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Alleviating Effect of *Rosa indica* Petal Extracts on Heat Stress Induced Male Infertility

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A B S T R A C T

Rosa indica is a perennial flower shrub which belongs to the family Rosaceae and genus rosa. From ancient times various rose preparations are used to treat sore throat, enlarged tonsils, as an antibacterial agent and there are few studies on its fertility enhancing properties too. The present study aims to evaluate the effect of aqueous and ethanolic extracts of *Rosa indica* petals on the serum levels of testosterone (ng/ml) and thus the semen parameters including Concentration (M/ML), motility (%) and morphology (%) on heat stress induced male wistar rats. In this experimental study, 30 adult male Wistar rats were used. The rats were randomly divided into 5 groups. First group was control (only with compressed food and adequate water), second group was Negative control (heat stressed), third group was positive control (heat stressed + treated with Quercetin), fourth and fifth group was heat stressed with 200 mg/kg of ethanolic and aqueous extracts respectively. After 21 days of treatment, blood samples were drawn after euthanasia by heart puncture from all the animals to measure the serum testosterone levels and Epididymal sperm was extracted to analyse various parameters. The collected data were analysed using ANOVA and found to be statistically significant and as follows. The group receiving heat treatment with ethanolic extract exhibited higher testosterone levels followed by increased semen concentration, motility and morphology. From this study it is apparent that the ethanolic extract of *Rosa indica* petals alleviate heat stress induced male infertility.

***Key words*:** Heat stress, Male infertility, *Rosa indica*, Sperm parameters, Testosterone

Fertility has drastically decreased in the last two decades, especially in men. Heat stress is one of the main factors responsible for decreased spermatogenesis due to oxidative stress [1]. Higher testicular temperature leads to increase in metabolism as a result decrease in spermatogenesis, increase in sperm damage in mice [1], men [2], bull [3-6] and ram [7-8]. Increase in ROS or decrease in antioxidant levels could happen after exposure to chronic heat stress. Antioxidant response would be immediate in acute situation, accomplished mainly by protein activation and on the other hand by gene activation and translation of few proteins in chronic conditions. Now a days, plants are a reliable source of alternate medicine due to various reasons like easy access, low cost, low side effects with sustainable effectiveness. Several plants are used to treat various diseases, including that of the reproductive system [9]. Plants are a rich source of modern medicine due to their abundance in phytochemicals like flavanoid, alkaloid, steroids, terpenoids, glycosides, phenols, glycoprotein etc. [10] which neutralises free radicles and toxins [11].

Rosa indica, a perennial shrub are a rich source of Vit c, anthocyanin, cyanidin 3,5 diglydiglycoside, kaempferol, arbinoside, galactoside, citronellol, terpenes, quercetin, geranoil [12-14] which makes it a candidate for treating various diseases [15]. Various rose preparations are used as tonics, astringents, laxatives, medicine to treat sore throat, tonsilitis, gall stones and also as an antibacterial agent [16]. Numerous studies shows that damask rose serves to decrease stress, relieve depression and trigger joy by stimulating central nervous system [17]. However very few studies have been carried out in context to the medicinal properties of *Rosa indica*. The aim of this study was to evaluate the alleviating effect of *Rosa indica* petal extracts on chronic heat stress induced male infertility.

MATERIALS AND METHODS

Chemicals

Solutes and solvents of analytical grade were used to carry out the present study.

Plant material

Rosa indica flowers collected from the local areas of Coimbatore, Tamil Nadu, was authenticated by the Head,

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Institute of Forest Genetics and Tree breeding, Coimbatore with authentication number 924/FECC/ID/IFGTB/2020.

Preparation of sample

The cleaned fresh petals of *R. indica* weighed to 100 g each was shade dried for 2 weeks. Pre weighed 100 g each of the petals were extracted with 500 ml of double distilled water and 100% ethanol respectively using mortar and pestle and incubated at 4℃ for 48 hours. After 48 hours each extract was filtered using cheese cloth and the filtrates lyophilized to get a coarse powder. The extract obtained was stored at 4℃ to carry out further studies.

Animals

After obtaining the Institutional animal ethical clearance from Ramakrishna College of Pharmacy, Coimbatore with approval number (COPSRIPMS/ IAEC/ PHD/ PROJECT/001/ 2020-2021), the study was conducted on 3 months old male Wistar rats weighing 180-240 g. Animals were housed under standard lab conditions of 12 hrs light and dark with free access to compressed food and water. The treatment was started after 7 days of acclimatization.

