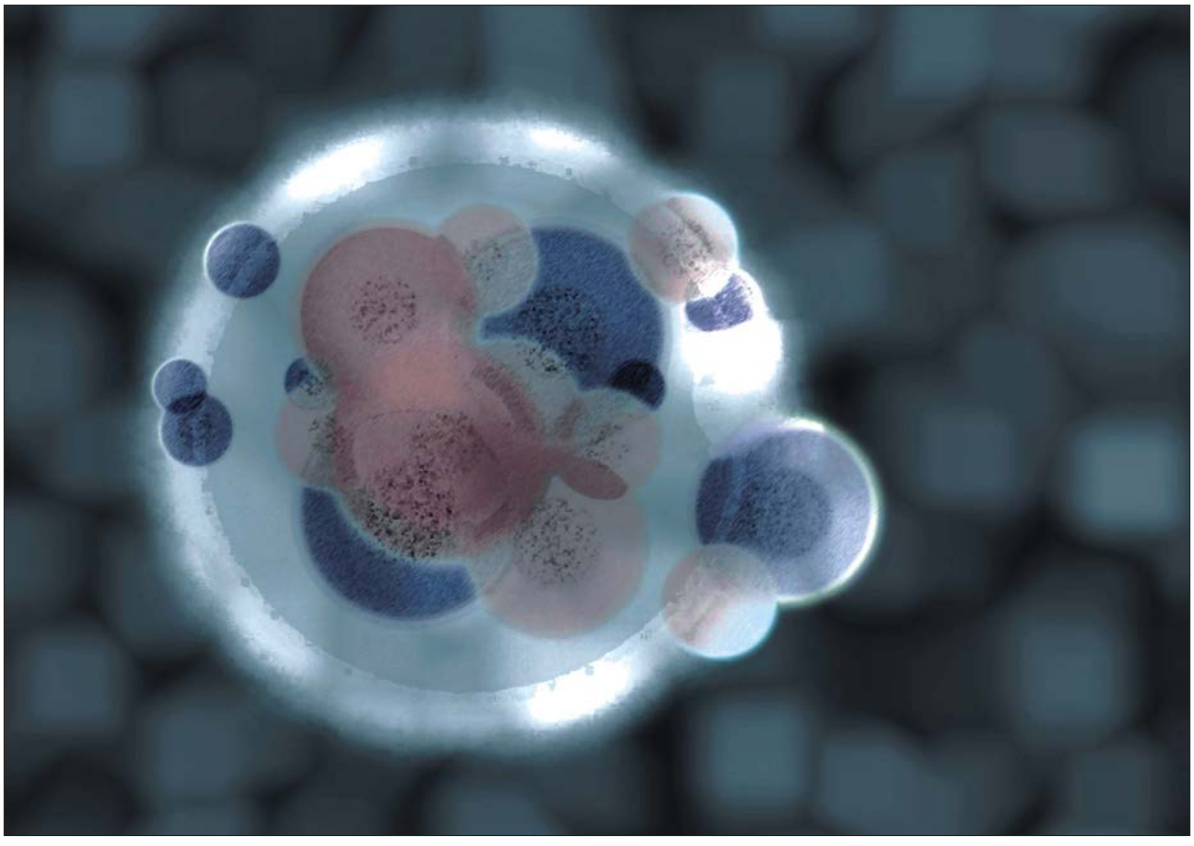

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 7 (3): 582-584, 2007
ISSN 1727-3048

Effects of Ethanolic Extract of *Capparis aphylla* (Roth.) on Testicular Steroidogenesis in Rats

[1]I. Sarathchandiran, [1]R. Manavalan, [2]M.A. Akbarsha, [2]B. Kadalmani and [1]P.K. Karar
[1]Department of Pharmacy, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India
[2]Department of Animal Science, Bharathidasan University, Trichirappalli-620 024, Tamil Nadu, India

Abstract: Ethanol extract of *Capparis aphylla* (Roth.) whole plant (EECA) was evaluated for possible testicular antisteroidogenic activity in mature male rat. The ethanol extract at the doses of 50, 100 and 200 mg kg^{-1} body weight (i.p) arrested the testicular steroidogenisis. The cholesterol and ascorbic acid content in testis were significantly elevated in treated rat. The extract also significantly inhibited the activity of Δ^5-3β-hydroxy steroid dehydrogenase (Δ^5-3β-HSD) and glucose-6-phosphate dehydrogenase (G-6-PD), the two key enzymes involved in testicular steroidogenesis. Results of this study suggested that the ethanol extract of whole plant of *Capparis aphylla* (Roth.) acts as an testicular antisteroidogenic agent.

