A Comparative Study of Effect of Alcoholic Extracts of Sapindus emarginatus, Terminalia belerica, Cuminum cyminum and Allium cepa on Reproductive Organs of Male Albino Rats

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Fruit extracts of *S. emarginatus, T. belerica* and seed extracts of *Cuminum cyminum* and *Allium cepa* (50 mg/day/rat) were fed orally to male albino rats for 60 days. The body weights were not affected but the weights of reproductive organs were decreased following the treatments. The sperm motility of cauda epididymis and sperm count of cauda epididymis and testis declined significantly leading to negative fertility test. Androgen dependent parameters (protein, sialic acid, fructose and ascorbic acid) were lowered, revealing reduction in the circulating androgen but on the contrary there was an increase in the testicular cholesterol level. Haemoglobin and Haematocrit values remained unaltered, indicating normal blood physiology of treated groups of animals. Serum biochemistry indicated an increase in the lipid parameters. The present study therefore, suggests that these extracts exert both antifertility and antiandrogenic activities. The overall data suggested *S. emarginatus* is superior to other three plant extracts in exerting the antifertility effects.

Introduction :

The genus *Sapindus* belongs to family *Sapindaceae* posses tremendous medicinal value. Since past, it is used as emetic, tonic, astringent, anthelmintic, for asthma, colic, diarrhea, cholera, tubercular glands and paralysis of limbs. The fruit is commonly used as a remedy for hair problems and also in preparation of shampoos. Gupta and Ahmad, 1990 have isolated Emarginatosides B and C from pericarp of the fruits of *Sapindus*. Bhargava, (1988), Talwar *et. al.*, (1995) have also reported antifertility effect of *Sapindus* species on mammals.

The genus *Terminalia* belongs to family *Combretaceae*, posses tremendous medicinal value. It is used as astringent, brain tonic, for cough, asthma, stomach, liver disorders, piles, leprosy, fever etc.

The fruit is one of the three constituents of the important Indian Ayurvedic preparation 'triphala'. Antifertility effects of *Terminalia* species have been reported on mammals (Setty *et. al*, 1976, 1977, Rao, 1989).

The genus *Cumin* belongs to family *Umblliferae*. The aromatic dried fruits commonly called cumin seed are used as spices and condiment. Abortifacient activity of the seeds has been investigated by few workers (Choudhury and Haq, 1980; Garg, 1976; Nadkarni and Nadkarni, 1954). Al-Khanis and Parmar (1988) reported the anti-implantation and abortifacient activity of the aqueous extract of *Cuminum* on female rats. Kant *et. al* (1988) have reported the oestrogenicity of *C. cyminum* seeds in ovariectomised rats.

The genus *Allium* belongs to family *Liliaceae*. Freshly pressed onion juice and solution of the extracted juice were found to contain heart stimulants, It also stimulates muscles of intestine and uterus, improves blood circulation. It is useful in fever, dropsy, hypertension, colic, hysterical fits, chronic bronchitis and other physical ailments.

A comparative study was undertaken to evaluate the antifertility effects of alcoholic extracts of *Sapindus emarginatus* (Aritha), *T. belerica* (Baheda) fruits *Cuminum cyminum* (Zeera) and *Allium cepa* (Onion) seeds in adult male rats.

Materials and Methods

Healthy, adult male albino rats (*Rattus norvegicus*) each weighing between 200 and 250 g were used for the experiments. The animals were maintained under standard husbandry conditions on a standard diet (Hindustan Lever Ltd., Bombay) and water was given *ad libitum*. The animals were exposed to 14 day light hours. The dried fruits of *Sapindus emarginatus*, *T. belerica* and seeds of *C. cyminum* and *A. cepa* were weighed, powdered and used for extraction of the phytodrug. The concurrent extraction on water bath was carried out for 16 to 18 h. using 50 % ethanol as a solvent. The extracts were filtered and dried in vacuo. The dried extracts were scrapped from glass petridishes and weighed. These crude drugs thus obtained were fed orally (50 mg/day/rat for 60 days) using distilled water as a vehicle. The control group received only vehicle for 60 days.

