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EFFECT OF β-SITOSTEROL ISOLATED FROM THE *BARLERIA PRIONITIS* ROOTS ON THE L-CARNITINE AND GYCEROPHOSPHOCHOLINE CONTENTS OF THE REPRODUCTIVE TISSUES OF MALE RATS

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ABSTRACT

Objective- *Barleriaprionitis* (*B.prionitis*), famous as the porcupine flower, has various medicinal properties in Ayurveda. Among the several phytochemicals, β- sitosterol (BS), is one of the nutrients present in *B. prionitis*. BS is a well-known natural sterol comprised by many herbal drugs. L-carnitine and Glycerophosphocholine (GPC) are one of the main reproductive parameters that reflex the reproductive capacity of a male. Therefore, in the present investigation, the effect of BS, isolated from *B. prionitis* roots was estimated on these parameters as well some other necessary reproductive indices because by reducing them we can construct an oral male contraceptive method which is essential in this era, as we are dealing with the extreme population explosion in India. **Methods-** BS was extracted using soxhelet extraction method from the roots of *B. prionitis* and administered orally to the male albino rats at the dose levels of 10 and 30 mg/kg body weight, the control group of rats was fed by olive oil in the same quantity, for the duration of 60 days. After completion of the experiment, rats were autopsied and their testes and Cauda Epididymides were dissected out. Sperm motility, sperm density, L-carnitine, GPC contents were measured in them. **Results-** All four parameters showed a significant dose dependent decline in the BS treated groups as compared to the control. **Conclusion-** These results are indicative that BS is an antifertility agent present in *B. prionitis* roots, as all four parameters above effect the fertility in male rats.

Keywords- Sterol, Sperm motility, L-carnitine, Phytochemicals, Soxhelet

INTRODUCTION

Population growth throughout the world, especially in rising developing countries like India is affecting tremendously our economic progress [1]. Therefore, priority should be given for the eradication of this population explosion [2].The alternatives available to men for fertility control are much more restrained compared to those of adult females. Continued efforts over the past three decades to develop additional methods of male contraception have made some substantial contributions in the area [3]. Although considerable progress has been realized in the ontogeny of a highly effective kind of synthetic contraceptive drugs for men, the most challenging pursuits are the search for fresher, more potent,cheaper and reversible methods with lesser side effects [4].That is why traditional herbal drugs from plant sources have received considerable interest for the control of men fertility.If we somehow limit the sperm motility and sperm count of the semen, this can lead to a lower capacity of sperms to fertilize the ova. Hence our main aim which is to check the population explosion can be achieved.

Among several medicinal plants, *Barleriaprionitis (B. prionitis)* L. of Acanthaceae family is significant and clearly accepted herb possessing healing & curative qualities. It is commonly known as Porcupine flower, Barleria, Kundan, Mullu guarantee, Pilikantashelio, etc. The flora is especially well recognized for caring for bleeding gums and toothache. Because of its anti odontalgic property, it is also recognized as 'Vajradanti' [5].

Extracts and isolated phytochemicals from this plant have been found to have a wide scope of pharmacological activities like antimicrobial [6], Anti-nociceptive,[7], antispermatogenic [8],antihelmintic, antioxidant [9], antidiabetic [10], anti-inflammatory [11], antiarthritic, cytoprotective, Antihelmintic [12], diuretic, antidiarrheal, and enzyme inhibitory activities without any toxic effects. The aqueous bioactive fractions are reported to possess hepatoprotective, antistress, and immuno restorative properties [13]. Male rats treated with isolated fractions of the *B. prionitis* root methanolic extract (100 mg/kg for 60 d) showed a significant reduction in spermatogenesis without affecting general body metabolism. Sperm motility and sperm density in cauda epididymides weresignificantly deteriorated. The population of various spermatogenic cells, such as primary spermatocytes, secondary spermatocytes, and round spermatids was declined significantly in treated animals [14]. This attracted us to isolate the active component of *B. prionitis*and to determine if this can be accountable for the antifertility activity in male rats. Herein we reported the isolation of β-sitosterol (BS) which is one of the most ubiquitous substances of plant extracts [15]. β-sitosterolhas been proven to be aharmless and nontoxic plant nutrient for sustaininggood health and safeguard against many severe health disorders and diseases. β-sitosterol posse's genotoxicity effect [16], antidiabetic [17-19], antibacterial [20], antimicrobial [21], antihelminthic and antimutagenic [22] activities. It is also utilized for the Prostatic cancer treatment [23].