Key words: *Capparis aphylla*, antifertility, Δ^5-3β-HSD, testicular steroidogenesis, G-6-PD, cholesterol, ascorbic acid

INTRODUCTION

During the past few decades sporadic attempts have been made by various investigators to develop male contraceptive agents from various antifertility plants available in their locality or in the market. Various medicinal plant extracts have been tested fro their antifertility activity both in male and female (Kamboj, 1988). Some of these plants had spermicidal effects, others caused reduction in the sperm counts and altered the mobility of the sperms. Some of them caused testicular change and altered hormone levels (Bhargava, 1984; Reddy *et al*., 1997). But so far no significant lead has been obtained by these studies. These results prompted us to screen various plants in our locality. Based on these trials one plant, *Capparis aphylla*, was selected for detailed studies.

Capparis aphylla (*C. decidua*) Roth (Capparidaceae) is a much branched shrubs without leaves or with very small leaves. Flowers are orange red in colour. *C. aphylla* is seen in South west, North west India and Tirunelveli district in Tamil Nadu, India. Bark of this plant is used as diaphoretic, whole plant is used in cough and asthma, fruits used in cardiac trouble. The plant consists capparine, capparilline, capparinine, n-pentacosane, n-triacontanol, β-sitosterol and 1-stachydrine, root bark contains spermidine alkaloid-capparisinine. This is the first attempt in antifertility research, on this plant (Asokkan *et al*., 1992).

MATERIALS AND METHODS

Plant material: Whole plants of *Capparis aphylla* were collected from Tirunelveli district of Tamilnadu, India. Taxonomic identification was made from botanical survey of medicinal plant unit, Government Siddha Medical College, Government of India, Palayamkottai, Tamilnadu, India. The whole plant was dried at room temperature, powdered by the mechanical grinder, sieved and stored for further used. The powder was soxhlated with 90% ethanol at 39°C. The extract was filtered and concentrated to dry mass by vaccum distillation. The semi dried material was diluted with n-butanol and water 1:1, the n-butanol soluble material was separated by separating funnel, dried under room temperature and powdered for further use.

Animal: Three months old Wistar strain male albino rat of 200 g body weight was procured from the Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. Rats were fed with standard pelled feed from Gold Mohur Laboratory animals feeds, Bangalore, India and water *ad libitum*. The experiment was performed under the guidance of the Ethical Committee, Annamalai University, Annamalai Nagar. The animals were housed in polypropylene cage under control environmental condition with provision of 12 h light and 12 h dark.

Materials: The ethanolic extract (dissolved in normal saline) of whole plant of *Capparis aphylla*, digitonin,

Corresponding Author: P.K. Karar, Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu, India
Tel: +0091-04144238627

cholestrol, nicotinamide adenine dinucleotide (NAD), Ascorbic acid, Dehydroepiandrosterone (DHEA), Nicotinamide adenine dinucleotide phosphate (NADP),Glucose-6-phosphate (G-6-P) (SIGMA CHEMICALS,USA), Chloroform LR, Acetone AR, Diethyl ether LR, Potassium hydroxide LR, Disodium hydrogen orthophosphate LR, Potassium dihydrogen phosphate LR, Glacial acetic acid, Acetic anhydride, Ethyl acetate, Sodium acetate (S.D. Chemicals, India), Ethanol (Bengal Chemicals and Pharmaceuticals Ltd., India), Phenolphthalein, Thiourea (Sarabhai M Chemicals Ltd., India),Sodium chloride (Basynth, India), Tris-HCl buffer (SRL, India), Metaphosphoric acid (E.Mark, Germany), 2,4-Dinitrophenyl hydrazine (Loba Chemicals, India), Concentrated bromine solution, Concentrated sulphuric acid (International Chemical Industry, February 20, 2007 India).

