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Phytochemical Investigation and Anti-Fertility Activity of Lichen *Parmelia perlata*

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***Abstract*- The present work deals with the investigation of the petroleum ether extract of lichen *Parmelia perlata*. The study led to the isolation of compounds- (I) Stigma-4,22-diene-3-one, (II) Stigmasterol, (III) 5,4´-Dihydroxy-3,6,7,3´,5´ pentamethoxy flavone, (IV) Benzyl-2,6-dimethoxy benzoate, (V) Benzyl-2,3,5,6-tetramethoxy benzoate. Compounds III-V have been isolated for the first time from this lichen. Also the anti-fertility activity of the crude extract was studied. *Parmelia perlata* exhibited anti-spermatogenic effect in treated rats. The pet-ether extract of *Parmelia perlata* decreased the fertility of male albino rats by 100%.There was a marked decrease in the number of primary and secondary spermatocytes and spermatids. A significant reduction was found in seminiferous tubular diameter and differential count of Leydig cells.**

***Keywords*- *Parmelia perlata*, 5,4´-dihydroxy-3,6,7,3´,5´ pentamethoxy flavone, benzyl-2,6-dimethoxy benzoate, benzyl-2,3,5,6-tetramethoxy benzoate, anti-fertility, male albino rats.**

I. INTRODUCTION

Parmelia perlata is a well known lichen of family Parmeliaceae. A lichen is an association of an alga and fungus, living together in a symbiotic relationship. *Parmelia perlata* is commonly called Stone flower or Chadila. In India it is mainly found in Himachal Pradesh and West Bengal. It is used as food, fodder and medicine. It is a good pain reliever and is used as a remedy for early healing of wounds. It cures many skin diseases and is considered to be an expectorant, astringent, resolvent, laxative, carminative and aphrodisiac. It is also used in treatment of fever, cough, dysentery and renal calculi. This lichen exhibits antimicrobial [1-2], antiviral [3], anti-tumor [4], antispasmodic [5] , antioxidant [6] and antipyretic [7] activities. Its hepatoprotective action [8] has also been reported. Phytochemical studies of *P.perlata* have led to the isolation of various chemical constituents such as atranorin, chloroatranarin, salazinic acid [9], lecanoric acid [10], imbricaric acid [11], lecanora. Two terpenes, parmelanostene and permelabdone [12] and usnic acid [13] have also been isolated from this lichen.

Encouraged by the presence of interesting chemical compounds in this lichen, we examined its petroleum ether extract. The study led to the isolation of five compounds- (I) Stigma-4,22-diene-3-one, (II) Stigmasterol, (III) 5,4´-Dihydroxy-3,6,7,3´,5´ pentamethoxy flavone, (IV) Benzyl-2,6-dimethoxy benzoate, (V) Benzyl-2,3,5,6-tetramethoxy benzoate. Compounds III-V has been isolated for the first time from this lichen. In pursuing our interest in its medicinal and bioactive properties, the anti-fertility activity of the extract was also studied.

II. MATERIALS AND METHODS

A) Plant material

The plant material *Parmelia perlata* (lichen) was collected from the hills of Himachal Pradesh (India). Identification of the lichen was done with the help of Department of Botany, University of Rajasthan, Jaipur, India and a voucher specimen was deposited at RUBL Herbarium, Jaipur (RUBL 3674).

B) *General experimental procedures*

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus and are uncorrected. TLC (both Qualitative and quantitative) was performed on aluminium sheet Kieselgel 60 F254 (E. Merck). For column chromatography silica gel (E. Merck, 60-120 mesh, 550 gm) was used. The IR spectra (with KBr pellets, ν in $cm^{-1)}$ were recorded on FTIR SHIMADZU 8400S spectrometer . The ^{1}H and ^{13}C NMR spectra (δ in ppm, *J* in Hz, in $CDCl_3$) were recorded at 300 MHz and 125 MHz on a Brucker NMR instrument, respectively, using TMS as an internal standard. FAB mass spectra were recorded on JEOL SX 102 /DA-6000 mass spectrometer using Argon/Xenon as FAB gas.

