Original Research Article

Antitesticular activities of different solvent fractions from hydro-methanol (2:3) extract of *Cuminum cyminum* in albino rat: A Comparative analysis

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A b s t r a c t

Currently available contraceptives are associated with adverse effects. So, search on safer agents in this purpose is one of the priority areas of WHO. Our previous study showed a significant antifertility effect of hydro-methanol extract of *Cuminum cyminum* Linn (Umbelliferae) in male albino rat. The main objective of this work isto search outthe potentfraction of hydro-methanol extract of seed of *Cuminum cyminum* in adult male albino rat for the development of herbal male contraceptive to reduce the bio-burden of phytomolecules. The n-hexane, chloroform, ethyl acetate, and n-butanol fractions of the hydro-methanol (2:3) extract of seed of *Cuminum cyminum* were administrated orally to male rat. Results showed the maximum antitesticular activity of chloroform fraction (CH-Fr) than other fractions included here. Treatment with CH-Fr fraction resulted a significant inhibition in spermiological parameters, activities of testicular androgenic key enzymes and antioxidative enzymes, levels of serum testosterone and seminal vesicular fructose, number of different generations of germ cells at stage VII of spermatogenic cell cycle and seminiferous tubular diameter (STD) along with significant increase in the level of testicular cholesterol in respect to the control. Significant upward and downward expression in Bax and Bcl-2 gene of male germ cells were indicated which focussed the sperm apoptotic enhancer activities of the fraction. The findings indicated that among the said four different fractions, the chloroform fraction of the hydro-methanol extract of the seed of *Cuminum cyminum* had most effective antitesticular activity.

Keywords: Hypotesticular activity; Apoptosis; *Cuminum cyminum;* Androgenesis; Oxidative stress; Spermatogenesis.

Introduction

Overpopulation is a global problem with serious implications for the future. Numerous methods are being used to reduce the total fertility rate in developing countries. Steroidal contraceptives are effective enough and accepted widely to serve the purpose. However, the safety of their prolonged exposure is controversial [1]. Therefore, now time is alarming us to think of some alternatives in the field of contraception. Hence, efforts are made to think back on our natural products [2]. The importance of plants as a source of antifertility drugs has been emphasized by many researchers [3]. Antifertility agents obtain from indigenous medicinal plants would be immense benefit especially to inhabitants of developing countries as the cost of these drugs would be within their means [4]. There are several medicinal plants like *Gossypium herbaceum* [5], *Alstonia macrophylla* [6], *Ricinus communis* [7], *Achyranthes aspera* and *Stephania hernandifolia* [8] known to posses male contraceptive properties either by suppressing spermatogenesis and or by spermicidal action on human or animal sperm. These plants had been chosen by their indigenous medicinal values. *Cuminum cyminum* is such plant locally known as jeera, which belongs to the family *Umbelifereae,* is one of the most widely used of spices. Cumin has proven hypolipidemic [9] and anti hyperglycimic activities [10]. The abortifacient activity of the seed has been investigated by few workers [11,12]. There are preliminary reports along with ours where male contraceptive effect of methanol extract of seed of *Cuminum cyminum* in adult male rats has been focused though molecular mechanism behind it and the study on effective fraction have not been conducted till now to find out the most effective phytomolucle(s) in this purpose [13].

Previously, we have reported that the hydro-methanol extract of *Cuminum cyminum*(HM-Ex-Cc) is the most potent extract for inhibiting the testicular activity [14]. The aim of this study was to investigate the most potent fraction having antitesticular activity out of the four fractions, namely n-hexane (NH-Fr), chloroform (CH-Fr), ethyl acetate (EA-Fr) and n-butanol (NB-Fr) obtained from the HM-Ex-Cc to minimize the bioburden of the phytomolecules on

physiological system and to develop a clue for herbal male contraceptive development.

Materials and methods

Plant materials

The fresh seed of *Cuminum cymunum* was collected from Garbeta area of Midnapore district, West Bengal, India, in January 2013. The collected seeds were identified in the Botany and Forestry department, Vidyasagar University, Midnapore, West Bengal. A voucher sample (VU/BMLSM/Cc-I/12) was deposited in the herbarium of the same department for ready reference.

Fraction preparation

These seeds were washed and dried at room temperature. The dried seeds were grind in electrical grinder to obtain a course powder. The powder material was repeatedly extracted three times with (2:3) hydro-methanol at room temperature. The extract was then concentrated under low pressure to yield a reddish brown extract. Then the obtained HM-Ex-Cc was partitioned between water and organic solvents of increasing polarities, to yield four new fractions including NH-Fr, CH-Fr, EA-Fr and NB-Fr.

