

ANTIFERTILITY ACTIVITY OF MEDICINAL PLANT EXTRACTS ON ALBINO RATS

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ABSTRACT

Population rise, traditional inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increase emphasis on the use of plant materials as a source of medicine for a wide variety of human ailments. Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant originated. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of India. Indian medicinal plants are widely used by all sections of the population and it has been estimated that over 7500 species of plants

are used by several ethnic communities. India possesses more than 500 tribal communities and even today, tribals and certain local communities in India practice herbal medicine to cure a variety of diseases and disorders. The antifertility activity revealed in the present study; the *Streblus asper* aqueous leaf extract (SAALE) and *Streblus asper* methanol leaf extract (SAMLE).

KEYWORDS: SGOT, SGPT, Sperm count, Antifertility activity.

INTRODUCTION

In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care.^[1,2] As a part of the strategy to reduce the financial burden on developing countries, it is obvious that

an increased use of drugs prepared from medicinal plants will be followed in the future.^[3,4] The Indian flora is extensively utilized as source of drugs mentioned in the traditional systems of medicine *Streblus asper* aqueous leaf extract (SAALE) and *Streblus asper* methanol leaf extract (SAMLE). Group – I -Control, Group – II- SAALE 200mg/kg, Group – III -SAALE 400mg/kg, Group –IV- SAMLE 200mg/kg, Group –V- SAMLE 400mg/kg. Route of administration of extract was by oral.

MATERIAL AND METHODS

Collection of plant material

Streblus asper plant were collected from the village area of Kesamudram, Warangal District, Telangana State. It was identified and authenticated by Prof. V.S. Raju (Retd.), Department of Botany, Kakatiya University, Warangal. The plant was stored in the herbarium of the lab by allocating voucher number (RPU/ZOO/SA/2012).

Preparation of extracts

The collected plant material leaves were dried in shade for about 15 days. The leaves were powdered with electrical grinder. The collected coarse powder then passed through No. 20 mesh and the fine powder was used for the extraction.

Maceration technique was employed to prepare the extract from leaf powder of the plant. Solvents like methanol and aqueous were used to get the extract. 50g of powder was taken in Stoppard conical flasks; it was mixed with 250ml of solvent and allowed for 24hrs at room temperature with random shaking. Then the filtrate-I was collected and the marc dissolved in 250ml of solvent for 24 hrs and collected the filtrate-II. Then the filtrates (I&II) were subjected to distillation to get extracts and stored in well closed amber glass containers at refrigerator temperature prior to use.

A total of 40 male albino rats were divided into 5 groups of eight (8) rats in each group. Group I served as the control while group II, III and IV, V were treated with aqueous (SAALE) and methanol (SAMLE) extracts of *Streblus asper* separately with 200mg/kg, 400mg/kg body weights for 21 days. Route of administration was by oral.

Group – I = Control.

Group – II = SAALE 200mg/kg (*Streblus asper* Aqueous leaf extract).

Group – III = SAALE 400mg/kg (*Streblus asper* Aqueous leaf extract).

Group –IV = SAMLE 200mg/kg (*Streblus asper* Methanol leaf extract).

Group –V = SAMLE 400mg/kg (*Streblus asper* Methanol leaf extract).

Sperm Motility and Count

To determine the sperm count and motility, cauda epididymis was teased in 20 ml of normal saline and one drop of the evenly mixed sample was applied to a Neubauer's counting chamber under cover slip. Motility expressed as percentage was determined by counting both motile and immotile spermatozoa per unit area. Ten fields of microscope were randomly selected and sperm motility was assessed on each field.^[24] The sperms were divided into motile, sluggish and immotile ones. Cauda epididymal sperm counts were made under high power microscope and expressed as million/ml of suspension.^[23]

$$\% \text{ Motility} = \frac{\text{Motile sperm}}{\text{Motile sperm} + \text{non-motile sperm}} \times 100$$

SEROLOGICAL PARAMETERS

The blood samples were centrifuged to separate serum and serum samples were used for serological tests. The serological tests were performed by using commercially available kits.

RESULTS

Final body weights and Reproductive organ weights

The final body weights of the treated groups were decreased than to the control group rats. The decreased body weights of group-II, III were more than to the groups – IV, V (Table No.1.1, Chart No.1.1).