C) *Extraction and isolation*

Shade dried lichen was powdered and extracted with petroleum ether on steam bath for 3 x 12 hrs. The crude extract, after removal of solvent, was obtained as a dark green, semi-solid mass (400 gm). The extract so obtained was chromatographed over silica gel column (of height 1.2 m with 5 cm diameter) packed with 900g silica gel. The column was eluted with different solvents in order of increasing polarity and the following compounds were isolated, purified and characterized.

i) 5,4´-Dihydroxy-3,6,7,3´,5´ pentamethoxy flavone (III)

This compound was isolated on elution of the column with chloroform and ethyl acetate in 3:1 ratio. After removal of solvent, a yellow crystalline solid was obtained. A single spot was seen on TLC examination. Its R_f value was found to be 0.32 in system [C_6H_6: CH_2Cl_2: Et_2O (1:1:1)]. The melting point of this compound was 179^0 C. IR (KBr, cm^{-1}): 3510 (-OH), 1660, 1600 (C=C-C=O). ^{1}H NMR (δ ppm, $CDCl_3$): 12.32 s (-OH), 7.45 s (H-2´, H-6´), 6.43 s (H-8), 4.01 s (-OCH_3), 3.96 s (-OCH_3), 3.95s (2 × OCH_3), 3.90s (-OCH_3). ^{13}C NMR (δ ppm, $CDCl_3$): 189 (C=O), 51.6 (3,6,7,3',5'-O$\underline{C}H_3$),106(C-2),162(C-3), 122 (C-1'), 127(C-2', C-6'), 159 (C-3' ,C-5'),162(C-4'), 104 (C-10), 110 (C-6), 132 (C-7), 128(C-5),194(C-8) and 150 (C-9). MS (m/z): 404 [M^+], 389 [M-Me]$^+$, 373 [M-OMe]$^+$, 359, 343, 331, 246, 118, 69, 57 etc.(for molecular formula $C_{20}H_{20}O_9$).

Isolation of compounds (IV) and (V)

On elution of the column with pure solvent ethyl acetate, two compounds were obtained which were further separated by preparative TLC using Petrol-Et_2O (7:3) mixture as solvent system.

ii) Benzyl-2,6-dimethoxy benzoate (IV)

This compound was isolated as colourless oil, 45mg and it showed a single spot on TLC plate. IR (KBr, cm^{-1}): 3000-2880 (C-H str.), 1750 (COOR), 1610, 1590, 1480, 1400, 1310, 1265 (C-O str.), 1125, 1080 cm^{-1}. ^{1}H NMR (δ ppm, $CDCl_3$): 7.44 dd (2 × Ar-H), 7.36 m (3 × Ar-H), 7.26 d (1 × Ar-H), 6.55 d (2 × Ar-H), 5.36 s (O-CH_2-Ph), 3.80 s (2× OCH_3). ^{13}C NMR (δ ppm, $CDCl_3$): 170.92 (C=O), 65.23 (-O-$\underline{C}H_2$-), 56.3(O$\underline{C}H_3$ at C-2 and C-6), 103(C-1') , 135.28 (C-2',C-6'), 127.34 (C-3',C-5'), 126.04 (C-4'),162.45 (C-1), 135.69 (C-2 and C-6),130.04 (C-4),102.22 (C-3 and C-5) . MS (m/z): 272 [M^+], 181 [M-CH_2-Ph]$^+$, 165 [$C_6H_3(OMe)_2CO]^+$, 149 [181-MeOH]$^+$ 91[$CH_2C_6H_5]^+$ for molecular formula $C_{16}H_{16}O_4$.