Animals

Thirty, healthy, sexually matured and active, male Wistar rats weighing 130 ± 10 g were used for the present investigation. The animals were acclimatized for two weeks, placed in their respective cages and housed in a well ventilated 'Animal House' under the standard environmental conditions i.e. temperature 22 ± 3 C; 12:12 h light and dark cycle; humidity: 45–50% with free access to tap water and standard laboratory rat feed. The research protocol was approved by the Institutional Ethic Committee (IEC), Vidyasagar University (IEC/3/C-3/14 dated 3/11/2014). All the animals were handled throughout the experimental period as per guideline of Committee for the Purpose of Control and Supervision of Experiments and Animals (CPCSEA) Govt. of India.

Experimental design

The initial body weight of all the thirty rats were recorded and divided into 5 groups consisting of 6 animals in each group. The duration of experiment was 28 days.
Group I: Rats of this group were orally fed with 0.5 ml of olive oil / 100 g body weight / day for single time.
Group II: Rats were treated with NH-Fr of HM-Ex-Cc at a dose of 20 mg / 0.5 ml olive oil / 100 g body weight / day for single time.

Group III: Rats of this group were administered for single time with CH-Fr of HM-Ex-Cc at a dose of 20 mg / 0.5 ml olive oil / 100 g body weight / day.
Group IV: Rats were treated with EA-Fr of HM-Ex-Cc for single time at a dose of 20 mg / 0.5 ml olive oil / 100 g body weight / day.
Group V: Rats of this group were subjected for the treatment with NB-Fr of HM-Ex-Cc at the dose of 20 mg / 0.5 ml of olive oil / 100 g body weight / day for single time.
After 24 hours of last treatment, the final body weights were recorded and the animals were sacrificed by light ether anaesthesia. Blood samples were collected from dorsal aorta by syringe and sera were separated by centrifugation at 3000 g for 10 minutes and stored at -20 C until used for testosterone and metabolic toxicity parameters assessment. Then, testes, epididymis and seminal vesicle were dissected out, trimmed off extraneous fat and weighed accurately on electronic balance. The organs' weights were expressed in terms of g / 100 g body weight. One testis was kept at -20 C for biochemical, genomic and proteomic studies, other testis was used for paraffin block preparation in connection with histological and histometric study. Epididymal fluid was collected from cauda for sperm count, motility and viability.

Sperm analysis

The microscopical count of spermatozoa was performed with haemocytometer following the standard method and expressed as the number of spermatozoa per ml of suspension [15]. The numbers of motile spermatozoa were counted under the microscope and the result was expressed as percentage after counting 100 spermatozoa in each field [15]. Sperm viability was assessed by nigrosin eosin staining method [15].

Tissue biochemistry

The testicular activities of Δ^5, 3β-hydroxysteroid dehydrogenase (HSD) and 17β-hydroxysteroid dehydrogenase (HSD) and the concentration of testicular cholesterol were determined using standard methods [16]. A portion of testis was homogenised in 0.1 (M) phosphate buffer (pH 7.4), and assayed for the activities of testicular catalase, peroxidase and the levels of conjugated diene (CD) and thiobarbituric acid reactive substance (TBARS) [17]. Seminal vesicular fructose level was estimated according to standard laboratory protocol [15].

Hormonal assay

Testosterone assay was performed by solid phase enzyme linked immunosorbent assay (ELISA). The reagents were obtained from Lilac Medicare (P) Ltd, Mumbai, India. The result was expressed as ng / ml [16].

Total RNA isolation

Total RNA was extracted from the testicular tissue with 'High Pure RNA Tissue Kit' (Roach Diagnostic Mannheiem, Germany) according to the manufacturer's recommendations. The purified total RNA was then reverse transcribed using "Trinscriptor first strand cDNA synthesis kit" (Roach Diagnostic). The resultant cDNA was then diluted 20-fold and kept at -20°C [18].

Quantitative real-time PCR (qRT-PCR) to determine the levels of gene expression

The sequences of all primers used in qRT-PCR were listed in Table 1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal control gene for qRT-PCR assay. Quantitative Real-Time PCR (qRT-PCR) was conducted with the SYBR Green qRT-PCR kit (Roach Diagnostic) on a Light Cycler 480 II Detection System (Roach diagnostic). The reactions were performed in a 20 μL volume mixture containing 10 μL SYBR Green I mixture, 1 μL primers (Foraward and Revers), 2 μL cDNA, and 7 μL sterile, distilled- deionized water. Primer specificity was assessed through melting curve analysis [18].

Table 1. Primer sequence and specific conditions used for PCR amplification of candidate genes.