Novelty and rationality of this study-

Among the various factors in male reproductive tissues which are responsible for the control of male fertility,here we have considered L-carnitine and GPCespecially because both of them are the marker parameters in the testes and epididymis to improve sperm motility. L-carnitine provides readily available energy to spermatozoa. This energy plays a key role in the sperm metabolism like sperm motility,maturation and spermatogenesis. This beneficial effect is mediated by the transport of long chain fatty acids across the inner membrane of mitochondria for utilization in metabolism through β-oxidation [24]. So as GPC has also been known to occur in low concentration in the water-soluble fraction of mammalian tissues. [25], first identified GPC as a major component in reproductive tracts of male rats. A recent survey has shown that GPC is probably a universal constituent of mammalian semen and that

in larger domestic animals the fluid contents of the epididymis are a rich source [26], there was a significant correlation between sperm motility, progression, and the GPC ratio [27].

In this backdrop current research is formulated to study if our active component BS that was isolated from the roots of *B. prionitis* can reduce the levels of testicular L-carnitine and GPC. If these two are reduced to a significant level we can limit the sperm dynamics and further male fertility too.

MATERIALS AND METHODS

Collection and authentication of the plant material

The plant material (roots) of *Barleriaprionitis* was collected from the hilly area of Ajmer, Rajasthan. The plant was taxonomically identified and authenticated by the Department of Botany, University of Rajasthan, Jaipur. (RUBL NO. 211575). A Voucher specimen was preserved in the reproductive physiology laboratory, Department of Zoology, University of Rajasthan, Jaipur for further verification.

Extraction and isolation of the compound

The shade dried plant material (1.5 kg) was finely powdered and extracted with methanol in a 5 liter round bottom flask for 72 h on a water bath. The extract was filtered hot and the solvent was removed by distillation under reduced pressure where a semi-solid dark gray mass (27 g) was obtained. The solvent free extract was chromatographed over silica gel column built in petroleum ether and eluted with increasing amounts of benzene. Elutes of 200 ml were collected each time, and the solvent was distilled on a water bath. The homogeneity of the fractions was examined by TLC on silica gel plates. The spots developed were visualized under UV light and then by exposure to iodine vapor. Similar fractions were combined and purified. Fractions eluted with petroleum ether-benzene mixture (80:20) yielded a white solid with an Rf value of 0.47 on TLC in petroleum ether-benzene (4:1). The structure of the isolated compound was established on the basis of elemental analysis and spectroscopic evidence (IR, UV, 1HNMR, 13CNMR, MS). Test for alcohol and steroid were done by using Salkowski reaction and Liebermann-burchard reaction [28].

Animals

Healthy and fertile male albino rats (Body weight: 150 to 200 g) of "Wistar strain" were used for the present investigation. All animal experiments were performed according to ethical guidelines suggested by the Committee for the Purpose of Control and Supervision of experiments on animals (CPCSEA), Ministry of Environment and Forest, Government of India (1678/GO/ a/12/CPCSEA Dated 09-01-2013).

Dose determination

Doses were determined using fixed-dose procedure. Briefly, BS was given at one of the four fixed doses (5, 50, 100, 2000 mg/kg BW) at a time to 5 male Wistar rats. On the basis of these experiments, two doses of BS (i.e. 10 and 30 mg⁄ kg Body Weight (BW)/ d) were used in subsequent experiments.

Experimental design

Animals were kept for 7 d for acclimatization prior to the experiment. After 15 d of acclimatization, they were randomly divided into 3 groups of 4 animals each. The daily dose of the compound was prepared and administered to each animal for 60 d. The treatment schedule of each group was as follows:

Group I: Animals of this group received 0.5 ml of olive oil/day for 60 d. (Olive oil-treated control)

Group II: Rats received BS in a dose of 10 mg/kg body weight daily for 60 d.

Group III: Rats received BS in a dose of 30 mg/kg body weight daily for 60 d.

Autopsy scheduled

After 24 h of last treatment, the final weight was recorded, and the animals were sacrificed using mild ether anesthesia.

Sperm characteristics and analysis of L-carnitine and GPC

Sperm motility and sperm density were done using the method described by Prasad et al. [29]. L-carnitine and GPC content was described using the methods by [27] and [30] respectively.