Experimental design

Treatment of Animals: Twenty four healthy Wistar albino male rats were selected for present study. The animals were equally divided into 4 groups containing 6 rats each and treated as follows:

Group I: Phosphate buffer saline (PBS) (5 mL kg^{-1} body weight)

Group II: Ethanolic extract of *Capparis aphylla* (Roth.) (EECA) dissolved in phosphate buffer saline (PBS) (50 mg kg^{-1} body weight)

Group III: Eethanolic extract of *Capparis aphylla* (Roth.) (EECA) dissolved in phosphate buffer saline (PBS) (100 mg kg^{-1} body weight)

Group IV: Eethanolic extract of *Capparis aphylla* (Roth.) (EECA) dissolved in phosphate buffer saline (PBS) (200 mg kg^{-1} body weight)

The phosphate buffer saline (PBS) and the different doses of ethanolic extract were administered intraperitoneally on daily for 18 days after 18 h of fasting. The rats were weighed before and after the commencement of the experiment. All the animals were sacrificed 24 h after the last dose and 18 h of fasting. Testis, cauda epididymis and adrenal glands were immediately dissected out, trimmed off from adherent fats and weighed. Sperm from cauda epididymis were released in phosphate buffer saline (PBS).

Biochemical estimation: Testicular tissues about 3 mg weight carefully homogenized in Potter Elvehjem homogenizer using chloroform: Ethanol mixture (2:1) and non-polar part was extracted out and total cholesterol content was estimated Abell *et al.* (1952).

About 5 mg of tissue was homogenized in Potter Elvehjem homogenizer using 2.5 mL ice cold 5% metaphosphoric acid and centrifuged for 20 min at 355 rpm then ascorbic acid content was calculated (Sierralta *et al.*, 1978).

About 3 mg of testicular tissues was again homogenized in Potter Elvehjem homogenizer using 0.5 M Tris-HCl (pH 8.3) and centrifuged. The activity of G-6-PD was estimated as described by Bergmeyer (1965).

About 2 mg of testicular tissues was homogenized in Potter Elevhjem homogenizer using 1 mL of normal saline and 1 mL of 0.1 M phosphate buffer (pH 7.4) and centrifuged. The activity of Δ^5-3 β-HSD was estimated as described by Nakajin *et al.* (1995).

Protein was estimated with Folin's phenol reagent and the activity of enzymes were expressed in unit per mg of protein as described by Lowry *et al.* (1951)

Statistical analysis: Statistical analysis was done by Student' t-test

RESULTS AND DISCUSSION

The results are summarized in the Table 1. The ethanol extract of whole plant of *Capparis aphylla* (Roth.) (EECA) at all the doses of 50, 100 and 200 mg kg^{-1} body weight significantly increased the level of total cholesterol and ascorbic acid contents of testicular tissues in treated rats. The activities of Δ^5-3β-HSD were inhibited significantly ($p<0.05$ by 50 mg and $p<0.001$ by both 100 and 200 mg). Similarly, the activities of G-6-PD were inhibited significantly ($p<0.01$ by 50 mg and $p<0.001$ by both 100 and 200 mg) by all the doses of whole plant of *Capparis aphylla* (Roth.) (EECA). From the data, it is evident that the drug treated rats resulted in, the cholesterol and ascorbic acid content is more than the Phosphate Buffer Saline (PBS) control treated rats and decrease in the Δ^5-3β-HSD and glucose-6-phosphate dehydrogenase activities in comparison to Phosphate Buffer Saline (PBS) treated rats.