Gene	Primer sequence	Anealine temperature (C)	Number of cycles	Amplification size (bp)
Bax	F-GTGAGCGGCTGCTTGTCT R-GTGGGGGTCCCGAAGTAG	58	35	73
Bcl-2	F-GTACCTGAACCGGCATCTG R-GGGGCCATATAGTTCCACAA	60	35	76
GAPDH	F-ACCACAGTCCATGCCATCSC R-TCCACCACCCTGTTGGCTGTA	58	35	452

Abbreviations: Bcl-2-B-cell lymphoma 2; GAPDH-glyceraldehyde-3-phosphate dehydrogenase; bp-base pair;

Western blot analyses

Frozen testes were thawed in 1.5 ml ice-cold RIPA (Radio Immuno Precipitation Assay) buffer per gram of tissue. After homozing the tissue using tissue homogenizer, lysates were centrifuged at 10,000 g for 10 min at 4°C. Western blotting was carried out as described [19]. To monitor equal loading of protein, β-actin was used. Densitometric analysis was performed with the help of image analysis software (lab works image analysis software, version 4.0; UVP Inc.).

Histological and histometric studies

Testes were fixed in Bouin's fluid, and were sectioned at the thickness of 5 μm as per standard protocol and stained with haematoxylin and eosin. Seminiferous tubular diameter (STD) was measured with the "Dewinter caliper pro 3.0 software". Quantitative analysis of gametogenesis was carried out at stage VII of seminiferous epithelial cell cycle according to the method of Leblond and Clermont [20]. Stage VII of seminiferous epithelial cycle was selected as quantative study of spermatogenesis

because it represent the condition of spermatogenesis as a whole as all varieties of germ cells are present at this stage [20].

Statistical analysis

Results were expressed as the mean ± S.E. (standard error of mean). Statistical differences between control and the test fractions were assessed by analysis of variance (ANOVA) followed by the 'Multiple Comparison Student's two tail t-test'. Significant deviation in the results was noted at the p-values less than 0.05 [21].

Results

Body weights and organo-somatic indices

No significant change was observed among the body weights of fraction treated rats as well as comparison with the vehicle control. Testiculo, seminal vesiculo and epididymal-somatic indices were significantly decreased in all the fraction treated groups in respect to vehicle treated control (Table 2). The values of these parameters were significantly reduced in CH-Fr treated group in respect to NH-Fr or EA-Fr or NB-Fr treated group (Table 2).

Table 2. Effect of four fractions of HM-Ex-Cc on body weight, testiculo-somatic, seminal vesiculo-somatic and epididymalo-somatic indices in albino rat (mean ± SEM, n= 6). ANOVA followed by 'Multiple Comparisons followed Student's two tail t-test'. Values with different superscripts (a, b, c) in each column differ from each other significantly, (p<0.05).

Groups	Body weight (g)		Relative organ weights (g /100 g body weight)		
	Initial	Final	Testis	Epididymis	Seminal vesicle
Vehicle (Olive oil control)	132.05±2.19[a]	145.17±1.75[a]	1.86±0.041[a]	0.55±0.032[a]	0.42±0.038[a]
NH-Fr treated	128.53±2.03[a]	142.06±2.07[a]	1.55±0.038[b]	0.44±0.044[b]	0.31±0.029[b]
CH-Fr treated	130.56±2.41[a]	149.24±2.17[a]	1.37±0.032[c]	0.32±0.026[c]	0.26±0.028[c]
EA-Fr treated	129.27±2.08[a]	141.53±2.05[a]	1.50±0.043[b]	0.48±0.034[b]	0.35±0.032[b]
NB-Fr treated	134.26±1.87[a]	146.85±1.75[a]	1.51±0.036[b]	0.47±0.033[b]	0.32±0.028[b]

Spermatogenic profile

Treatment with different fractions using NH-Fr or CH-Fr or EA-Fr or NB-Fr resulted a significant diminution in sperm count, motility and viability as compared with the vehicle treated control group (Figure-1). Among the fractions treated groups, the values of the said parameters were decreased significantly in CH-Fr treated group in respect to others (Figure-1).
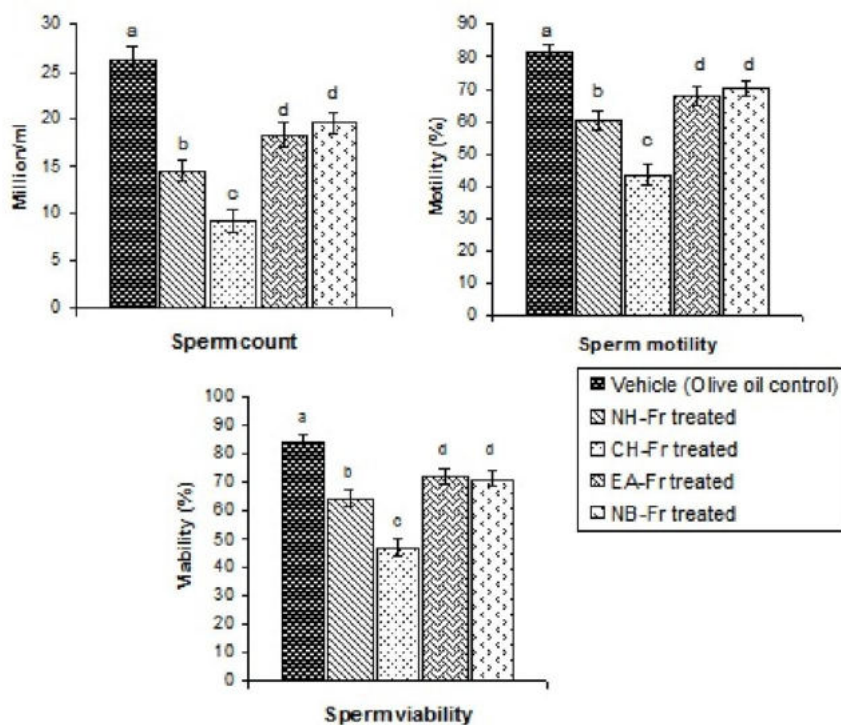