RESULTS

- **Sperm motility and sperm density**

  Sperm motility and Sperm density were significantly reduced in treated groups i.e., 10 mg and 30 mg/kg BW BS treated groups of rats, as compared to control. (Table-1).

- **L-carnitine**

  There was a significant decrease in the L-carnitine content of epididymis due to the treatment of 10 and 15 mg/kg BW of BS ($p<0.01$) from *Barleriaprionitis*roots, when compared to control. (Table-1).

- **GPC**

  GPC contents of epididymis also showed similar dose dependent decline as stated above for L-carnitine.

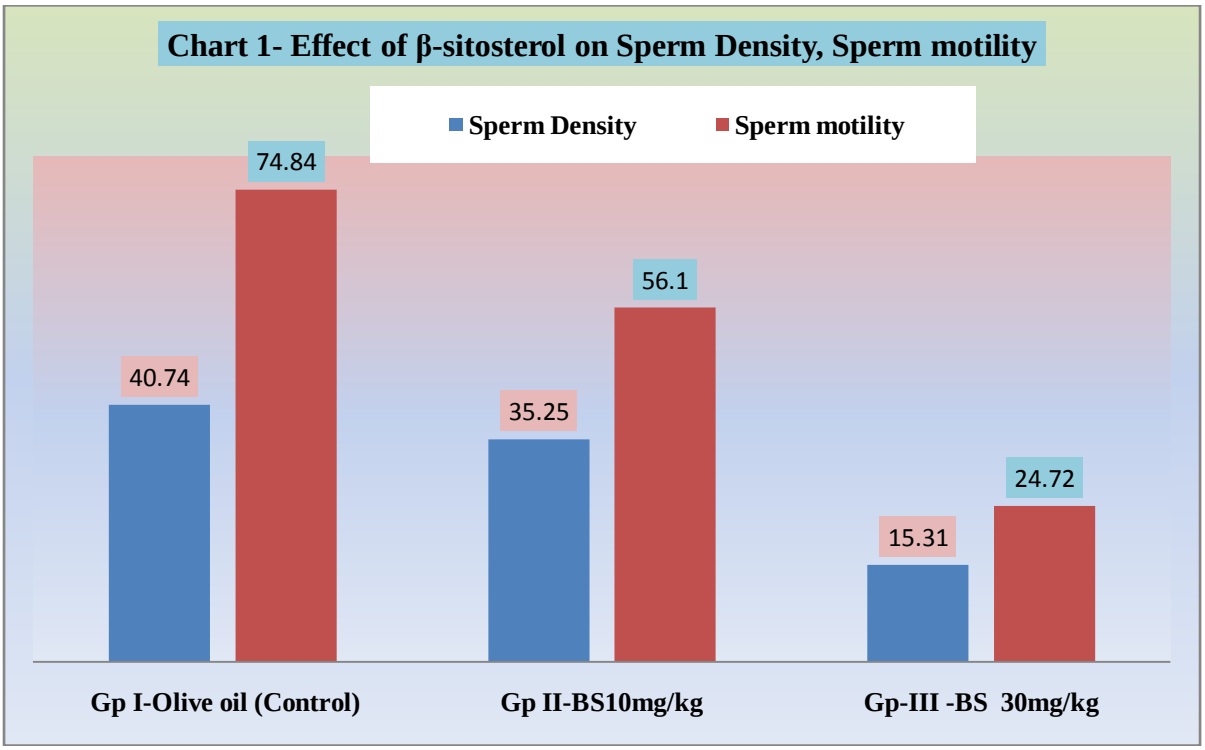
  GPC levels were significantly reduced in the 10 ($p<0.01$) and 30 ($p<0.001$) mg/kg BW of BS treated groups, comparatively to control. (Table-1).