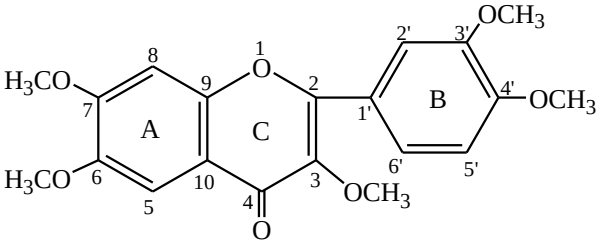
iii) Benzyl-2,3,5,6-tetramethoxy benzoate (V)

It was isolated as colourless oil, 30 mg and it showed a homogenous behaviour on TLC plate. IR (KBr, cm^{-1}): 2900-2880 (C-H str.), 1735 (-COOR), 1595, 1380, 1280 (C-O str.), 1130, 1100, 1080 cm^{-1}. $^{1}HNMR$ (δ ppm, $CDCl_3$): 7.47 d (2 × Ar-H), 7.35 m (3 × Ar-H), 6.75 s (1 × Ar-H), 5.39 s (Ph-CH_2-O), 3.85 s (2× OMe), 3.74 s (2 × OMe). ^{13}C NMR (δ ppm, $CDCl_3$): 178 (C=O), 67 (-O-$\underline{C}H_2$-), 56.4 (O$\underline{C}H_3$ at C-2, C-3, C-5, C-6), 104(C-1') , 136.38 (C-2',C-6'), 126.04 (C-3',C-5'), 128.34 (C-4'),168.30 (C-1), 145.02 (C-2 and C-6),165.32 (C-4),130.04 (C-3 and C-5). MS (m/z): 332 [M^+], 317 [M-Me]$^+$, 289 [317-CO]$^+$, 225 [$C_6H(OMe)_4CO]^+$,197 [225-CO]$^+$,91 [-$CH_2C_6H_5]^+$ for molecular formula $C_{18}H_{20}O_6$.

RESULTS AND DISCUSSION

Compound (III): For this a highly abundant molecular ion peak was obtained at m/z 404 in the mass spectrum. A base peak at m/z 389 resulted due to the loss of methyl

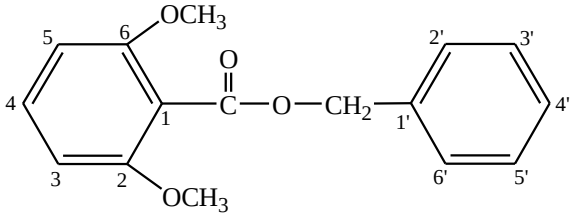
radical from molecular ion. Other prominent peaks were 373 $[M\text{-}OMe]^+$, 359, 343, 331, 246, 118, 69, 57. From this spectral data its molecular formula was found to be $C_{20}H_{20}O_9$. It's IR Spectrum (KBr, cm^{-1}) revealed the presence of hydroxyl group (3510 cm^{-1}) and conjugated carbonyl moiety (1660, 1600 cm^{-1}). The ^1H NMR spectrum (δ ppm, $CDCl_3$) confirmed the presence of chelated hydroxyl group (C-5) by the appearance of the diagnostic signal at δ 12.32. A sharp singlet integrated for two protons (H-2´ and H-6´) of ring C and a singlet due to one proton (H-8) of ring A appeared in the aromatic region at δ 7.45 and δ 6.43 respectively. Large singlets at δ 3.90, δ 3.95, δ 3.96 and δ 4.01 corresponded to the five methoxy groups in the molecule. The ^{13}C NMR spectrum (δ ppm,$CDCl_3$) corroborated the presence of 20 carbons ranging from δ 46 to δ 194. The value at δ 189 was assigned to the carbonyl (C=O) moiety at C-4. The presence of alkene (C=C) carbons at C-2 and C-3 was ascertained by signals at δ 106 and δ 162.The absorption signal for carbon of the methoxy groups appeared at δ 51.6. The carbons of ring B appeared at δ 122 (C-1'), 127(C-2', C-6'), 159 (C-3', C-5') and 162 (C-4'). Similarly, the ring A carbons showed signals at δ 104 (C-10), 110 (C-6), 132 (C-7), 128 (C-5), 194(C-8) and 150 (C-9). With the help of above spectral data, compound (III) was identified as 5,4´-dihydroxy-3,6,7,3´,5´ pentamethoxy flavone. The spectral data was compared with the literature values. This is the first report of the presence of this compound in this lichen.



