inability of a male to make a fertile female pregnant, also for a minimum of at least one year of unprotected intercourse. The male is solely responsible for about 20% and is a contributing factor in another 30% to 40% of all infertility cases (Esteves *et al.,* 2011).

As male and female often co-exist, it is important that both partners are investigated for infertility and managed together. Overall, the male factor is substantially contributory in about 50% of all cases of infertility (Cates *et al.*, 1998). There are several reasons for male fertility to occur including both reversible and irreversible conditions. Other factors that could influence each of the partners could be their age, medications, surgical history, exposure to environmental toxins, genetic problems, and systemic diseases. The key purpose for evaluating male infertility is to identify his contributing factors, offer treatment for those that are reversible, determine if he is a candidate for assisted reproductive techniques (ART) and provide proper counselling for irreversible and untreatable conditions (Barak & Baker, 2000). In rare cases, male infertility could be a herald to a more serious condition. This is an additional reason to do a comprehensive evaluation of the male partners of infertile couples; so that any significant and underlying medical conditions can be identified and treated (Shih *et al.,* 2019). Of greater concern is the widely reported general trending, over recent decades, of reductions in sperm counts globally. The average sperm counts in 1940 were 113 million/mL but dropped to 66 million/mL in the 1990s. While the exact causes are not known, contributing factors are thought to be increasing long-term exposure to environmental toxins as well as improved global medical care, which paradoxically allows more men with marginal health to participate in reproductive activities. There is also the possibility that the reported decrease in sperm counts might merely reflect differences in laboratory techniques, inconsistent laboratory criteria, and varying counting methods (Esteves *et al.,* 2011). Infertile men appear to have an increased risk of developing cancer compared to the general population. This risk is highest in azoospermic men. For example, it has been reported that 5% to 8% of patients with testicular cancer have azoospermia (Winters & Walsh, 2014).

Globally, infertility affects approximately 13 to 15% of all couples, while one in five is unable to achieve pregnancy in the first year (Winters & Walsh, 2014). Approximately 10-15% of couples demonstrate primary infertility and of these a male fact is identified in approximately 50% of the cases (Winters & Walsh, 2014). It has been reported severally in literatures suggesting a possible decline in human semen quality during the last 50-60 years (Esteves *et al.,* 2011). The burden of infertility is generally worse in the developing world due to the constrained medical resources and the high cost of treatment as well as cultural fears, taboos, and stigmas (Carlsen *et al.,* 2003). Agarwal *et al.*, estimated the overall pure male factor infertility could range between 2.5% and 12%. In North America, the estimated male infertility rate is between 4.5% - 6%, while it's 9% in Australia and could be as high as 8% to 12% in Eastern Europe (Esteves *et al.,* 2011).

A study by Bayasgalan *et al.,* (2004) estimated the cause of infertility due exclusively to a male factor at 25.6% (Esteves *et al.,* 2011). A similar study conducted by Thonneau *et al.,* found that among the French population, a prevalence of 20% of all infertility was due exclusively to a male factor (Winters & Walsh, 2014). Similarly, Philippov *et al.,* (1998) used a WHO questionnaire in Western Siberian to show a rate of 6.4%, while in Nigeria, Ikechebelu *et al.,* found a male infertility prevalence of 42.4% (Evers, 2002). Of greater

concern is the widely reported general trending, over recent decades, of reductions in sperm counts globally; the average sperm counts in 1940 were 113 million/mL but dropped to 66 million/mL in the 1990s. While the exact causes are not known, contributing factors are thought to be increasing long-term exposure to environmental toxins such as smoke and medications like zenobiotics/antibiotics (sulphonamides) (Esteves *et al.*, 2011). The current study is aimed at assessing effect of *Mimosa pudica* on infertility of wistar rat induced with sulphonamide.

Materials and Methods

Study area

This study was carried out at Lead City University (LCU), Ibadan Oyo State. Lead City University is the first University. It is Located at Toll Gate Area, Off Oba Otudeko Ave, 200255, Ibadan Oyo State, Nigeria.

Sample Analysis

From a five (5) ml of blood collected from Wistar Rat, the following parameters were tested after spinning and separation of the sample. Testosterone, Prolactin, Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Total Sperm Count.

Research Design

Seven (7) groups was used for the experiment. Each groups contains six (6) male Wistar rats which was divided into 7 groups. Aqueous extract of *Mimosa pudica* leaf was prepared in the laboratory and dose was calculated based on the rat's body weight.