The vital reproductive organs like testis, cauda epididymis, seminal vesicles weights were also decreased in the extract treated rats of group-II, III the effect of the SAALE was more on the reproductive organs weights than to the SAMLE. (Table No.1.2 & 1.3, Charts No. 1.2 & 1.3).

SEROLOGICAL PARAMETERS

The SGOT values of the all groups were presented in the table No. 1.4, Chart No. 1.4. The normal values of SGOT were observed in the group-I. The SGOT values were slightly increased in the group- II and III. The fluctuated values of SGOT were observed in the group –IV, V.

The SGPT levels were marginally altered in all extracts treated rats. The changes of the SGPT values of group - II and III were reported in the Table No.1.4, Chart No. 1.4. The normal SGPT values were observed in the group-IV and V. (Table No. 1.4, Chart No.1.4).

SPERM COUNT AND MOTILITY

Sperm count of the group-II, III rats were reduced than to the group of control, the lowered levels was also recorded in the group– IV, V. But the sperm count of group IV and V was not much decreased than to the group-II, III. (Table No.1.5, Chart No.1.5).

The motility of sperms in the extract treated rats was observed in the Table No. 1.5, Chart No. 1.5. The sperm motility of the group- II and III were declined than to the control group. The sperm motility was not much affected in the SAMLE treated group IV and V than to the SAALE treated group- II, III.

Table: 1.1- Body weight parameters.

GROUP	BODY WEIGHT (G)
Group-I (Control)	223.12± 8.32
Group-II (SAALE 200mg/kg)	217.50± 7.35 ^{ns}
Group-III (SAALE 400 mg/kg)	216.88± 6.34 ^{ns}
Group-IV (SAMLE 200mg/kg)	220.63± 7.46 ^{ns}
Group-V (SAMLE 400 mg/kg)	218.13± 6.31 ^{ns}

All values were expressed in mean± SD, with n=8, the values were analyzed with one way ANOVA followed by Dunnett multiple comparison test. **p<0.01 compare to group-I, *p<0.05 compare to group-I, ns= Not significant compare to group-I.



Table: 1.2 -Cauda epididymis and seminal vesicle weights.

GROUP	Cauda epididymis (mg)	Seminal vesicle(mg)
Group-I (Control)	208.40±6.91	481.27± 5.55
Group-II (SAALE 200mg/kg)	198.34±7.29 ^{ns}	397.59± 5.73**
Group-III (SAALE 400 mg/kg)	180.65±7.84**	294.34± 6.01**
Group-IV (SAMLE 200mg/kg)	206.03±7.67 ^{ns}	387.46± 7.58**
Group-V(SAMLE 400 mg/kg)	192.04±7.53**	379.49± 7.75**

All values were expressed in mean±SD, with n=8, the values were analyzed with one way ANOVA followed by Dunnett multiple comparison test. **p<0.01 compare to group-I, *p<0.05compare to group-I, ns= Not significant compare to group-I.

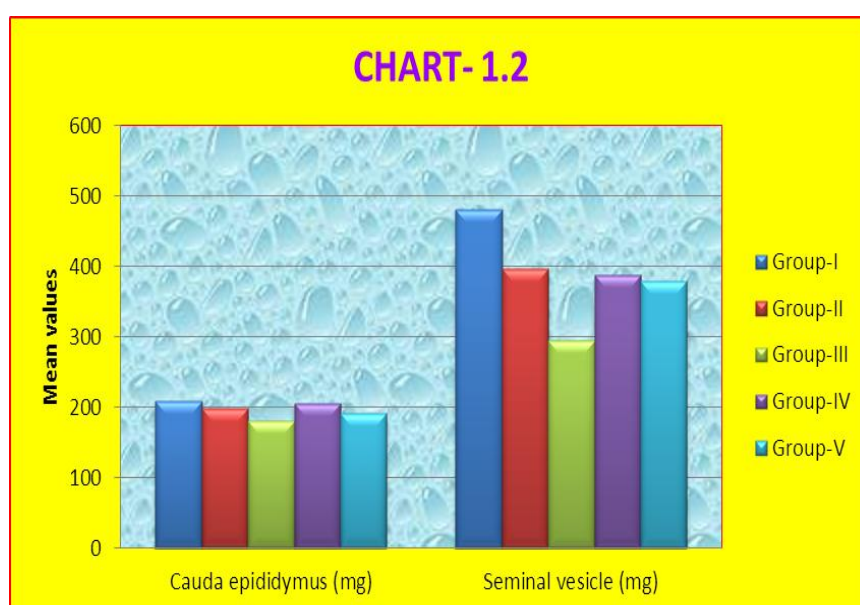


Table: 1.3 - Testis weights.