5-Hydroxy-3,6,7,3',4'-pentamethoxy flanone

Fig.1. Compound III

Compound (IV) : The mass spectrum of this compound exhibited a significant molecular ion peak at m/z 272. Other prominent fragment ion peaks appeared at m/z 181 $[M\text{-}CH_2Ph]^+$, 165 $[C_6H_3(OMe)_2CO]^+$ (base peak) and 91$[C_6H_5\text{-}CH_2]^+$ etc. From this spectral data its molecular formula was confirmed as $C_{16}H_{16}O_4$. . In the IR spectrum (KBr, cm^{-1}) the absorption bands at 1750, 1610, 1590 cm^{-1} revealed the presence of an aromatic ester function. Other significant absorption bands appeared at 3000-2880 (for C-H stretching), 1265 (for C-O stretching). The ^1H NMR spectrum (δ ppm,$CDCl_3$) displayed the presence of a double doublet at δ 7.44 for the two ortho aromatic protons and a multiplet at δ 7.36 corresponded to the three aromatic protons of the monosubstituted benzene ring. The signal for the three aromatic protons of the trisubstituted benzene ring appeared as a doublet at δ 7.26 for one proton and another doublet at δ 6.55 for remaining two protons. The benzylic proton gave a singlet at δ 5.37. For the two methoxy groups a singlet integrated for six protons appeared at δ 3.8 ppm. In $^{13}CNMR$ spectrum (δ ppm,$CDCl_3$) the presence of carbonyl moiety was confirmed by the appearance of a signal at δ 170.92. Signal for oxymethylene carbon was obtained at δ 65.23 and the signal for the two methoxy carbons appeared at δ 56.3. The aromatic carbons of the benzyl moiety gave absorption signals at δ 103(C-1'), 135.28 (C-2',C-6'), 127.34 (C-3',C-5') and 126.04 (C-4'). The absorption signals for the aromatic carbons of the other ring appeared at δ 162.45 (C-1), 135.69 (C-2, C-6), 130.04 (C-4), 102.22 (C-3 and C-5). With the help of these spectral data this compound was identified as benzyl-2, 6-dimethoxy benzoate. The spectral data was compared with reported values. This compound is being reported for the first time in this lichen.

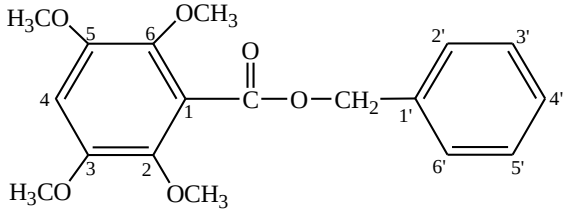


Benzyl-2,6-dimethoxy benzoate

Fig.2. Compound IV

Compound (V) : The mass spectral study of this compound revealed a highly abundant molecular ion