A total of forty-two (42) male wistar Rats were used as the sample size. Six (6) were induced with sulphoanamide, six (6) was induced with sulphoanamide and 100mg of the *Mimosa pudica* was given, (6) were induced with sulphoanamide and 200mg of *Mimosa pudica* extract given, six (6) were induced with sulphoanamide and 300mg of *Mimosa pudica* extract was administered, and the last six (6) were given only 100mg of *Mimosa pudica* extract.

Group 1: Rats were given food and water only: this was negative control
Group 2: The rats were induced with sulphoanamides (3000mg)
Group 3: Rats were induced with sulphoanamide (3000mg) + a standard drug (silymarin 210mg) this is used as positive control
Group 4: Sulphoanamide (3000mg) + 100mg of the plant extract (*Mimosa pudica*)
Group 5: Sulphoanamide (3000mg) + 200mg of plants extract (*Mimosa pudica*)
Group 6: Sulphoanamide (3000mg) + 300mg of plants extract (*Mimosa pudica*)
Group 7: was given 100mg of the plants extract (*Mimosa pudica*).

Laboratory Method and Principle of immune assay

This was study conducted in Lead City University, Ibadan, Oyo State. Nigeria. Different groups of wistar rats were induced with different ranges of sulphonamides and Silymarine with extracts of *Mimosa pudica*. Thereafter, blood and semen were properly collected respectively for Hormonal Assay (Follicle stimulating hormone FSH, luteining hormone LH, prolactin and testosterone) and semen analysis/culture.

Sperm Motility

The motility test was conducted by placing a drop of the sperm cells suspension on a clean grease free slide, covered with a cover slip and examined. A total of 42 spermatozoa were counted and the percentage of motility were recorded. To determine the sperm count, a 1;10 dilution of the sperm suspension was made in physiological saline and the capillary tube was used in collecting a portion and charged into Neubauer haemocytometer. The cells in the appropriate ruled areas of the counting chamber were counted. Sperm viability test was conducted by mixing one drop (10-15 micro litre) of 0.5% eosin with one drop of semen on a slide, after two minutes, the preparation was examined microscopically using x10 objective to focus and x40 to count the percentage of viable and non-viable spermatozoa.

Sperm Count and Morphology

In assessing sperm count and morphology, a thin smear of the liquefied well mixed semen was made on a clean grease free slide and fixed with 95% ethanol for 5-10 minutes and then allowed to air dry. The smear was washed with sodium bicarbonate formation to remove any mucus that may be present. It was then rinsed several times, and stained with dilute carbonfuchsin (1 in 20) loeflers methylene blue was used to counterstain the

smear for 2 minutes. It was then washed with water, drained and allowed to air dry. Using x100 objective, 100 cells were counted and the percentage showing normal morphology and the percentage showing that appears abnormal was recorded. Sample was also examined for appearance, liquefaction, viscosity, PH and cellular elements other than spermatozoa.

Data management and Analysis

Information was extracted using a data collection sheet designed for the purpose. The data were coded and analysed using statistical package for social sciences (SPSS) version 21.0. This consisted of initial univariate and bivariates analyses, and then multivariate logistics regression analysis to identify the independent determinant of abnormal semen parameters in male infertility. Test of statistical significance was based on 95% confidence interval and $p<0.05$ using chi square test with fisher exact correction where applicable.

Results and Discussion

Table 4.1 indicates the effects of drugs induced on sperm counts, morphology and motility on the Wister rats. Rats that were administered plant extract of 100mg only have the highest sperm count of 58 X 10^6 and rats that were administered Sulphoanamides are with the lowest sperm count 18 X 10^6.

For the morphology, rats of the control group and those that were administered plant extract of 100mg only have the highest normal cell of 80 while the rats that were administered Sulphoanamides were with the lowest normal cell of 42. For the motility, rats of the control group were most actively motile (85) and rats that were administered Sulphoanamides are the least actively motile (10).

The entire plant of *Mimosa pudica* is very useful for various functions such as biological and pharmacological activities. The plant is crushed and used to relive itchiness and itch related diseases (Jha, 2017). The traditional medicine has originated with the infliction of diseases in the human as the natural products including plants which can easily reach of humans.