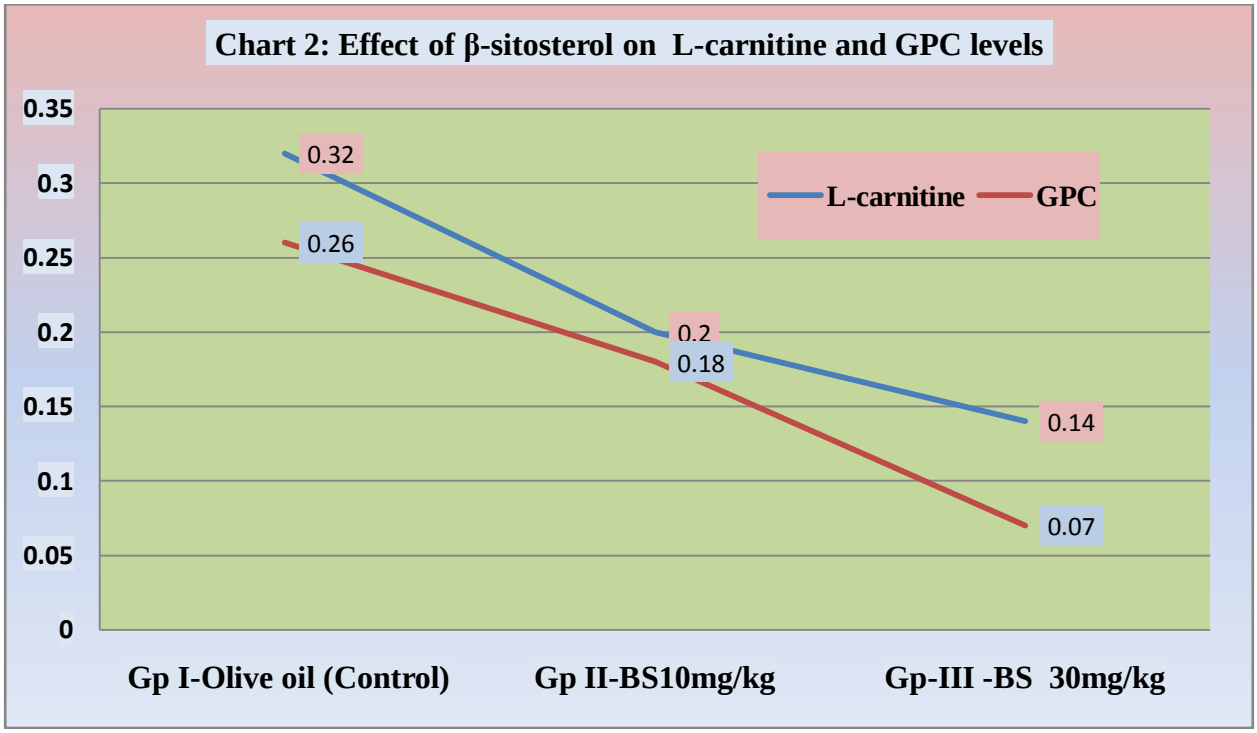
Table 1: Effect of β-sitosterol on sperm Density, sperm motility, L-carnitine and GPC levels of male albino rats.

Groups	Treatment	Sperm Density (Millions/ml)	Sperm motility (%)	L-carnitine (g/dl) (Epididymis)	GPC (mmol/l) (Epididymis)
Group I	Olive oil (Control)	40.74 ± 1.02	74.84 ±0.67	0.32 ±0.04	0.26 ±0.02
Group II	BS 10mg/kg	35.25* ±1.36	56.10** ±3.65	0.20** ±0.03	0.18** ±0.03
Group III	BS 30mg/kg	15.31*** ±0.56	24.72*** ±1.56	0.14** ±0.05	0.07*** ±0.04

Duration: 60 d; Values are ± S.E.M.; Four animals were maintained in each group
*Level of significance- ns- non significant, *p<0.05, **p<0.01, ***p<0.001, when compared to control*

Chart: Effect of β-sitosterol on sperm Density, sperm motility, L-carnitine and GPC levels of male albino rats.





DISCUSSION

Plants and their products constitute the fundamental portion of Medicare. Comparatively few attempts have been made to study the effects of plant products on male

reproductive system and fertility. For a communal perception of fertility control, we led to study the effect of *B.prionitis* active constituent i.e., BS which was isolated from its roots, on the male fertility and some marker parameters of male reproductive tissues.