In our earlier studies, the ethanolic extract of *Capparis aphylla* (Roth.) showed reduction in the number of spermatozoa (sperm count) and their motility, which is due to inhibition of androgenic synthesis and as a result there is reduction in weights of testis and accessory reproductive organs. This idea was further strengthened by the accumulation of cholesterol and ascorbic acid, the principal precursor for the formation of androgens in biogenic pathway in the testis. The further

Table 1: Effect of ethanol extract of *Capparis aphylla* (Roth.) whole plant (EECA) on testicular cholesterol, ascorbic acid, Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) and glucose-6-phosphate dehydrogenase (G-6-PD) content in rats (mean±SD, n = 6)

Treatment design	No. of treatment days	Cholesterol (mg g^{-1} tissue)	Ascorbic acid (mg g^{-1} tissue)	Δ^5-3β-HSD (U mg^{-1})	G-6-PD (U mg^{-1})
PBS (5 mL kg^{-1} b.w i.p)	18	87.32±4.50	145.44±5.32	8.77±0.17	26.96±0.27
EECA (50 mg kg^{-1} b.w i.p)	18	102.24±5.22*	170.46±6.78*	6.04±0.22*	20.12±0.31
EECA (100 mg kg^{-1} b.w i.p)	18	132.41±6.34**	188.32±7.81**	5.10±0.21**	15.50±0.16**
EECA (200 mg kg^{-1} b.w i.p)	18	169.32±4.24***	205.48±10.41***	4.04±0.12***	11.81±0.12***

PBS = Phosphate buffer saline, b.w = body weight, i.p = intraperitoneal, EECA = Ethanol extract of *C. aphylla*. *p<0.05. **p<0.01, ***p<0.001 significantly different form phosphate buffer saline control

support to this proposition obtained from the diminished values of Δ^5-3β-HSD and glucose-6-phosphate dehydrogenase activities in testis. It is well documented that Δ^5-3β-HSD is a key enzyme involved in androgen biogenesis. Knorr successfully established that Δ^5-3β-HSD is an important enzyme in the production of steroid hormone. McKerns have shown that gonadotrophins through the activation of glucose-6-phosphate dehydrogenase metabolism in pentose phosphate pathway increased the rate of formation of NADPH essential for hydroxylation reaction in the formation of the steroid hormones from cholesterol.

CONCLUSIONS

On the basis of present findings and experimental data, it may be concluded that the ethanolic extract of *Capparis aphylla* (Roth.) whole plant exhibited inhibition of testicular steroidogenesis in male rats thereby acting as an antifertility agent.

REFERENCES

Abell, L.L., B.B. Levy, B.B. Brodie and F.E. Kendall, 1952. A simplified method for the estimation of cholesterol in serum and demonstration of its specificity. J. Biol. Chem., 95: 357.

Asokkan, L.V., K.K. Kokkar and O.J. Chakar, 1992. Glossary of Indian Medicinal Plants with Active Principles. Part-I CSIR-New Delhi.

Bergmeyer, H.U., 1965. In: Methods of Enzymatic Analysis. 2nd Edn., Academic Press, New York, pp: 744-751.

Bhargava, S.K., 1984. Effects of plumbagin on reproductive functions in male dog. Ind. J. Exp. Biol., 22: 153-156.

Kamboj, V.P., 1988. A review of Indian medicinal plants with interceptive acitivity. Ind. J. Med. Res., 87: 336-355.

Lowry, O.H., D.J. Rosesnburg, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin-phenol reagent. J. Biol. Chem., 193: 265-275.

Nakajin, S., T. Nakajima, M. Uchida, S. Ohno and M. Shinoda, 1995. Direct expression of pig testicular 3 α/β (20 β)-hydroxysteroid dehydrogenase in *Escherichia coli*. J. Steroid Biochem. Mol. Biol., 54: 257-264.

Reddy, M.C., D. R.K. Murthy and S.B. Paril, 1997. Antispermatogenic and androgenic activities of various extracts of *Hibiscus rosasinesis* in albino mice. Ind. J. Exp. Biol., 35: 1170-1174.

Sierralta, W., A.J. Truitt and P.W. Jungblut 1978. Studies on the involvement of lysosomes in estrogen action, I. Isolation and enzymatic properties of pig endometrial lysosomes. Hoppe Seylers Z Physiol. Chem., 359: 517-528.