Experimental design

3 months old male Wistar rats each weighing 180-240 g were randomly allocated to 5 groups, each containing 6 animals. The study group is as follows:

Group 1- Control - Water and Food only
Group II- Negative control- Heat treatment
Group III- Positive control- Heat treatment + 50 mg/kg Quercetin
Group IV- Heat treatment + 200 mg/kg Aqueous extract
Group V- Heat treatment + 200mg/kg Ethanolic extract

Heat stress was induced from day 8 of treatment for 6 consecutive days by immersing hindlegs, tail and scrotum in 43℃ thermostatically controlled water bath for 30 minutes (Naushin). Both the extracts and Quercetin were dissolved in 0.5% carboxy methyl cellulose and administered through intra gastric gavage. After 21 days of treatment animals were euthanised using 100 mg/kg ketamine intra peritoneally and Blood collected immediately by cardiac puncture. Epididymis was removed and sperm collected from the cauda epididymis using MOPS buffer.

Serum testosterone measurement

Serum was collected by centrifuging blood at 2000 rpm for 10 minutes and stored at -20℃ till further study. Serum testosterone was measured using Electrochemiluminescence immunoassay (ECLIA) kit by Roche Diagnostics GmbH, Mannheim as per manufacturer's instruction.

Sperm analysis

The cauda epididymis was dissected from all the study animals, washed with Sodium bicarbonate buffer, several incisions made using scalpel blade in the epididymal tail, gentle pressure applied and incubated for 15 minutes at 37℃ to allow the spermatozoa to swim out from the reproductive duct (Nichi m from evaluating lasting 28). After incubation the following parameters were studied including sperm concentration, motility and morphology using improved Neubauer hemocytometer by diluting sperm suspension in the diluting solution (50g/L Sodium bicarbonate) in distilled water (1:20). 10 µl of the diluted sample was put into the Neubauer hemocytometer and number of sperm were counted under a light microscope (Nikon Ts100, Tokyo, Japan) and expressed as Million/ml. Sperm motility (as Rapidly progressive, Slow progressive and sluggish) was counted and expressed as % motility. Sperm morphology was assessed from air dried smear prepared by mixing 10 µl of the sperm sample with equal amount of Eosin Nigrosin stain under oil immersion and expressed as % normal forms.

Statistical analysis

The data obtained were analysed using one way ANOVA and a $P<0.05$ was considered statistically significant

RESULTS AND DISCUSSION

Testosterone level

The total testosterone (ng/ml) was highest in the positive control group (animals with heat treatment + Quercetin) followed by heat stressed animals treated with 200 mg/kg ethanolic extract and then by heat stressed animals treated with 200 mg/kg aqueous extract with a significant P value of 0.01.

Epididymal sperm parameters

Heat stressed animals treated with 200mg/kg ethanolic extract and 200 mg aqueous extract showed a comparably higher sperm parameters including sperm concentration, motility and morphology to the group treated with quercetin. The group treated with heat stress alone had least semen parameters which could be witnessed from the graphs below:

Table 1 Testosterone level (ng/ml) with a significant $P<0.01$

	Mean(ng/ml)	St D	Std Error	F stat	p value
Control	0.53	0.15	0.087	59.88	0.01
Negative control	0.46	0.22	0.128		
Positive control	1.88***	0.12	0.069		
Aqueous extract	0.69	0.1	0.057		
Ethanolic extract	1.63**	0.11	0.068		

***Highest level of testosterone; **Comparably higher level

Table 2 Sperm concentration (%) with significant $P<0.05$

	Mean(ng/ml)	St D	Std Error	F stat	p value
Control	7.2	0.8367	0.3742	72.34	0.05
Negative control	4.8	0.8367	0.3742		
Positive control	11.8***	0.8367	0.3742		
Aqueous extract	5.6	0.5477	0.2449		
Ethanolic extract	10.2**	0.8367	0.3742		

***Highest level of testosterone
**Comparably higher level

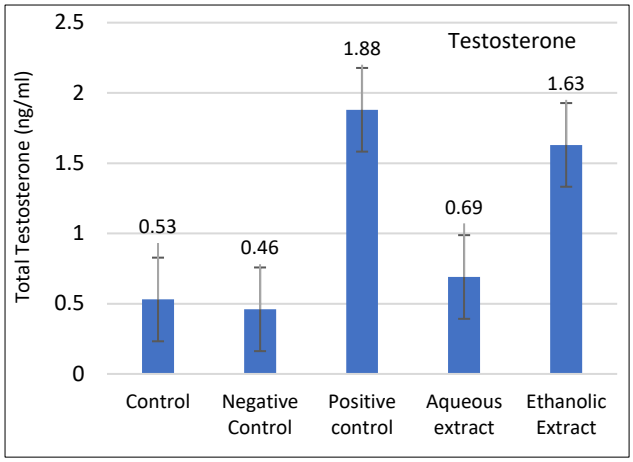

Fig 1 Testosterone levels (ng/ml).
Positive control (Quercetin) showing the highest testosterone levels followed by ethanolic extract treated group