After the completion of the treatments the fertility test was done. Normal cycling estrous or proestrus females (two) were caged with one treated male. Mating was confirmed next day either by observing the presence of spermatozoa in the vaginal smear or by the formation of vaginal plug. The female rats were kept separate for 22 days at laboratory conditions. The number of females delivered and the number of litters were recorded. Fertility test was taken as positive if the females delivered else it was taken as negative.

On day 61 the animals were autopsied and blood was extracted from heart. The serum was separated and used for serum biochemistry. Reproductive tissues (testis, epididymis, vas deferens, seminal vesicle, ventral prostate) and vital organs (liver, kidney, heart, adrenal) were blotted free of blood, weighed and used for tissue biochemistry.

The following parameters were studied: Sperm count and motility (Prasad *et. al.*, 1972), protein (Lowery *et. al.*, 1951), sialic acid (Warren, 1959), glycogen (Montgomery, 1957), fructose (Foreman *et. al.*, 1973), cholesterol (Zlatkis *et. al.*, 1953), ascorbic acid (Roe and Kuether, 1943), acid phosphatase and alkaline phosphatase (Fiske and Subbarow, 1925).

In all the analysis, a minimum of six replicates was done for each tissue and parameter. The results were analyzed statistically using Student's t-test.

Results :

The data revealed that the body weights of rats were not much altered after the treatment of all the four extracts; however, in all the treated groups a general decrease in the reproductive organ weights was observed in relation to the control. A significant reduction was observed in weights of testis (p<0.01), epididymis (p<0.001), seminal vesicle (p<0.01) and ventral prostate (p<0.001) in *S. emarginatus* treated animals. The treatment of *T. belerica* also resulted to a significant decrease in the weights of testis (p<0.001), and ventral prostate (p<0.001), vas deferens (p<0.01), seminal vesicle (p<0.001) and ventral prostate (p<0.001), epididymis (p<0.001). *C. cyminum* treated group showed a significant reduction in weights of testis (p<0.001), and seminal vesicle (p<0.001). Significant reduction in weights of testis (p<0.001), and seminal vesicle (p<0.001). Significant reduction in weights of testis (p<0.001), and seminal vesicle (p<0.001).

epididymis (p<0.001), vas deferens (p<0.05), seminal vesicle (p<0.001) and ventral prostate (p<0.05) was also observed in *A. cepa* treated group of animals. The weights of vital organs were not affected in all the treated groups (Table-1).

The cauda epididymal sperm suspension of control rat revealed that the sperms were actively motile, showing forward progression. The spermatozoa motility of all the extract treated rats showed that the sperms were sluggishly motile without any forward progression. The percent motility after treatments with the four extracts declined significantly (p<0.001). A significant decrease was also noticed in the sperm density of cauda epididymis (p<0.001) and testis (p<0.001) of all the extract treated rats (Table-2).

The fertility test was 100% negative in *S. emarginatus*, 75% negative in *T. belerica*, 67% negative in *C. cyminum* and 60% negative in *A. cepa* treated groups of animals (Table-2).

The protein concentration of reproductive organs was lowered after the treatments of the fruit/ seed extracts. A significant reduction in the protein contents of testis (p<0.001), cauda epididymis (p<0.01) and ventral prostate (p<0.001) was observed following the treatment of *T. belerica* extract. Similar reduction in protein concentration of testis (p<0.01) and cauda epididymis (p<0.01) was observed after C. cyminum seed extract. A. cepa seed extract treatment also showed a significant reduction in the protein contents of testis (p<0.01) and cauda epididymis (p<0.001) (Table-3). The testicular and cauda epididymal sialic acid concentration of all the extract treated animals decreased significantly. The fructose concentration of seminal vesicle was diminished significantly in *S. emarginatus*, *T. belerica* (p<0.001) and C. Cyminum, A. cepa (p<0.05) treated animals respectively (Table-3). The cholesterol content of adrenal increased significantly (p<0.001) in all the four groups of treated animals. The testicular cholesterol content was also increased after the treatments of fruit/seed extracts of four plants. The increase was significant (p<0.001) in S. emarginatus, C. cyminum and A. cepa treated groups (Table-3).