According to the estimation of World Health Organization 80% of the world's population including all developed and developing countries use plants and natural products for their healthcare (Jha, 2017). Forty-two (42) abino wistar rats were used for the study and answers to the research question were generated and the objectives achieved. The rats were divided into 7 groups (6 in each group), acclimatized for 14 days then the administration lasted for 14 days. The mechanism of action and the effects of *Mimosa pudica* on the fertility damaged, may be due to its antioxidant properties which involve flavonoids and phenols, the phytochemical act as antioxidant by scavenging 2,2-Diphenyl-1-picrylhydrazyl and nitric oxide free radicals (Jha, 2017).

In this study, there is a significant effect of *Mimosa pudica* on sulphonamide-induced male infertility and its ability to reverse damage caused. This is in agreement with research conducted by Shih *et al.*, (2019) on a variety of traditional medicinal applications with biochemical properties (flavonoids, glycosides, alkaloids, terpenoids, quinines, phenol, tannins) as an active antioxidant that reverse infertility cases among male. Ren *et al.*, (2001) reported herbal plants with *Mimosa pudica* properties has subtances (flavonoids, glycosides, alkaloids, quinines, coumarins, phenol) with multi-biological/clinical properties and its ability to reverse infertility related cases.

Rats that were administered plant extract of 100mg only have the highest sperm count of 58 x 10^6 and rats that were administered Sulphoanamides are with the lowest sperm count 18 x 10^6. For the morphology, rats of the control group and those that were administered plant extract of 100mg only have the highest normal cell of 80 % while the rats that were administered Sulphoanamides were with the lowest normal cell of 42%. For the motility, rats of the control group were most actively motile 85% and rats that were administered Sulphoanamides are the least actively motile 10%.

This study is similar to the outcome of the study reported by Hwang *et al.*, in (2004) after exposing guinea pig to 2,3,7,8 –tetrachlorodibenzodioxin (TCDD) and treated them with Ginseng (herbal medicine) which main constituents is Flavanoids and Saponin. His result showed that ginseng has protective and therapeutic effect on testicular damage by eliminating free radicals. This is attributed to the very phytochemicals present in them (flavonoids and saponin). Kumar *et al.*, also in 2013 using swiss albino rat also reported that the epididymis sperm count was increased in the panax ginseng (PG) group for 56 days compared with the control group by increasing the glial cell-derived neurotropic factor (GDNF) messenger and Ribonucleic acid (mRNA) by 24.1% and 25.2% respectively which is a reflection of the phytochemicals present in the plant (Panax ginseng).

Table.1 Effects of Drugs Induced on Sperm Counts, Morphology and Motility of Wister Rats

Group/ Characteristics	Control	Sulphoanamides	Sulphoanamide & Silymarin	Sulphoanamide & Plant (100mg)	Sulphoanamide & Plant (200mg)	Sulphoanamide & Plant (300mg)	Plant extract (100mg)
Sperm count	56 X 10^6	18 X 10^6	51 X 10^6	20 X 10^6	37 X 10^6	57 X 10^6	58 X 10^6
Normal cells	80	42	78	44	46	75	80
Abnormal cells	20	58	22	56	54	25	20
Actively motile	85	10	80	20	57	80	82
Moderately motile	10	2	15	20	13	10	10
Dead & Immotile	5	88	5	40	30	10	8

Table.2 Effects of Drugs Induced on Hormonal Assays of Wister Rats

Group/ Characteristics	Control	Sulphoan-amides	Sulphoanamide & Silymarin	Sulphoanamide & Plant (100mg)	Sulphoan-amide & Plant (200mg)	Sulphoanamide & Plant (300mg)	Plant extract (100mg)	F	P-value
Testosterone	19.3±2.2	3.7±1.6	20.0±1.4	11.0±1.8	17.3±1.0	20.0±1.4	17.8±1.7	84.533	<0.001*
Prolactin	7.8±2.6	25.0±2.4	20.0±2.1	20.0±2.1	14.3±0.8	10.8±1.5	8.0±1.3	71.747	<0.001*
LH	6.0±0.7	3.8±1.2	5.0±1.1	5.0±1.1	6.0±0.6	6.0±0.9	6.7±0.5	6.621	<0.001*
FSH	9.0±1.8	0.9±0.1	11.0±0.9	11.0±0.9	10.7±1.2	12.2±1.2	8.8±1.2	67.079	<0.001*