Figure 1. Comparative analysis of sperm count, sperm motility and sperm viability in control and different fractions treated groups of HM-Ex of C. cyminum. Data are expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c, d) differ from each other significantly; p<0.05 (ANOVA followed by Multiple Comparison followed Student's two tail t-test).

Testicular Δ^5, 3β-HSD and 17β-HSD

Testicular Δ^5, 3β-HSD and 17β-HSD activities were decreased significantly in all the fractions treated groups in comparison with the vehicle-treated control group (Figure. 2). No significant alterations in the activities of these enzymes were noted among the NH-Fr, EA-Fr and NB-Fr treated groups. Activities of these two androgenic key enzymes were significantly inhibited in the CH-Fr treated group in respect to the other fractions treated group (Figure 2).
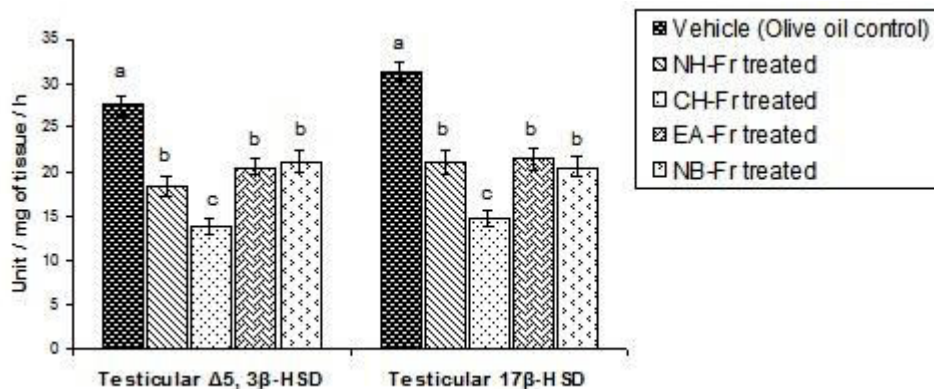
Figure 2. Effect of four different fractions of HM-Ex C. cyminum on the testicular Δ5, 3β-HSD and 17β-HSD activities in rat. Data are expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c) differ from each other significantly; p<0.05 (ANOVA followed by Multiple Comparison followed Student's two tail t-test).

Testicular cholesterol, serum testosterone and seminal vesicular fructose

Significant diminution in the levels of serum testosterone and seminal vesicular fructose along with increase in the level of testicular cholesterol were noted in all the fractions treated groups as compared with the vehicle-treated control group (Table 3). After CH-Fr treatment, serum testosterone and seminal vesicular fructose levels were significantly reduced and testicular cholesterol level was significantly increased in comparison to NH-Fr or EA-Fr or NB-Fr treated group (Table 3). Though, no significant difference in the levels of said parameters was noted when comparison was made among the NH-Fr, EA-Fr and NB-Fr treated groups (Table 3).

Table 3 Comparative analysis in the levels of serum testosterone, testicular cholesterol and seminal vesicular fructose, activities of SGOT and SGPT in mature albino rat after treatment with NH-Fr or CH-Fr or EA-Fr or NB-Fr of HM-Ex *C. cyminum* (mean ± SEM, n = 6). ANOVA followed by 'Multiple Comparisons followed Student's two tail t-test'. Values with different superscripts (a, b, c) in each column differ from each other significantly (p<0.05).

Groups	Serum testosterone (ng / ml)	Testicular cholesterol (mg / g of tissue)	Seminal vesicular fructose (µg / g of tissue)
Vehicle (Olive oil control)	12.58 ± 0.6[a]	31.28 ± 1.22[a]	0.092 ± 0.005[a]
NH-Fr treated	9.27 ± 0.8[b]	38.16 ± 1.48[b]	0.062 ± 0.004[b]
CH-Fr treated	6.32 ± 0.5[c]	44.31 ± 1.51[c]	0.034 ± 0.005[c]
EA-Fr treated	8.92 ± 0.8[b]	37.75 ± 1.62[b]	0.057 ± 0.003[b]
NB-Fr treated	9.06 ± 0.5[b]	39.72 ± 1.58[b]	0.060 ± 0.004[b]

Oxidative stress assessment in testis

Testicular catalase and peroxidase activities were decreased significantly in all fractions treated groups when compared to their vehicle treated control group. Significant decreased activities in catalase and peroxidase were noted in NH-Fr or CH-Fr treated group when compared with EA-Fr or NB-Fr treated group. However, the significant decrease in these enzyme activities was noted in CH-Fr treated group in respect to NH-Fr treated group. No significant difference in the activities of catalase and peroxidase were noted when comparison was made between EA-Fr and NB-Fr treated groups (Figure 3).