GROUP	Testis weights (G)
Group-I (Control)	1.44±0.24
Group-II (SAALE 200mg/kg)	1.23±0.24 ^{ns}
Group-III (SAALE 400 mg/kg)	1.16±0.13*
Group-IV (SAMLE 200mg/kg)	1.39±0.26 ^{ns}
Group-V(SAMLE 400 mg/kg)	1.37±0.14 ^{ns}

All values were expressed in mean±SD, with n=8, the values were analyzed with one way ANOVA followed by Dunnett multiple comparison test. **p<0.01 compare to group-I, *p<0.05compare to group-I, ns= Not significant compare to group-I.



Table: 1.4 - Serological parameters - sgot and sgpt.

GROUP	SGOT (U/L)	SGPT(U/L)
Group-I (Control)	29.77±3.10	30.10±3.37 ^{ns}
Group-II (SAALE 200mg/kg)	32.13±3.02 ^{ns}	33.45±4.96 ^{ns}
Group-III (SAALE 400 mg/kg)	31.02±6.18 ^{ns}	33.27±3.08 ^{ns}
Group-IV (SAMLE 200mg/kg)	28.05±5.75 ^{ns}	32.04±3.67 ^{ns}
Group-V(SAMLE 400 mg/kg)	31.89±3.85 ^{ns}	29.35±2.65 ^{ns}

All values were expressed in mean±SD, with n=8, the values were analyzed with one way ANOVA followed by Dunnett multiple comparison test. **p<0.01 compare to group-I, *p<0.05 compare to group-I, ns= Not significant compare to group-I.

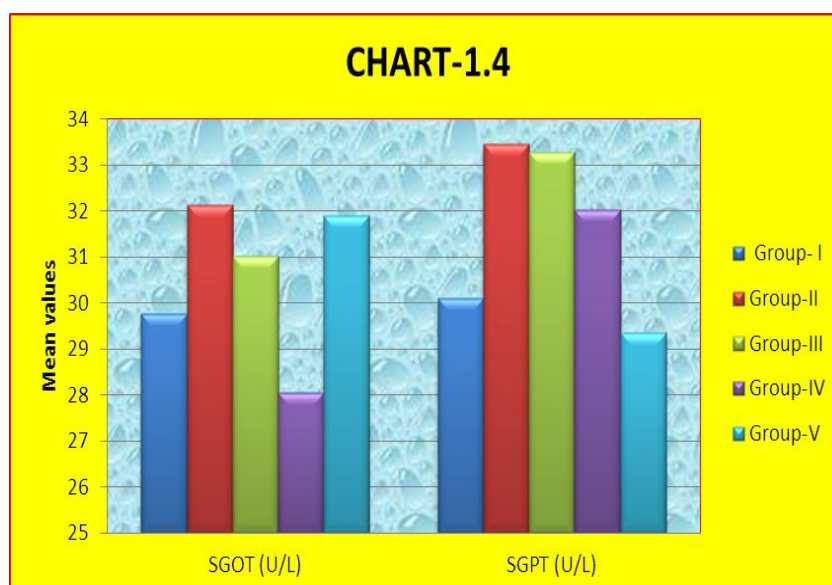
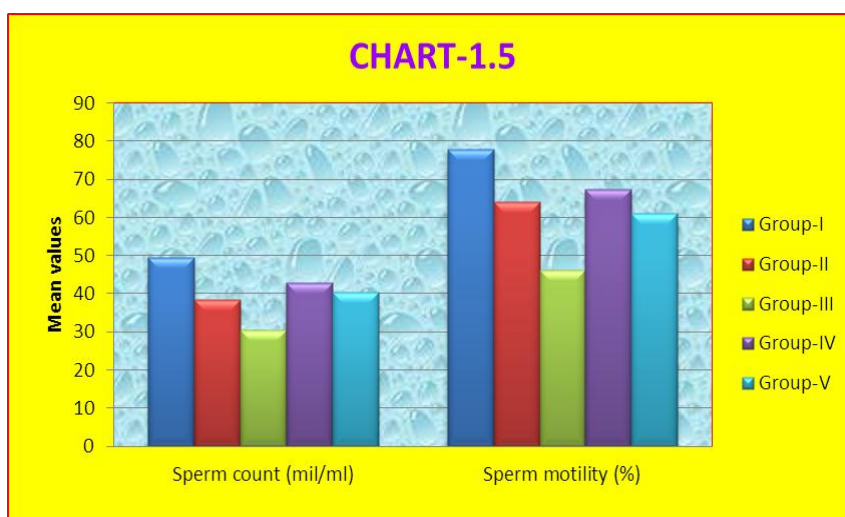