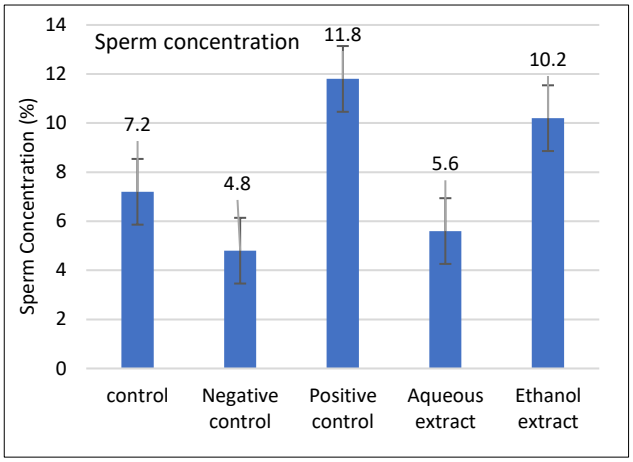

Fig 2 Sperm Concentration (m/ml).
Positive control showing the highest sperm concentration followed by ethanolic extract treated group

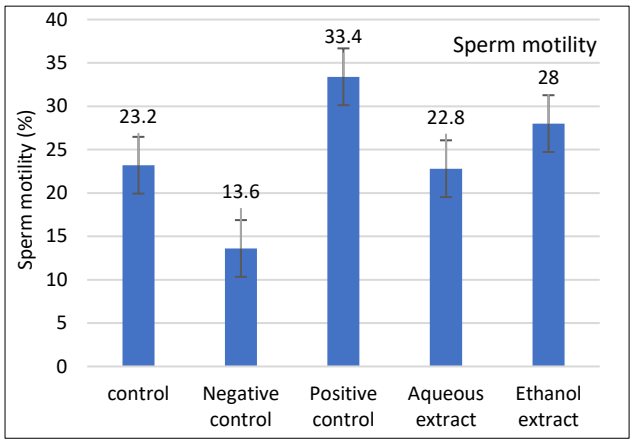

Fig 3 Sperm motility (%). Positive control showing the highest % of sperm motility followed by ethanolic extract treated group

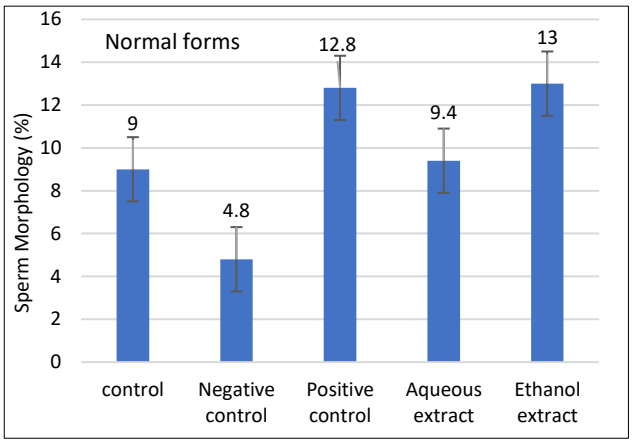

Fig 4 Sperm morphology (%). Ethanolic extract treated group showing the highest % of normal forms followed by positive control

Table 3 Sperm motility (%) with a significant P<0.01

	Mean(ng/ml)	St D	Std Error	F stat	p value
Control	23.2	1.6432	0.7348	99.25	0.01
Negative control	13.6	1.6733	0.7483		
Positive control	33.4***	1.6733	0.7483		
Aqueous extract	22.8	2.0494	0.9165		
Ethanolic extract	28**	1	0.4472		

***Highest level of testosterone
**Comparably higher level

Table 4 Normal forms (%) with a significant P<0.05

	Mean(ng/ml)	St D	Std Error	F stat	p value
Control	9	2.1213	0.9487	19.1498	0.05
Negative control	4.8	1.3038	0.5831		
Positive control	12.8	1.6432	0.7348		
Aqueous extract	9.4	1.5166	0.6782		
Ethanolic extract	13	1.8708	0.8367		

***Highest level of testosterone
**Comparably higher level

The present study shows that heat stress damaged the testicular structures including the leydig cells thus exhibiting reduction in the serum total testosterone level and semen parameters. Whereas the oral administration of ethanolic extract of *ROSA indica* petals alleviated the effect of heat stress on the animals and thus by improving the spermatogenesis. From the above results it could be justified that the ethanolic extract of *Rosa indica* petals, rich in antioxidants reduces oxidative stress in testes, increase the leydig cell activity thus by increasing the serum testosterone and there by spermatogenesis.

CONCLUSION

It could be concluded that *Rosa indica* is a best candidate and a cost effective alternate to treat male infertility caused due to oxidative stress as a result of increased testicular heat.

Ethical approval: *Author declares that the Institutional ethical approval number was obtained and mentioned COPSRIPMS/ IAEC/ PHD/ PROJECT/001/ 2020-2021.*

Conflict of interest: *Authors declare that there are no conflicts of interest.*

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