Figure.1 The average weight of wistar rat for each group

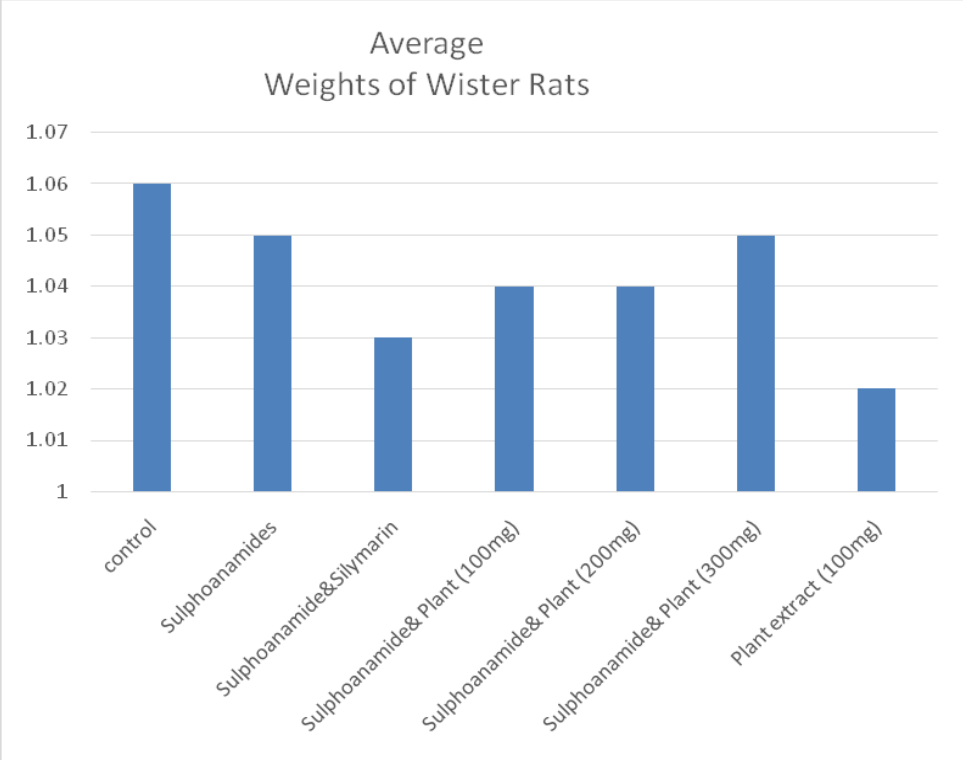


Figure.2 Morphology of wistar rats

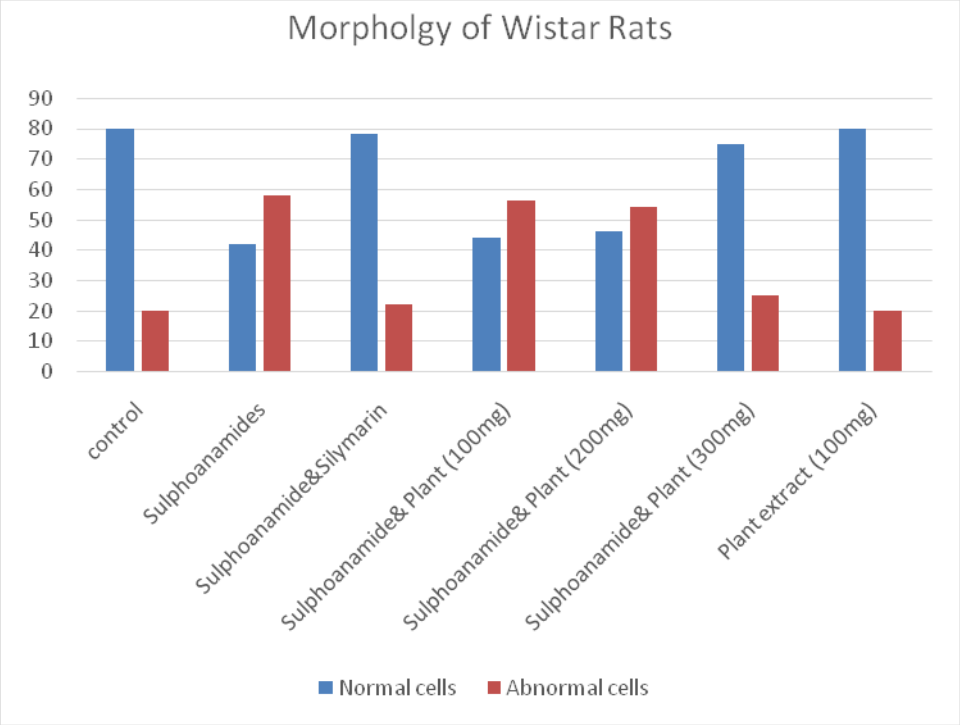


Figure.3 Motility of wistar rat

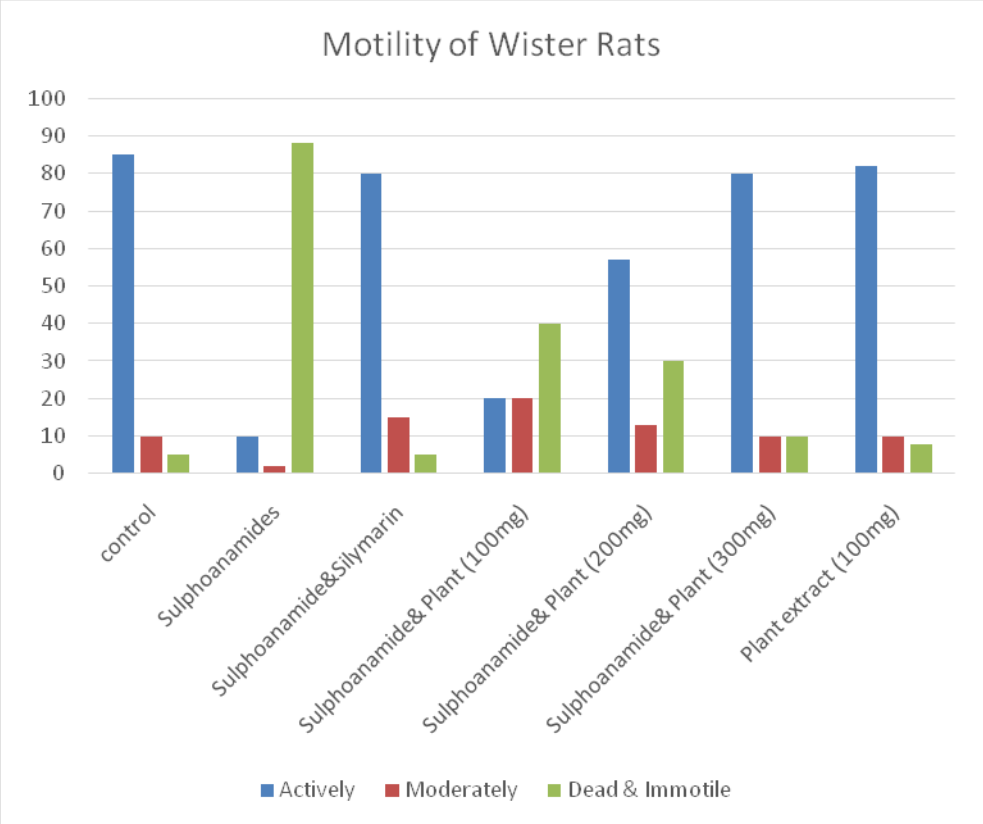


Figure.4 Total cell count of wistar rat

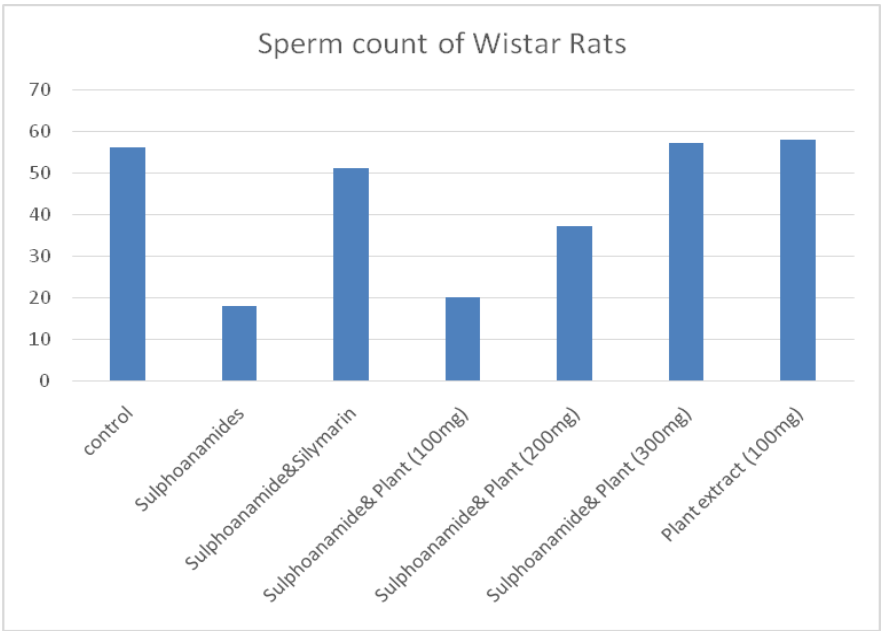


Figure.5 Testosterone level in wistar rat

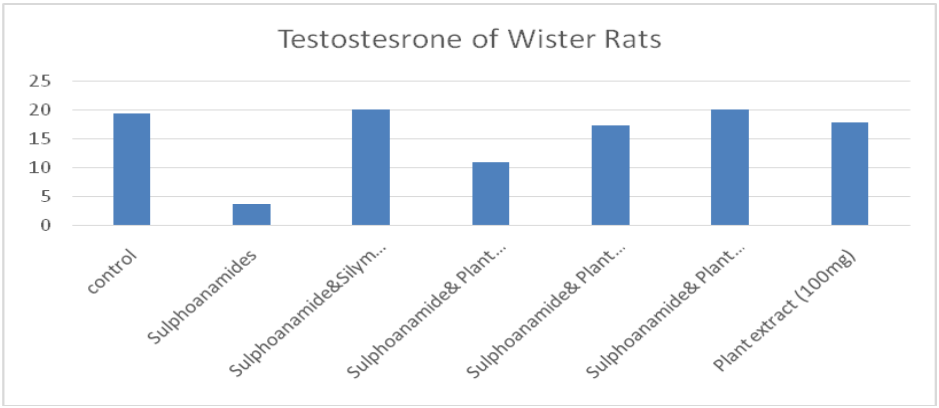