The glycogen concentration of testis was reduced in *S. emarginatus*, *C. cyminum* and *A. cepa* treated animals as compared to control, whereas an increase was observed in the testicular glycogen level in *T. belerica* treated

group. *S. emarginatus* treatment resulted in a significant reduction in the liver glycogen concentration (p<0.001). *C. cyminum* and *A. cepa* treatments also reduced the liver glycogen concentration. On the contrary, an increase in the concentration of liver glycogen was evident following the *T. belerica* treatment (Table-4). Treatment of the fruit/seed extracts resulted in reduction in the ascorbic acid concentration of testis and cauda epididymis. This decrease was more significant (p<0.001, p<0.01 respectively) in *S. emarginatus* treated group of rats (Table-4). A significant decrease in the acid phosphatase content of ventral prostate was observed in the *S. emarginatus*, *T. belerica* (p<0.01) and *C. cyminum* (p<0.05) treated groups. Reduction was also evidenced in the concentration of alkaline phosphatase content of ventral prostate of alkaline phosphatase content of alkaline phosphatase content of alkaline phosphatase content of ventral prostate of all the treated groups (Table-4).

Haemoglobin and Haematocrit values remained unaltered, indicating normal blood physiology of all the group of animals, however, *S. emarginatus* treatment resulted in increased serum cholesterol concentration (p<0.01). The concentration of VLDL, triglycerides, albumin, A/G ratio was decreased. Treatment of *T. belerica* also caused a significant increase in the serum cholesterol concentration (p<0.01) while concentration of VLDL (p<0.05), triglycerides (p<0.05), albumin, A/G ratio decreased. The protein and globulin concentration of serum was increased to some extent in both the treated group of animals (Table-5).

Treatment of *C. cyminum* and *A. cepa* also caused a significant increase in the serum cholesterol concentration (p<0.001, p<0.01 respectively) while the concentrations of VLDL (p<0.05, p<0.01), triglycerides (p<0.05, p<0.01), albumin (p<0.01, p<0.05), A/G ratio (p<0.01, p<0.05) respectively, were significantly declined (Table-5).

Discussion :

Oral administration of alcoholic extract of *S. emarginatus*, *T. belerica* (fruits) and *C. cyminum*, *A. cepa* (seeds) for 60 days, brought about a decrease in the weights of reproductive organs indicating that the circulating level of androgen was not enough to maintain the weights of the reproductive organs, however, the weights of vital organs were not much affected.

The structural and functional integrity of reproductive tissues depends on the circulating androgen (Chinoy *et. al.*, 1982), and therefore, any small change in androgen content may result in reductions in the weights of the reproductive organs.

The negative fertility test may be attributed to decreased spermatozoa density and motility of cauda epididymis. Moreover, the spermatozoa were sluggishly motile and therefore, unable to fertilize the normal cycling females. These data clearly indicate the spermicidal action of these crude alcoholic extracts.

The decline in androgen dependent parameters (protein, sialic acid and fructose) suggest a reduction in androgen levels (Prasad and Rajlakshmi, 1976) except the cholesterol level which increased in both testis and adrenal as a result of treatment. A decrease in the above androgen dependent parameters supports the fact that the circulating androgen level is depleted as a result of the treatment. The requirement of cholesterol for the normal activity of the testicular gland has been well established (Biswas and Deb, 1966), therefore, increased concentration of testicular and serum cholesterol may be correlated with its non-utilization in the system. Haemoglobin and Haematocrit values remained unaltered, indicating normal blood physiology of all the group of animals.

The mechanism of action of these extracts is probably by selective androgen deprivation to epididymis, thereby affecting sperm motility and metabolism. The androgen deprived effect of the extracts is evident by the significant increase in the testicular cholesterol content by this treatment. It is further substantiated by significant fall in levels of seminal vesicle fructose in these animals.