Thiobarbituric acid reactive substance (TBARS) and conjugated diene (CD) are the main end products of lipid peroxidation. After treatment with CH-Fr, levels of TBARS and CD in testis were elevated significantly in comparison to the NH-Fr or EA-Fr or NB-Frtreated group as well as the vehicle treated control group. After NH-Fr or EA-Fr or NB-Fr treatment, significant elevation in the levels of these parameters was noted in respect to vehicle treated control group (Figure. 3). However the levels of these two parameters were elevated significantly in NH-Fr treated group in respect to EA-Fr or NB-Fr treated group (Figure. 3). No significant change in the levels of CD and TBARS were noted between EA-Fr and NB-Fr treated groups (Figure. 3).
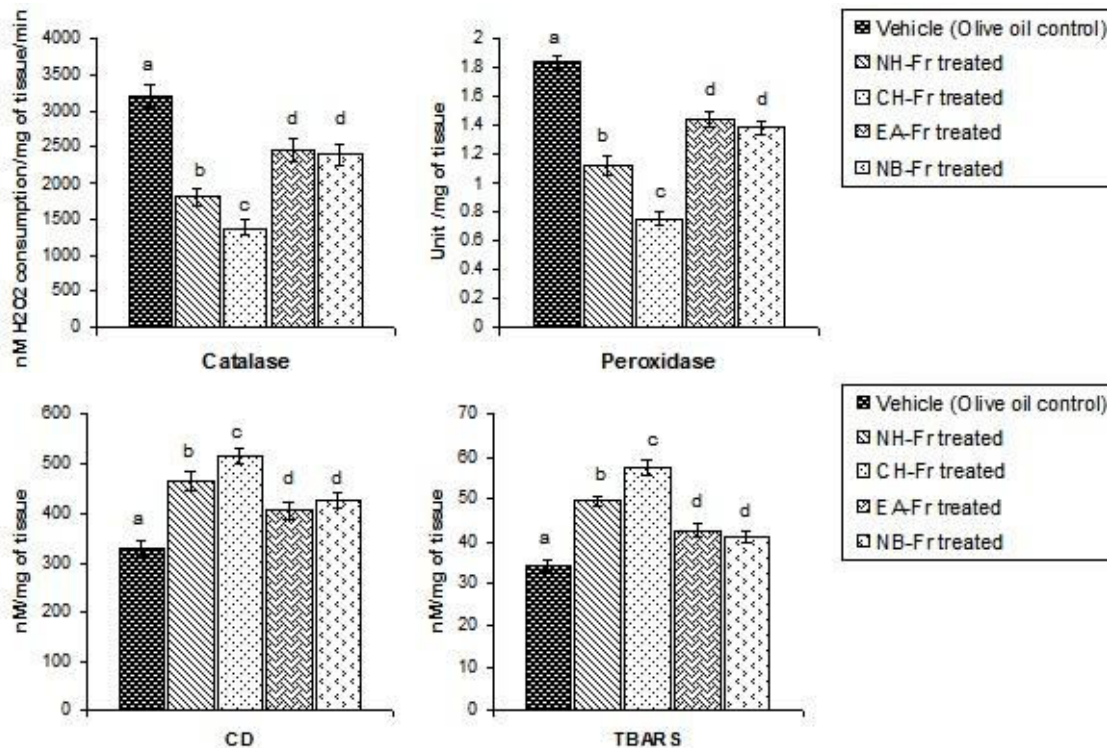
Figure3. Effect of different fractions of HM-Ex C. cyminum on the oxidative stress sensors i.e. catalase and peroxidase activities and the levels CD and TBARS in rat testis. Data are expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c, d) differ from each other significantly; p<0.05 (ANOVA followed by Multiple Comparison followed Student's two tail t-test).

qRT-PCR study of Bax and Bcl-2 genes

Transcript levels of Bax, and Bcl-2 were analysed of testicular tissue. After CH-Fr treatment, the up regulation in the expression of Bax gene and down regulation in the expression Bcl-2 gene in testicular tissue were observed in respect to the NH-Fr or EA-Fr or NB-Fr treated group as well as the vehicle treated control group.

