



## PROTECTIVE EFFECT OF *ROSA CANINA L.* ON STRESS INDUCED REPRODUCTIVE CHANGES IN FEMALE RATS

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### ABSTRACT

Here we evaluate the aqueous extract of *Rosa canina L.* hips in treating female reproductive dysfunctions. Restraint stress (for 3h/day for 28 days) was used as stressor to induced changes in reproductive dysfunctions. Rats were pretreated with 100mg/kg body weight and 200mg/kg b.w. for 15 days with Aqueous extract of *Rosa canina L.* hips and were continued for next 28 days along with induction of stress. Assessment of its effectiveness was done by observing changes in estrous cycle and weight of the organs studies. *Rosa canina L.* treated rats showed significant changes in the different phases of estrous cycle and restored to normal. Improvement in the weight of ovaries, uteri, liver and decrease in the weight of adrenal glands were also seen when compared with stressed control. From the experimental studies, aqueous extract of *Rosa canina L.* hips at two different doses showed promising improvement in treating female reproductive dysfunctions induced by restraint stress. The above activity may be due to the presence of various secondary metabolites like ascorbic acids, tocopherols, bioflavonoids, tannins, flavonoids, alkaloids and other constituents present in the Aqueous extract of *Rosa canina L.* hips

**Keywords:** *Rosa canina L.* hips; Restraint stress; Estrous cycle; organ weight.

### INTRODUCTION

Reproductive failure is a significant concern in both developed and developing countries because of its high importance in every family.<sup>1</sup> Infertility is defined as the failure to conceive a recognized pregnancy after 12 months of unprotected intercourse. Among such couples, causative factors are found in about 30-40% in females, 10-30% in males and in 15-30% of cases, both partners have detectable abnormalities.<sup>2</sup>

High stress perception is a risk factor for PCOS, anovulation, severe premenstrual pain, menstrual disturbance, pregnancy outcomes including preterm delivery and low birth weight, as well as postpartum depression and early onset of perimenopause.<sup>3</sup> All of the above causes are due to the suppression of gonadotrophic hormones, activation of sympathoadrenomedullary system and oxidative stress.<sup>4</sup>

Rose hip, the pseudo-fruit of *Rosa canina L.* (Rosaceae), consists of an urn-shaped receptacle with numerous achenes inside. Fresh rose hip is rich in vitamin C and is widely used for food production. Both fresh and dried rose hips are frequently used as an ingredient of fruit and herbal teas.<sup>5</sup> Rose hip extracts are nowadays used in traditional folk medicine as diuretic, laxative, for kidney and lower urinary tract disorders, arthritis, gout, fever, colds and for vitamin C deficiency.<sup>6</sup>

Due to its popularity as a medical remedy, *Rosa canina L.* has become a popular research subject for researchers, as well. Researchers have shown that the utilization of *Rosa canina L.* as a remedy in traditional folk medicine comes from its high content of phenolic compounds and minerals. In particular *Rosa canina L.* is a great source of ascorbic acids, tocopherols, bioflavonoids, tannins, pectin, minerals, aminoacids, flavonoids, unsaturated and polysaturated fatty acids, phospholipids, minerals, gallactolipids, and carotenoids.<sup>7</sup>

The aim of this study was Protective effect of *Rose Hip* on stress induced reproductive changes in female rats because rose hip rich vitamin-C so it has good anti-oxidant activity.

### MATERIALS AND METHODS

**Plant:** The fresh hips of *Rosa canina L.* was collected from Agra, Uttar Pradesh, in September 2012, identified by Dr. R. K. Sharma, Botany department of Rajasthan University, Jaipur. (Voucher No. RU2178)

**Preparation of ethanolic extract:** The hips of *Rosa canina L.* was chopped into small pieces and dried under shade at room temperature. The dried hips was powdered and passed through coarse sieve (10/44). The dried, powdered of *Rosa canina L.* hips (200g) was extracted with water (500ml) for 20h in a Soxhlet extractor till complete exhaustion. Aqueous extract was concentrated at 40°C to obtain dark brown residue. Yield was 10% w/w with respect to dried power. Preliminary phytochemical studies showed the presence of ascorbic acids, tocopherols, bioflavonoids, tannins, pectin, minerals, aminoacids, flavonoids, unsaturated and polysaturated fatty acids, phospholipids, minerals, gallactolipids, and carotenoids.<sup>7</sup>