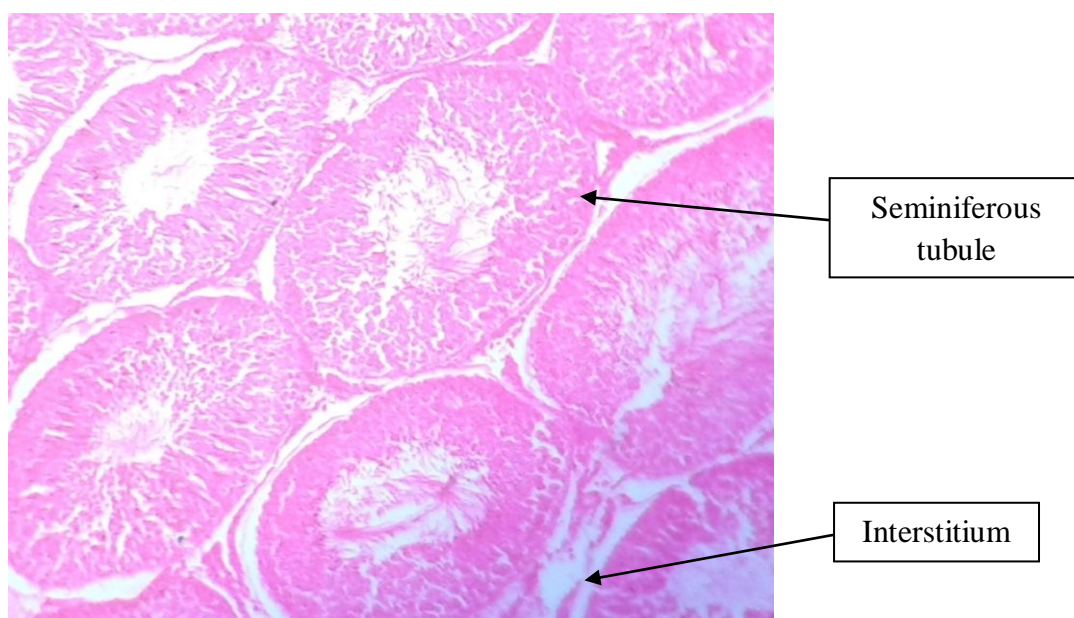
Table: 1.5- Sperm count and sperm motility.

GROUP	SPERM COUNT (mil/ml)	SPERM MOTILITY (%)
Group-I (Control)	49.44±4.76	77.76±5.52
Group-II (SAALE 200mg/kg)	38.35±5.30**	64.22±7.56**
Group-III (SAALE 400mg/kg)	30.47±6.22**	46.12±5.34**
Group-IV (SAMLE 200mg/kg)	42.99±4.44 ^{ns}	67.39±5.73**
Group-V (SAMLE 400 mg/kg)	40.38±5.81**	60.93±4.14**

All values were expressed in mean±SD, with n=8, the values were analyzed with one way ANOVA followed by Dunnett multiple comparison test. **p<0.01 compare to group-I, *p<0.05 compare to group-I, ns= Not significant compare to group-I.