Up regulation in the expression of Bax gene and down regulation in the expression Bcl-2 gene in testicular tissue was also noted in NH-Fr or EA-Fr or NB-Fr treated group in respect to the vehicle treated control group (Figure. 4). No significant difference was noted in Bax and Bcl-2 gene expression in testicular tissue when comparison was made among the NH-Fr, EA-Fr and NB-Fr treated groups (Figure. 4).
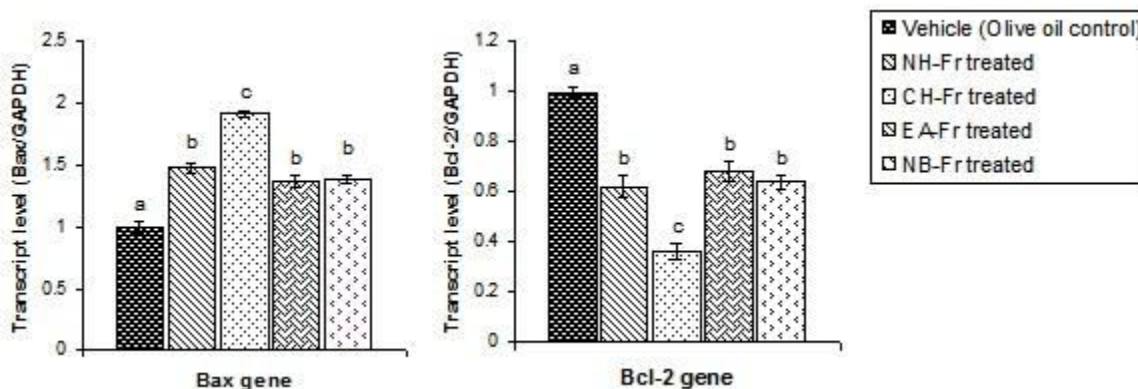

Figure 4. Gene expression study of testicular Bax and Bcl-2 by qRT-PCR in different fractions of HM-Ex of *C. cyminum* treated groups and the control group. Data are expressed as mean ± SEM, n=6. Bars with different superscripts (a, b, c) differ from each other significantly; p<0.05 (ANOVA followed by Multiple Comparison followed Student's two tail t-test).

Western blot of Bax and Bcl-2 proteins

Pro-apoptotic Bax protein synthesis was significantly increased and anti-apoptotic Bcl-2 protein synthesis was significantly decreased in testicular tissue, in NH-Fr or CH-Fr or EA-Fr or NB-Fr treated rats in respect to the vehicle treated control group. The significant

elevation in Bax protein level and diminution in Bcl-2 protein level in testicular tissue were noted in CH-Fr treated group in respect to NH-Fr or EA-Fr or NB-Fr treated group (Figure. 5). However no significant differences in the levels of these two apoptotic proteins were noted when comparison was made among the NH-Fr, EA-Fr and NB-Fr treated groups (Figure-5).
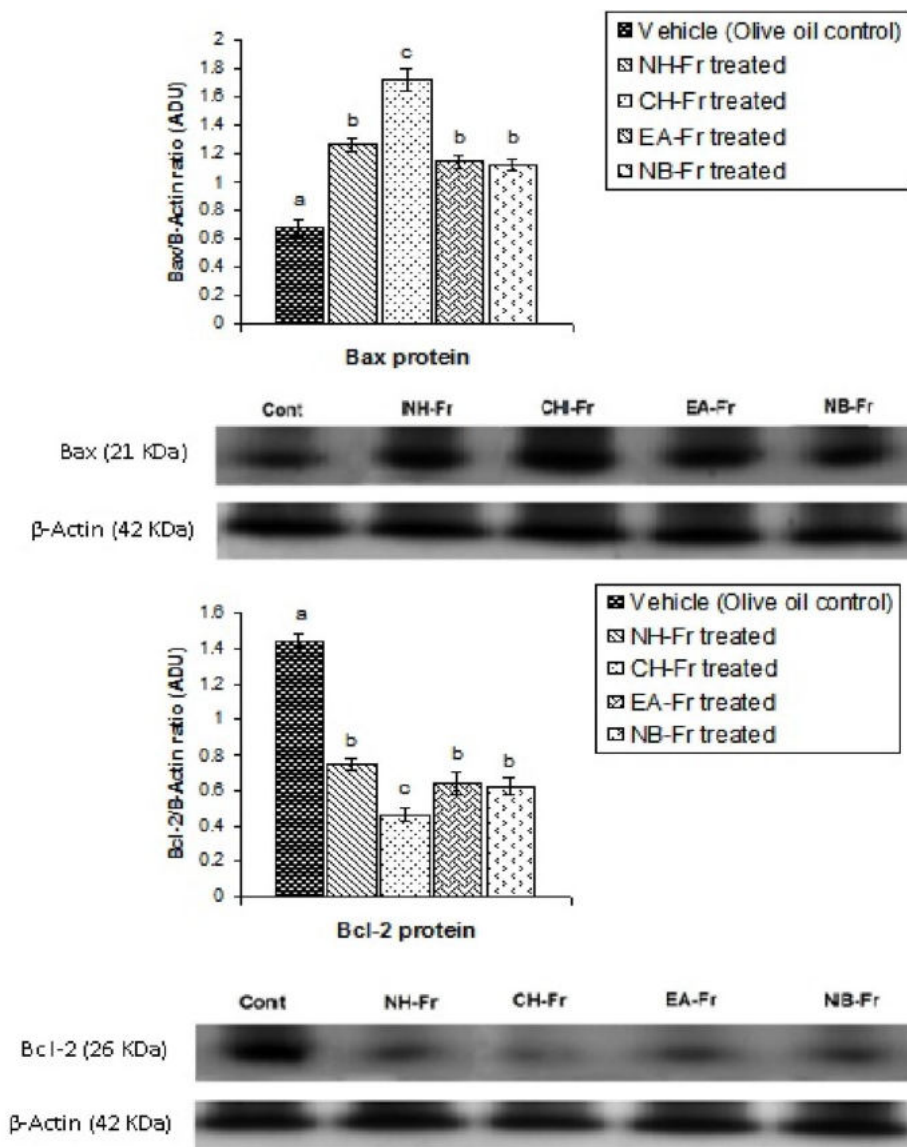