The results of this study revealed a marked decrease in the sperm motility as well as sperm density in a dose-dependent manner with BS. Sperm characteristics are considered as an imperative reproductive index as they account for the process of spermatogenesis and fertility. The observation of low sperm count in BS treated animals is associated with testosterone suppression, which is consistent with previous findings [31-33]. Lower sperm concentration might be an indicator of spermatogenesis suppression. Another reason for this low sperm count is the generation of reactive oxygen species which further leads to the oxidative stress in the reproductivetissues. Inanother study impairment in testicular gametogenesis and steroidogenesis was observed because of the treatment of alpha-tocopherol-succinate (provitamin-E) which showed similarities with our experiment [34]. As well as, testicular weight reduction may also be one of the reasons for the reduction in the sperm count [35]

A Significant decrease in sperm motility was also observed in the present study, which stresses that BS have the straight influence on mature and stored sperms in Epididymis. Sperm motility may be decreased due to alteration in energy metabolism of sperms. Rich and consistent supply of energy in the form of ATP isrequisite for the sperms tosustain motility in the male and female reproductive tract. The initiation of the flagellar movement of sperm is dependent on the phosphorylation of a contractile protein dynein. Brokaw [36] described in their studies that after phosphorylation, the dynein ATPase is activated. The energy released by the hydrolysis of ATP converted to force, causes the microtubules to slide past one another. Interference with enzymatic reactions involved in uncoupling of oxidative phosphorylation may be a reason for reduced sperm motility by BS treatment. This can lead to the incompetence of the spermatozoa to reach the Fallopian tubes and fertilize the egg, thus causing sterility [37].

Highly polar compounds like Carnitines have extensive distribution around nature [38]. L-Carnitine, which is a quaternary amine, is present in testis and epididymis and play a key role in sperm metabolism by providing readily available energy for the spermatozoa, which is necessary for an optimum sperm motility, maturation and spermatogenesis. β-oxidation which occurs in mitochondria is a process in which long chain fatty acids are metabolized into acetyl-CoA, which enters the citric acid cycle, and NADH and $FADH_2$, which are co-enzymes used in the electron transport chain, both of these processes are necessary for the ATP generation. Due to the negative charge present on the membrane of mitochondria long chain fatty acids cannot penetrate it, so a carnitine shuttle (have L-carnitine as a key factor) is utilized, through that fatty acids are transported across inner mitochondrial membrane [39]. β-oxidationwhich occurs in mitochondria is a process in which long chain, fatty acids are metabolized into acetyl-CoA, which enters the citric acid cycle, and NADH and $FADH_2$, which are co-enzymes used in the electron transport chain. Due to the negative charge present on the membrane of mitochondria long chain fatty acids cannot penetrate it, so a carnitine shuttle (have L-carnitine as a key factor) is utilized, through that fatty acids are transported across the inner mitochondrial membrane. So, this reflects that L-carnitine is an essential component in the male reproductive system to provide energy to the spermatozoa. BS from *B. prionitis*may hinder carnitine biosynthesis by inhibiting enzymes like 4-N-trimethylaminobutyraldehyde dehydrogenase, which is necessary for carnitine synthesis. This type of similar result is also found in a study, where carnitine acetyltransferase was inhibited by mildronate [40].

Fertilizing capacity and maturation of spermatozoa occurs when they reach to epididymis through testes. There, they also attain motility. The organic fraction of human seminal plasma contains phosphate esters, particularly glycerylphosphorylcholine(GPC),

phosphorylcholine (PCh), and inorganic phosphate (ip). GPC is found in relatively high concentrations in the semen of many male animals, including man. GPC is synthesized by the epithelial cells of the epididymis, apparently under testosterone control [41]. Consequently, it has been suggested that GPC might be a useful indicator of epididymal function as it occurs in a significantly higher concentration in epididymis than in any other part of the male reproductive system [42]. BS isolated from *B. prionitis*is a primary steroid used in traditional medicine, which is structurally similar to cholesterol. It may inhibit the conversion of cholesterol to testosterone by blocking the side chain cleavage of cholesterol by CYP11A(a mitochondrial cytochrome P450 oxidase) or any other step of testosterone biosynthesis [43]. As testosterone levels are reduced, dependent GPC levels also get a decline. Further, these reduced GPC levels affect the functioning of the epididymis, which leads to defects in the sperm maturation process. These effects cumulatively reduce the sperm motility and density. As an outcome to this fertilizing capacity gets declined.

CONCLUSION-

According to the results of this study, it can be concluded that BS isolated from the roots of *Barleriaprionitis,* affects the male fertility in a dose dependent manner, by reducing sperm motility, sperm density, L-Carnitine and GPC levels. As these four parameters mainly reflects the functioning of male reproductive tract. So, BS can be used for the development of oral male contraceptive, but further studies should be planned to check the reversibility of the treatment and exact mechanism at the molecular level.

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CONFLICT OF INTERESTS

Declared none.

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