peak at m/z 332 in addition to other important peaks at 317 $[M\text{-}Me]^+$, 289 $[317\text{-}CO]^+$ and 91 $[C_6H_5CH_2]^+$ (base peak) etc. These values suggested its molecular formula to be $C_{18}H_{20}O_6$. In the IR spectrum (KBr, cm^{-1}) the absorption bands at 1735 and 1590 cm^{-1} confirmed the presence of an aromatic ester function. Other significant absorption bands were found at 2900-2880 (for C-H stretching), 1280 (for C-O stretching). The ^1H NMR spectrum (δ ppm, $CDCl_3$) displayed a sharp singlet at δ 6.57 ppm for the lone aromatic proton of the pentasubstituted benzene ring. A broad doublet at δ 7.47 and a multiplet at δ 7.35 corresponded to the two ortho and three aromatic protons of the phenyl ring respectively. A singlet δ 5.39 was assigned to the benzylic protons. The four methoxy groups appeared at δ 3.74 and 3.85 as singlets, each integrated for 6 protons. The $^{13}CNMR$ spectrum (δ ppm,$CDCl_3$) showed absorption signal at δ 178 for carbonyl group (C=O) and for the oxymethylene carbon an absorption signal appeared at δ 67.The presence of methoxy carbons was ascertained by the presence of signal at δ 56.4. Signals for the aromatic carbons of the benzyl moiety appeared at δ 104 (C-1'), 136.38 (C-2', C-6'), 126.04 (C-3',C-5'), 128.34 (C-4'). The aromatic protons of the other ring were identified by the presence of signals at δ 168.30 (C-1), 145.02 (C-2, C-6), 130.04 (C-3,C-5). The absorption signal for C-4 appeared at δ 165.32. From these spectral studies it was concluded that this compound is benzyl-2,3,5,6-tetramethoxy benzoate. The spectral data was compared with literature values. This is the first report of the presence of this compound in this lichen.



Benzyl-2,3,5,6-tetramethoxy benzoate

Fig.3. Compound V

The characterization of compound (I) stigma-4,22-diene-3-one and compound (II) stigmasterol was done on the basis of IR, ^1HNMR, mixed m.p.s. and co-TLC with authentic samples.

III. ANTIFERTILITY ACTIVITY OF *Parmelia perlata* ON MALE ALBINO RATS

Experimental study

Adult proven fertile colony bread Wistar rats weighing 150-175 g, were maintained in poly propyline cages with rat feed (Hindustan Lever Ltd.) and top water ad *libitum.* The animals were divided into one control group and one experimental group of 10 rats each. The routine dose of the plant extract was freshly dissolved in 0.5 ml of distilled water and administrated to test rats- 100 mg/kg per day. The study was approved by the ethical committee of the Department of zoology, University of Rajasthan.

Fertility test

The mating tests were performed from days 55 to 60. Male rats from control and treated groups were caged overnight with proestrous females in the ratio of 1:2 for normal mating. Presence of sperm in the vaginal smear confirmed positive mating and the day was taken as an index of Day-I of gestation. The implantation sites of mated females were checked after 2 weeks by laparotomy. Autopsy were done on mating males.

Blood and serum study

The animals were scarified using light ether anesthesia after 60 days. Blood was collected through cardiac puncture and serum was separated. The RBC and WBC count [14], Haemoglobin [15] and hematocrit [16] were analysed with routine methods. The serum was also tested for protein, cholesterol, HDL-cholesterol, phospholipid and triglyceride.

Sperm analysis

Sperm motility was evaluated in cauda epididymides and sperm density in cauda epididymides and testes by the method [17].

Tissue Biochemistry and Histology

The testes, epididymides, seminal vesicle and ventral prostate were dissected out and weighed. Fresh tissue from testis and accessory sex organs were processed for the biochemistry estimation of glycogen, protein, sialic acid, fructose and cholesterol. Beside they were fixed in Bouins's fluid, embedded in paraffin, sectioned at 5-6 µm and stained with hematoxylin and eosin for histological examination.

Quantitative study

Various testicular cell components i.e. spermatogonia, sertoli cells, preleptotene, pachytene, secondary spermatocyte and rounded spermatids were quantitatively analyzed using 800x. Interstitial cell types such as mature, fibroblasts, degenerating Leydig cells, were calculated by applying a differential cell count, which were statistically verified by the binomial distribution [18]. The Leydig cell nuclear area and sertoli cells nuclear area were also measured at 800x and 80x, respectively. Serum testosterone levels were assessed from samples using radioimmuno assay method [19].