Figure 5. Representative Western blot analysis of (A) Bax and (B) Bcl-2 protein expression patterns in experimental rat testis after 28 days treatment of different fraction of HM-Ex of *C. cyminum* and their semiquantitative densitometric analysis after normalising the results with housekeeping protein, β-Actin. Data are expressed as mean ± SEM; n = 6. Bar with different superscripts (a, b, c) differ from each other significantly, p < 0.05 (ANOVA followed by Multiple Comparison followed Student's two-tail t-test).
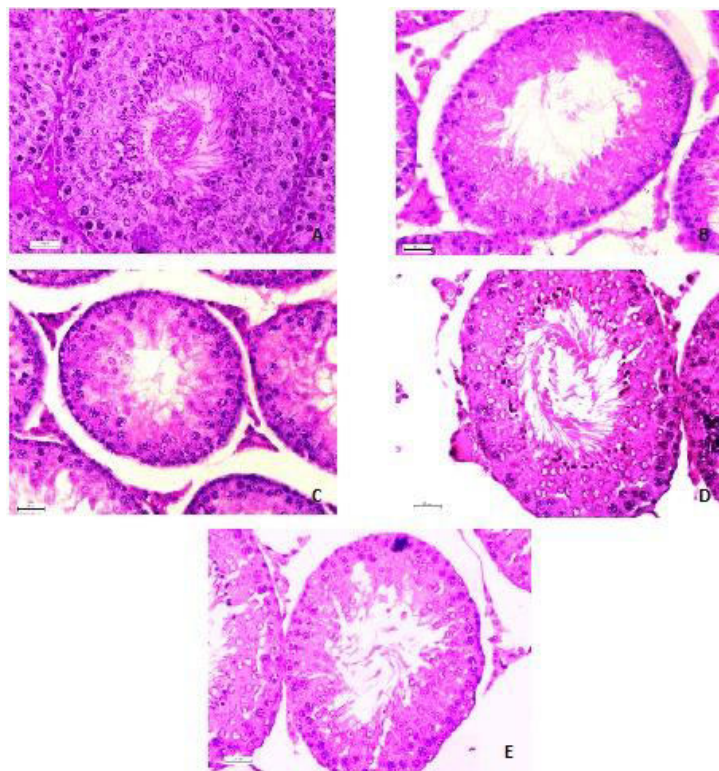
Plate 1. Histology of testis 400X (Hematoxyline Eosin Stain). A representative microphotograph of a control (A), NH-Fr (B), CH-Fr (C), EA-Fr (D) and NB-Fr (E) treated rat. Control group rat shows normal spermatogenesis at stage VII and STD. Different fraction treated groups shows significant diminution in the number of different generations germ cells at stage VII and STD in respect to control group.

Quantification of germ cell at stage VII and Seminierous Tubular Diameter (STD)

Quantative study of hormone sensitive germ cells at stage VII of spermatogenic cell cycle and STD revealed a significant diminution in the numbers of ASg, pLSc, mPSc and 7Sd after the treatment with NH-Fr or CH-Fr or EA-Fr or NB-Fr treated group in comparison to the vehicle treated control group (Table 4). Diminution in the numbers of the above mentioned germ cells and STD were significantly marked when animals were treated with CH-Fr in comparison with NH-Fr or EA-Fr or NB-Fr treated group. However, no significant difference in the above mentioned germ cells numbers and STD were noted among the NH-Fr, EA-Fr and NB-Fr treated groups (Table 4).

Table 4. Effect of different fractions of HM-Ex of seed of *C. cyminum* on quantification of different generations of germ cells at stage VII of spermatogenic cycle and STD in albino rat (mean ±SEM, n = 6). ANOVA followed by 'Multiple Comparison followed Student's two tail t-test'. Values with different superscripts (a, b, c) in each column differ from each other significantly (p<0.05).