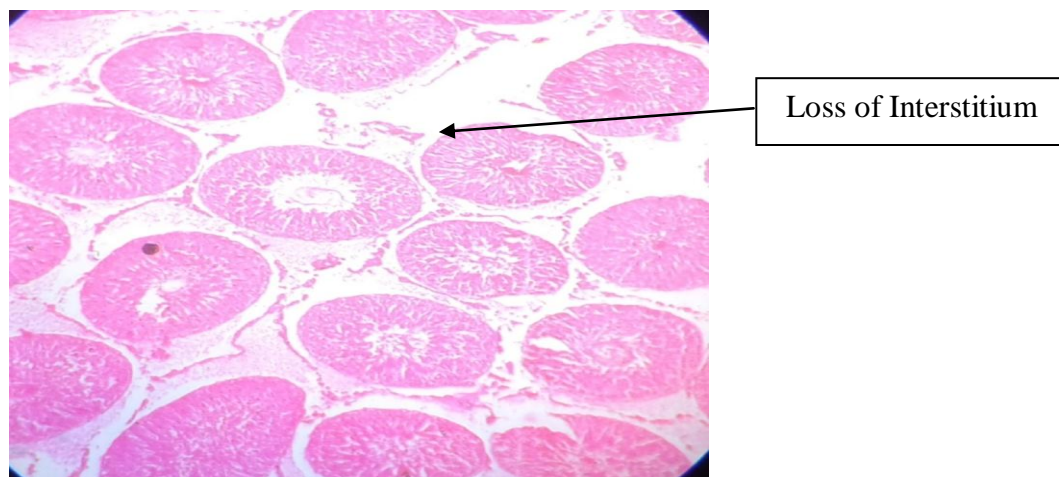


CROSS SECTION OF TESTIS (GROUP-I), Fig. 1.CONTROL (10X)



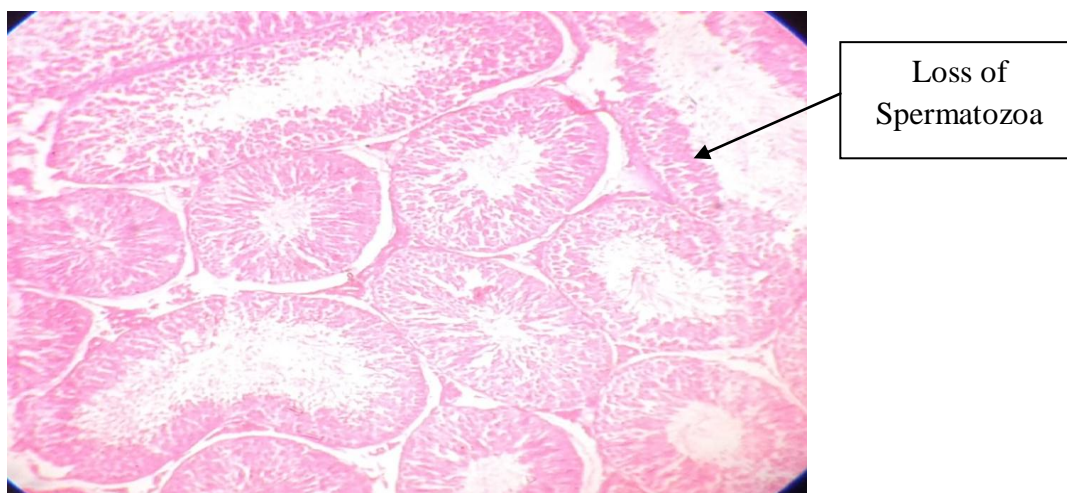
Normal histology is seen in the cross section of testis with seminiferous tubules. Seminiferous tubules are with full of different spermatogonial cells and spermatozoa. The interstitium is also seen.

CROSS SECTION OF TESTIS (GROUP-II), Fig. 2. SAALE 200 mg/kg (10X)