Statistical analysis

The data were expressed as mean ±standard error of mean (SEM) and student's 't' test was used to assess the statistical significance. The statistical significance level was set at $p<0.01$ and $p<0.001$.

RESULTS

Reproductive organ weights

A significant ($p<0.01$) reduction in the weight of testes, epididymides, seminal vesicles, and ventral prostate was observed in the ether extract of *Parmelia perlata* (Table-I).

Sperm Dynamics and fertility

The number of spermatogonia, spermatocyte and spermatids were significantly reduced ($p<0.01$) with the *Parmelia perlata* extract. The preleptotene, secondary spermatocyte and step-19 spermatids were decreased by 59.07%, 65.36%, 52.45% respectively and the mature Leydig cells were also reduced by 41.57%. At this dose level, Leydig cell nuclear area and cytoplasmic area, as well as the cross sectional surface area of sertoli cells, were significantly reduced ($p<0.01$) when compared to controls. The sperm density was significantly reduced in the testes ($p<0.01$) (Table-II) and (Table-IV).

Biochemical changes

The cholesterol content of the testes was significantly ($p<0.01$) increased while the protein and fructose content was significantly reduced ($p<0.01$) after following treatment with *Parmelia perlata* extract (Table-III).

Blood and serum biochemistry

The RBC and WBC counts, haemoglobin, haematocrit, blood sugar and the serum protein, cholesterol, triglyceride, phospholipid and HDL-cholesterol levels were within the normal range. Serum testosterone level was decreased.

DISCUSSION

Oral administration of *Parmelia perlata* reduces the weight of testis, and secondary sex organ [20]. Marked decrease in the sperm cell counts specially the number of secondary spermatocyte and rounded spermatid also reduces the testis weight [21]. Reduced protein content may be another reason for low sperm density as the growth rate of any organ is proportional to its protein content [22]. Reduction in Leydig cells nuclei diameters and disintegration of Leydig cells always lead to decrease in the androgen level [23]. Reduced nuclear area also manifest the impairment of Leydig cells function and decrease the number of mature Leydig cells. Deformations of Leydig cells indicate the insufficiency of those cells to synthesize testosterone [24-25].

The reduction in sperm density and motility in cauda epididymides is an important predictor of sperm fertilizing ability [26] and also suggest low level of testosterone to epididymis and therefore affect epididymal function [27]. The impaired epididymal function may also be due to reduced activity of the testes, which affects the normal passage to testicular's fluid into epididymis, this is also confirmed by decreased epididymal weight.

Results also show low counts of sertoli cells and some structural changes in sertoli cells after administration of *Parmetia perlata* extract. The reduction in number of secondary spermatocyte and spermatids reflected non-availability of androgen binding protein (ABP) from Sertoli cells [28]. ABP is essential to maintain intra testicular androgen concentration and transformation of advance stages of sperm cells. Meiotic and post meiotic sperm cells were highly sensitive to androgen concentration [29] and the alternation of androgen level in testis may affect the transformation of spermatocyte to spermatids.

The decreased level of sialic acid content might alter the structural integrity of acrosomal membrane, which ultimately affects the metabolism, motility and fertilizing capacity of spermatozoa [30]. *Parmelia* extract administration caused a decreased level in fructose concentration, which shows the lack of energy source in female body during capacitation.

In conclusion, the present study shows that the 100 mg/kg /day for 60 days of *Parmelia perlata* petroleum ether extract of male albino rats affects their reproductive efficiency by alteration in the spermatogenic activity without adverse toxicity.

IV. CONCLUSION

Lichens have tremendous medicinal values which are due to the presence of various phytochemical constituents. With the help of chromatographic techniques, we isolated three compounds (III- V) for the first time from this lichen. The medicinal values imparted to the lichen by these compounds can be studied by future researchers. To investigate the biological activity of this lichen, the anti-fertility activity of the pet.ether extract was examined which revealed the anti-spermatogenic activity of the extract without adverse toxicity. The study suggests further scope for research on different biological activities of *Parmelia perlata*.