Groups	ASg	pLSc	mPSc	7Sd	STD 400(µm)
Vehicle (Olive oil control)	1.82 ± 0.14^a	17.82 ± 0.59^a	18.25 ± 0.88^a	66.52 ± 1.29^a	576.35 ± 16.25^a
NH-Fr treated	1.28 ± 0.16^b	13.77 ± 0.61^b	14.22 ± 0.51^b	51.38 ± 1.42^b	418.27 ± 12.75^b
CH-Fr treated	0.72 ± 0.18^c	9.76 ± 0.78^c	10.02 ± 0.48^c	37.15 ± 1.28^c	351.48 ± 18.32^c
EA-Fr treated	1.30 ± 0.16^b	14.67 ± 0.63^b	15.08 ± 0.72^b	55.25 ± 2.09^b	436.37 ± 15.22^b
NB-Fr treated	1.32 ± 0.18^b	14.86 ± 0.81^b	15.24 ± 0.48^b	56.15 ± 1.82^b	455.27 ± 14.36^b

Discussion

The body weight of the fractions treated rats remained unchanged throughout the experiment. Body weight examination was done to obtain the necessary health information of the animals for interpretation of reproductive effect [22]. The significant diminution in the relative weights of testiculo-somatic, seminal vesiculo-somatic and epididymo-somatic indices was noted in CH-Fr treated

group in respect to the other fractions treated groups. This may be due to inhibition in testosterone synthesis as testosterone is one of the prime regulators of sex organs growth and development [23]. The fraction specific testicular inhibitory function of the *C. cyminum* seeds has been further supported here by the significant diminution in epididymal sperm profile, number of different generations of germ cells of spermatogenic cell cycle at stage VII and STD which may also supports the diminution in testosterone synthesis [24]. The testicular steroidogenic key enzyme activities were significantly decreased in CH-Fr treated group in comparison to the other fractions treated groups. This is also supported by the inhibition in testicular androgenesis as Δ^5, 3β-HSD and 17β-HSD are the key enzymes for androgenesis [25]. The fraction specific inhibition in testicular androgenesis was also reflected here from the testicular cholesterol assay as well as plasma testosterone level. As cholesterol is the precursor of androgens [26] and testosterone is the final product of androgenesis [26]. So, the significant diminution in the plasma testosterone along with the elevation in testicular cholesterol after treatment with CH-Fr in comparison to other solvent fractions treated groups also support that the CH-Fr is the effective solvent fraction out of four solvent fractions used here in relation to the inhibition in testicular steroidogenesis.From the quantification of seminal vesicular fructose it has been revealed that the most significant reduction in this parameter was noted when the animals were subjected to CH-Fr treatment. The inhibition in seminal vesicular fructose level may be due to inhibition in testicular androgenesis as fructose quantity in seminal plasma is regulated by testosterone [27].

To find out its direct inhibitory effect on testicular function by imposing oxidative stress, activities of important antioxidant enzymes such as testicular catalase and peroxidase, and the quantity of end products of free radicals like CD and TBARS in testis were measured. After evaluation of oxidative stress in testis it has been observed that the fraction prepared using chloroform as solvent have a significant oxidative stress imposing effect on testicular tissue in comparison to other fractions. The result of these oxidative stress parameters also supports that the CH-Fr is the most effective solvent fraction out of the four solvent fractions used here. This is also supported by a significant reduction in the sperm motility and viability after CH-Fr treatment in comparison to the other fractions.

The spermatogenesis and androgenesis process are also influenced by the oxidative stress linked apoptosis process. High level of testicular apoptosis was established after CH-Fr treatment following qRT-PCR and western blot study. The Bcl-2 family of proteins that contains both proapoptotic (such as Bax) and anti-apoptotic (such as Bcl-2) members constitute a critical, intracellular check point that determines a cell's susceptibility to apoptosis [28]. The potential modulators of germ cell apoptosis are Bcl-2 and Bax proteins [29]. Treatment with CH-Fr has been shown to increase in the expression of pro-apoptotic proteinBax, while reducing in the expression of Bcl-2, an anti-apoptotic protein suggesting their important roles on apoptotic cell death and other spermiological sensors.

So, from the above findings it may be concluded that the CH-Fr of HM-Ex-Cc has better anti testicular activities and may contain more active ingredient(s) having contraceptive activity than the other solvent fractions.

To explain the antitesticular activity of the CH-Fr in the present study, two possible hypotheses may be proposed. One may be the active ingredient (s) present in the said fraction may alter the pituitary-testicular hormonal milieu which results inhibition in testicular activities. The alternative hypothesis is that the effective ingredient (s) may induce oxidative stress in testicular tissue leading to generation of free radicals which result diminution in sperm functions by activation of germ cells apoptosis.

Acknowledgement

Conflict of interests

The authors have declared that there is no conflict of interest

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