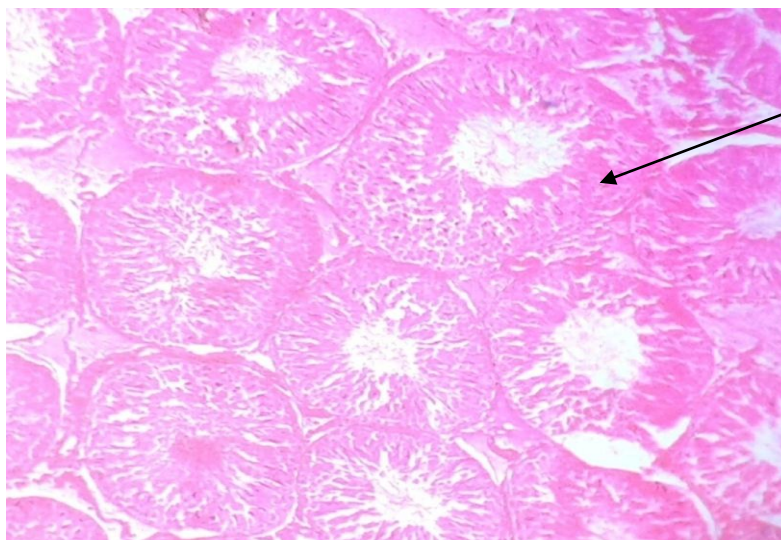


The loss of interstitium and dislocated seminiferous tubules are visible in the cross section of testis.

CROSS SECTION OF TESTIS (GROUP-III), Fig.3. SAALE 400 mg/kg (10X)



The degenerated spermatogonia, loss of spermatozoa are clearly visible in the seminiferous tubules of the testis cross section.

CROSS SECTION OF TESTIS (GROUP-IV), Fig. 4. SAMLE 200 mg/kg (10X)

Mild loss of
Spermatozoa

Seminiferous tubules with developed spermatogonial cells are visible. But some of the seminiferous tubules lost the spermatozoa mildly.

CROSS SECTION OF TESTIS (GROUP-V), Fig. 5. SAMLE 400 mg/kg (10X)

Loss of
Interstitium

The integrity among the seminiferous tubules is lost due to the loss of interstitium. The seminiferous tubules are fully with the spermatogonia cells and spermatozoans.

DISCUSSION

The final body weights were decreased in the extracts treated albino rats. The reduced testicular weights were also noticed in the extracts administered albino rats. The reduced testicular weights might be because of the direct effect of the extract on the histological

architecture of the testis. The increase or decrease of an organ weight after the administration of a compound indicates the toxic effect of the extract.^[5,6,7,8]

The decreased vital reproductive organ weights were observed in the extract treated rats. The reduced weights of cauda epididymis, seminal vesicles, were seen in the SAALE treated rats. The similar results were observed in the *Ocimum gratissimum* ethanolic extract administered male mice.^[9,10,11,12,13,14,15,16]

The serum parameters like SGOT, SGPT, ALP, explains the side effects of the extract. The results explain the slight elevated levels of these enzymes in the serum samples of the SAALE treated male rats. Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotics. Toxic effect of the extract causes the necrosis to liver cells and allow to release of liver marker enzymes into circulation. There is no much effect of SAMLE on male albino rats.

The reduced sperm count, motility were noticed in the SAALE treated male rats. The effect of SAALE was more than to the SAMLE treated rats. The decrease of sperm count might be because of the altered gonadotrophins (LH, FSH) which are necessary for the normal sperm production, development and maturation.^[17] The rats administered *Colebrookia oppositifolis* extracts also showed the similar results.^[18]

The reduced sperm count, motility might be observed because of the degeneration of seminiferous tubules and Leydig cells.^[7]

The reduction of spermatogenesis may be due to insufficient amount of testosterone. Similar results were reported by^[19] in nickel treated rats.

It was also reported that the deformed Leydig cells unable to synthesize the testosterone which reduces the sperm count.^[20] The reports of also noticed that Leydig cells function directly affects the spermatogenesis.^[10]

As the sperm count, motility have a direct effect on the fertility.^[22] The present investigation also revealed that the sperm count & motility were reduced in SAALE administered male albino rats. This may be due to an alteration in the enzymatic activities of the oxidative phosphorytic process required for ATP production which in turn is necessary for the

forward movement of spermatozoa.^[21] The effect of SAALE on sperm count and motility observed more than to the SAMLE.

The decrease of sperm motility caused by chemical agents has already reported by^[22] Seminal vesicles secrete a substance which directly stimulates sperm motility and antigen that seems to present immune response against spermatozoa.^[22]

CONCLUSSION

The present study is related to antefertility activity screening of medicinal plant (*Streblus asper*). The fertility activity of the SAALE (*Streblus asper* Aqueous leaf extract) treated rats showed increased levels of SGOT, SGPT. The fertility activity of the SAALE treated rats showed reduced number of sperms, motility. The results were explaining the antifertility activity of *Streblus asper*.

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