**Animals:** Experimental study was carried out using adult female Wistar Albino rats weighing between 170-200g. The animals were housed in polypropylene cages. The cages were maintained clean and hygienic. Animals were acclimatized in light and temperature controlled room with a 12-12h dark-light cycle, temperature 25±2°C and humidity 50±5%. The rats were fed with commercial pelleted rat feed and water *ad libitum*. The animal caring and handling were done according to the CPCSEA guidelines. The Institutional Animal Ethics Committee (IAEC/APPC/08/12)

**Dose selection:** Two doses (100mg/Kg and 200mg/Kg) of aqueous extract of *Rosa canina L.* Hips (AERC) were selected as previously reported.<sup>8</sup>

**Restraint stress model:** Animals with regular estrous cycle were selected and divided into four groups, six animals in each group. Animals were individually placed in a plastic restrainer (21cm in length x 6cm in diameter) with ventilated sliding door for 3h/day for 28 days.<sup>9,10,11</sup>

**Group I-** Vehicle control - distilled water, orally (5ml/kg body weight) for 28 days.

**Group II-** Restraint stress (RS) (3h/day) for 28 days.

**Group III and IV-** Rats were pretreated with aqueous extract of *Rosa canina L.* Hips (100mg/kg b.w., p.o. and 200mg/kg b.w., p.o. respectively) for 15 days prior to the starting of Restraint stress and was continued for another 28 days along with induction of stress from 16th day.

**Group III, IV** animals were subjected to Restraint stress for 3h/day after half an hour of administration of the aqueous

extract of *Rosa canina L.* Every day, immediately after the stress session, vaginal smears were examined in all the groups.<sup>12</sup> After the last stress session on 28th day all animals were sacrificed by cervical dislocation. Liver, ovaries, uteri, adrenal glands were isolated and weighed.

## RESULTS

**Table- 1: Effect of Aqueous extract of *Rosa canina L.* (AERC) hips on mean number of days in different phases of estrous cycle (28 Days) in rats.**

Groups	Proestrous	Estrous	Metestrous	Diestrous
Vehicle control	4.16 ±0.16	5.83 ±0.16	7 ±0.25	11 ±0.44
RS (for 3h)	15.66 ±0.22 <sup>***a</sup>	2.83±0.30 <sup>***a</sup>	1.66±0.21 <sup>***a</sup>	8±0.28 <sup>***a</sup>
RS + AERC (100mg/kg)	7.16±0.30 <sup>***b</sup>	4.16±0.16 <sup>***b</sup>	5±0.25 <sup>***b</sup>	11.66±0.21 <sup>***b</sup>
RS + AERC (200mg/kg)	5.66±0.21 <sup>***b</sup>	5.33±0.21 <sup>***b</sup>	5.83±0.54 <sup>***b</sup>	11.16±0.39 <sup>***b</sup>

Values are expressed as Mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnett's "t" test. Number of animals in each group n = 6. a Comparison made with vehicle control group; bComparison made with CRS group \*\*\*P<0.001; \*\*P<0.01

Stressed animals showed significant changes (P<0.001) in estrous cycle when compared to vehicle control. Prior and continued treatment with aqueous extract at both doses of *Rosa canina L* along with application of stress, the change in the different phases of estrous cycle were less compared to RS group (Table 1).