Figure.6 Prolactin level in wistar rat

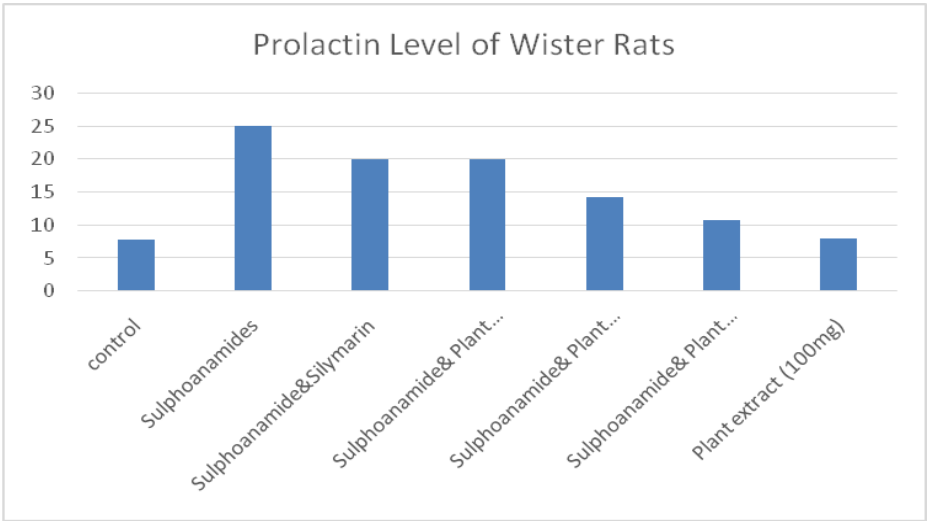


Figure.7 LH level in wistar rat

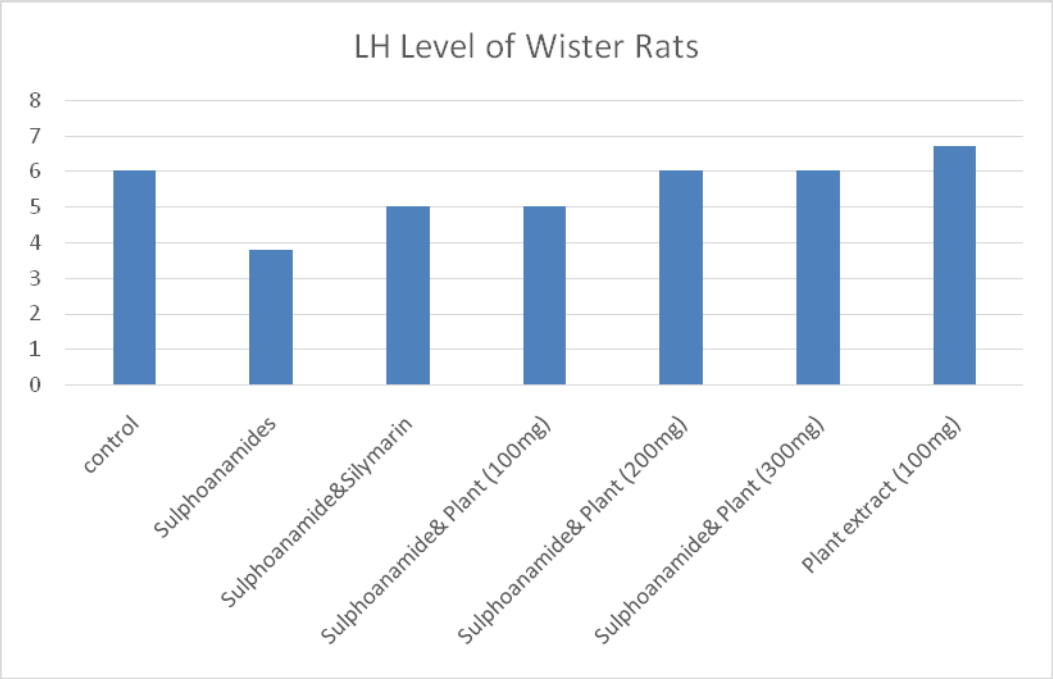
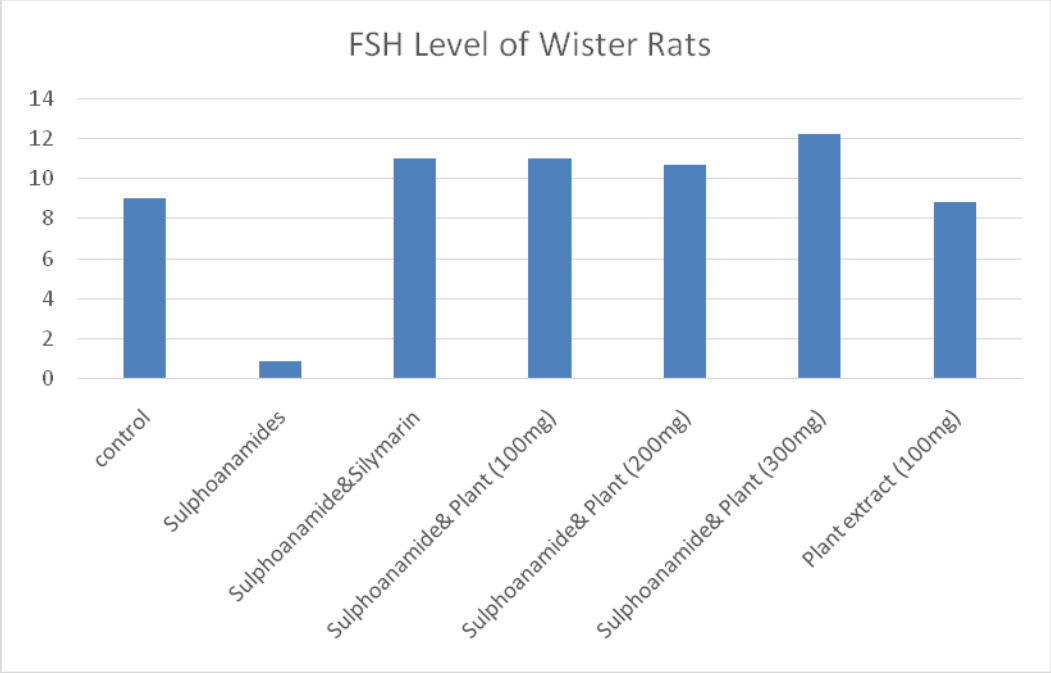


Figure.8 FSH level in wistar rat



Rats induced with sulphoanamides showed a low level of testosterone compared to group one which is a negative control. However, 100 mg, 200 mg, 300 mg of the extract elevated the testosterone level. This is in agreement with study of Nouri *et al.*, in 2009 where he reported effect of *Daucus carota* (herbal medicine) that contains Tanin, flavonoids, steroid as phytochemical constituent on benzene toxicity in swiss albino Rat. The study showed increase in testosterone level after administration of the plant containing these

phytochemicals showing enhancing effect on the spermatogenesis hormone. In consonance, this study is in agreement with the work of Akdogan *et al.*, in 2004 on the significant effects of herbal peppermint tea on plasma testosterone and testicular tissue in rat, he reported that consumption of 6g of green tea in 600ml of water for 7days could increase plasma testosterone and improve the post exercise increase in lipid hydroperoxidase in humans.