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Table I

Effect of *Parmelia perlata* extract on testicular cell population in rats

	Testicular cell counts (number/10 cross section)					
	Sertoli cell	**Spermatogonia**	**Preleptotene**	**Pachytene**	**Secondary Spermatocytes**	**Step-19 Spermatids**
Control (distilled water, 0.5 ml)	2.81 ± 0.02	6.87 ± 0.02	19.95 ± 1.9	29.29 ± 0.73	48.1 ± 0.6	34.75 ± 0.8
Parmelia perlata 200 mg/day per rat	1.31** ± 0.0828 (-53.3)	3.037** ± 0.2088 (-55.8)	7.597** ± 1.169 (-61.9)	11.669** ± 0.6073 (-60.19)	20.630** ± 3.640 (-57.12)	7.076** ± 3.02 (-79.6)

All values are Mean ± S.E.M. (n=10); ns: non significant; Level of significance ** P<0.001 vs control Percent variations Vs control in parentheses

Table II

Effect of *Parmelia perlata* extract on body weight, organ weights together with seminiferous tubule and Leydig cell nuclear diameter

	Body wt. (g)	**Organ Weight (mg/100g body weight)**				**Seminiferous tubule diameter (μm)**	**Leydig cell nuclear area (μm)**
Control (distilled water 0.5 ml)	240 ± 1.4	1345 ± 4.7	529.5 ± 1.2	605.7 ± 1.2	308.5 ± 2.02	268 ± 9	11.1 ± 0.02
Parmelia perlata 200 mg/day per rat	230^{ns} ± 5.8	975** ± 17	404.4 ± 9	156.94** ± 31	115.97** ± 5.1	200.4** ± 10.4	5.32 ± 16**

All values are Mean ± S.E.M (n=10); ns: non significant; Level of significance** P<0.001 vs control

Table III

Effect of *Parmelia perlata* extract on tissue biochemistry in rats

	Protein (mg/g)				Sialic acid (mg/g)				Glycogen (mg/g)	Cholestrol (mg/g)	Fructose (mg/g)
	Testes	**Cauda epididymides**	**Seminal vesicles**	**Ventral prostate**	**Testes**	**Cauda epididymides**	**Seminal vesicle**	**Ventral prostate**	**Testes**	**Testes**	**Seminal vesicles**
	mg/g										
Control	178.8 ± 0.5	268.7 ± 3.6	186 ± 3.4	162.4 ± 0.18	4.64 ± 0.16	5.34 ± 0.15	4.2 ± 0.09	5.2 ± 0.23	4.85 ± 0.6	7.82 ± 0.11	4.24 ± 0.04
Parmelia perlata	132.56** ± 1.48	204.41** ± 1.73	134.04** ± 5.9	141.45** ± 2.96	3.88* ± 0.14	3.66** ± 0.06	3.80^{ns} ± 0.16	3.72** ± 0.12	2.01 ± 0.01	15.15** ± 0.6	3.51** 0.073

All values are expressed as mean ± S.E.M. (n=10); ns=non significant; Level of significance * P<0.01; ** P<0.001 vs control.

Table IV

Effect of *Parmelia perlata* extract on sperm motility, concentration and fertility in rats

	Sperm motility (%) cauda epididymides	**Sperm density (million/ml)**		**Fertility**
		Testes	**Cauda epididymides**	
Control	74.1 ± 2.38	4.15 ± 0.21	52.2 ± 2.66	100%(+ve)
Parmelia perlata	29.85 ± 1.15**	2.25 ± 0.1**	11.8 ± 1.28**	100%(+ve)

All values are mean ± S.E.M.; levels of significance ** P<0.001