**Table- 2: Effect of Aqueous extract of *Rosa canina L.* (AERC) hips on different organ Weights (g/100 g of body weight) in female rats**

Groups	Ovaries (g/100g of b.w) (Mean ± SEM)	Uteri (g/100g of b.w) (Mean ± SEM)	Adrenal glands (g/100g of b.w) (Mean ± SEM)
Vehicle control	0.041±0.001	0.275±0.01	0.011±0.0009
RS (for 3h)	0.027±0.004 <sup>***a</sup>	0.070±0.009 <sup>***a</sup>	0.019±0.0003 <sup>***a</sup>
RS + AERC (100mg/kg)	0.037±0.001 <sup>***b</sup>	0.196±0.004 <sup>***b</sup>	0.016±0.0001 <sup>***b</sup>
RS + AERC (200mg/kg)	0.041±0.002 <sup>***b</sup>	0.213±0.01 <sup>***b</sup>	0.013±0.0002 <sup>***b</sup>

Values are expressed as Mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnett's "t" test. Number of animals in each group n = 6. aComparison made with vehicle control group; bComparison made with CRS group. \*\*\*P<0.001; \*\*P<0.01

Table 2 showed significant changes (P<0.001) in the organ weights of stressed rats. Animals pretreated with aqueous extract of *Rosa canina L* at both doses reduced stress induced changes significantly.

## DISCUSSION

Restraint stress produces psychological and physiological stress and was chosen as stress inducer to produce female reproductive dysfunctions.<sup>13</sup> Aqueous extract of *Rosa canina L.* hips was evaluated for female fertility improving activity in stressed rats using restraint stress models. The estrous cycle in rats involves many histological, physiological and morphological and biochemical changes within the ovary. During the estrous cycle the maturation and ovulation of preovulatory follicles takes place under the combined and balanced influence of ovarian and extra ovarian hormones. Any imbalance in these hormones leads to irregularity in the function of ovary and changes in the duration of estrous cycle.<sup>14</sup> Similarly oxidative stress also causes damage during oocyte maturation and ovulation.<sup>15</sup>

Restraint stressed rats showed a significant increase in the mean number of days in proestrous phase and decrease in estrous, metestrous and diestrous phases indicating the arrest of follicular development at the initial stages leading to nonmaturation of the follicles. Non availability of matured follicle indicates decrease estrous and metestrous phases. This disruption in the growth and differentiation of preovulatory follicles may be due to the non-availability of steroidal hormones, which are essential for their maturation and differentiation; local estrogen produced by granulosa cells, imbalanced endogenous steroid and protein hormones or due to the generation of free radicals like Reactive Oxygen Species (ROS). Aqueous extract of *Rosa canina L* treated groups showed significant decrease in the mean days of proestrous phase which indicates the development of follicles and significant increase in the mean days of estrous,

metestrous and diestrous phases when compared with Restraint stressed rats showing antagonizing effect against stress. It also indicates the maturation of the follicles, formation of Graafian follicles and corpus luteum which may be due to the increased secretion of either gonadotrophic or steroidal hormones or both or reversal of the stress induced oxidative stress.<sup>16</sup>

Ovaries are considered to be an aggregate of three endocrine tissues, the stroma, the follicle and the corpus luteum. The weight of these tissues constitutes the net weight of ovaries. The decrease in ovarian weights in stressed rats clearly indicated that there was no development of the follicles and hence decreases in activity of stroma, follicle and corpus luteum due to non-availability of either gonadotrophic or steroidal hormones or both<sup>17</sup> or due to oxidative stress. Aqueous extract of *Rosa canina L* treated groups showed a significant prevention in the loss of weight of ovaries which may be due to the availability of gonadotrophic or steroidal hormones or due to combating oxidative stress.

A significant decrease in uterine weight was also seen in stressed rats due to the non-availability of the hormones required for the development of uterus. Aqueous extract of *Rosa canina L* treated groups showed a significant prevention in the loss of weight of uterus which may be due to uterotrophic effect.

The increase in weight of adrenal glands in stressed rats may be due to the active involvement of the Hypothalamic Pituitary Adrenal (HPA) axis and sympathetic activation, which is highly responsive to stress. The adrenal hypertrophy takes place in response to the secretion of Adrenocorticotropic Hormone from the pituitary for

increased corticosterone from cortical cells to combat stress.<sup>18</sup> Aqueous extract of *Rosa canina L* treated groups showed significant decrease in the weight of adrenal glands which may be due to reversal of stress-induced adrenomedullary response to decreased production of corticotropic hormone.<sup>18</sup>

## CONCLUSION

Thus aqueous extract of *Rosa canina L* at both doses is useful in relieving stress induced female reproductive disorders.

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