The inherent flavonoids and Tanins are responsible for this. The study of Takeuchi *et al.*, (2014) reported Effects of herbal medicine (*Mimosa* properties) on total and free serum testosterone levels recruiting 38 participants with low testosterone and a significant increase after treatment with herbal medicine with mimosa properties.

Rats induced with sulphonamides showed high level of Prolactin compared to the negative control while the group containing 100mg, 200mg, 300mg of aqueous *Mimosa pudica* extract significantly decreased the level of prolactin. This study is in agreement with Chen *et al.*, (2008) on effects of herbal medicine on the serum prolactin of schizopheric patient with a significant regulation due to antioxidant properties of the herb. Vance *et al.*, in 2006 reported use of herbal medicine in management of hyperprolactinemia, the study showed a significant decrease in blood prolactin and further 50% decreased when treated with herbal medicine consisting of flavonoids.

The follicle stimulating hormone (FSH) of the sulphoanamide group showed a low level compared to the negative control group and 100mg, 200mg, 300mg of aqueous *Mimosa pudica* extract significantly elevated the FSH. Kaviarasan *et al.*, on a study experimented on rat in 2007 described herbal plant (*Trigonella foenum-graecum*) as an antioxidant and antiradical containing flavonoids and polyphenols which helps to regulate elevated FSH in infertility cases.

This study is also in agreement with study reported in Australia by Stankiewicz *et al.*, (2007) on the positive effect of complementary medicine with antioxidant properties in regulation of plasma follicle stimulating hormone and usage in infertility cases. In consonance, Gundiza *et al.*, (2003) reported that 100mg/kg/48hr of saffron herbal plants (*Crocus sativus*) increases the FSH level in mice after administration; again, this is accredited to the flavonoids and other phytochemicals present in the plant.

Rats of the sulphoanamides group showed a low level of LH compared to the negative control group. However, 100mg, 200mg, 300mg of the extract elevated the LH. In support of this study, Esteves *et al.*, (2011) demonstrated that consumption of Salep root extract (40 mg of salep extract in 200ul distilled water which characterized into saponins) improves spermatogenesis and the sexual organ in male mice by increasing the LH levels so that it can increase leydig cells proliferation. Another study by Modaresi *et al.*, (2008) demonstrated that Saffron (*Crocus sativus*) increases LH of serum in mice by consumption of 100mg/kg dosage during 20 days.

However, this study is not in support of Tena-sempere *et al.*, (2009) that reported mimosine, (one of the constituents of *Mimosa pudica*) as a substance that inhibits testosterone, prolactin, LH, FSH from adult rat testis *in vitro*. The effects of *Mimosa pudica* may be due to its antioxidant and antitoxic properties (Pande, 2017). Fertility damage from sulphoanamide may represent a part of a spectrum of hypersensitivity due to free radicals present in the sulphoanamide.

Despite the effects of the sulphoanamide and the changes it incurred on the rats, the pharmacodynamics ability of *Mimosa pudica* as stated by Takeuchi *et al.*, (2014) is proven in this study, *Mimosa pudica* at all levels or doses significantly receded sulphoanamide toxicity in this study.

According to the researcher, simultaneous administration of the leaf extract *M. pudica* along with the toxin ethanol in rats showed a considerable protection against the toxin-induced oxidative stress and fertility damage as evidenced by a significant increase in antioxidant activities (Parasuraman *et al.*, 2019). The effects of *Mimosa pudica* with proposed application to prevent and treat male infertility have been critically reviewed in this present study. The plant exhibit antioxidant and antitoxic ability due to its phytochemical's properties; this includes flavonoids, saponins, tanins, phenol, cousmarin. It has the potent capacity to reverse sulphanamide-induced male infertility in wistar rat. Therefore, the plant is an indispensable medicinal herb in Andrology.

Conclusion

This study shows the effectiveness of the plant *Mimosa pudica* has the ability to reverse the damage caused by sulphonamide induced infertility due to the ability to increase antioxidants enzymes that act as defense mechanism against reactive oxygen species and can

effectively scavenge free radicals. It is strongly recommended that *Mimosa pudica* at 100mg, 200mg, 300mg should be introduced as a traditional/ herbal drug especially for the regulation and control of male infertility.

Conflict of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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Authors' Contributions

The entire study procedure was conducted with the involvement of all authors.

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