Thus the present investigation shows that the *S. emarginatus, T. belerica* (fruits), *C. cyminum* and *A. cepa* (seeds) extracts exerts antifertility and antiandrogenic activity in male albino rats. The data also suggests that *S. emarginatus* can be a potent antifertility agent in comparison to the other three plant extracts.

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Effect of Sapindus,	Terminalia,	Cuminum	and Allium	on Reproductive	Organs

		Reproductive Organ (mg)					Vital Organ (mg)				
Group	Initial	Final	Testis	Epididymis	Vas	Seminal	Ventral	Liver	Heart	Kidney	Adrenal
					deferens	Vesicle	Prostate				
Control	202.50 ± 5.45	$\begin{array}{c} 212.50\\ \pm \ 4.51\end{array}$	641.09 ± 12.50	243.13 ± 4.86	42.05 ± 1.50	$\begin{array}{c} 207.21 \\ \pm 8.33 \end{array}$	123.92 ± 3.82	3139.64 ± 181.23	253.76 ± 8.43	287.99 ± 4.36	9.41 ± 0.44
<i>S.emarginatus</i> (50 mg/day/ rat for 60 days)	$\begin{array}{c} 166.70 \\ \pm \ 18.00 \end{array}$	196.00 ± 17.04	545.16 ^b ± 26.87	203.39 ^a ± 5.78	40.94 ± 0.89	149.67 ^b ± 14.28	95.41 ^b ± 1.34	3310.54 ± 105.45	270.07 ± 6.27	331.22 ± 14.13	11.23 ± 0.62
<i>T. belerica</i> (50 mg/day/ rat for 60 days)	286.25 ±3.35	256.25 ±1.94	549.35 ^a ± 12.19	161.95 ^a ± 9.20	$37.30^{b} \pm 0.325$	128.97ª ± 8.00	97.27ª ± 5.05	2588.60 ± 336.73	258.63 ± 5.82	333.26 ± 5.20	13.36 ± 0.94
<i>A.cepa</i> (50 mg/day/ rat for 60 days)	212.50 ± 8.84	189.00 ± 7.78	439.03 ^a ± 8.62	208.21 ^a ± 2.88	33.41° ± 2.73	127.43 ^a ± 4.27	95.69 ^c ± 0.93	3711.11 ± 61.09	264.29 ± 3.94	354.78 ± 16.22	$\begin{array}{c} 10.07 \\ \pm \ 0.69 \end{array}$
<i>C.cyminum</i> (50 mg/day/rat for 60 days)	237.50 ± 5.38	250.00 ± 17.38	451.08 ± 6.23	192.75ª ± 1.86	41.24 ± 0.80	153.73 ^a ± 4.82	122.45 ± 3.29	2871.11 ± 118.17	287.52 ± 2.23	378.28 ± 13.43	$\begin{array}{c} 10.58 \\ \pm \ 0.89 \end{array}$

Table-1Body (g) and organ weights (mg/100 g body wt) in control, S. emarginatus, T. belerica, A. cepa and C. cyminum
treated group of rats.

Values are mean \pm S.E.

- a = P < 0.001
- b = P < 0.01
- $c \quad = \quad P < 0.05$

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	Sperm Motility (%)	Sperm d (million	Fertility (%)	
Group		Cauda epididymis	Testis	
Control	66.73 ± 1.33	54.50 ± 0.49	5.27 ± 0.09	90-100% +ve
S.emarginatus (50 mg/day/rat for 60 days)	26.59 ± 1.67^{a}	11.69 ± 2.48^{a}	1.45 ± 0.14^a	100% -ve
<i>T. belerica</i> (50 mg/day/rat for 60 days)	38.84 ± 2.36^{a}	18.17 ± 3.39^{a}	1.43 ± 0.29^a	75.0% -ve
A.cepa (50 mg/day/rat for 60 days)	45.87 ± 0.16^{a}	16.50 ± 0.29^{a}	1.43 ± 0.11^{a}	60 % - ve
<i>C.cyminum</i> (50 mg/day/rat for 60 days)	35.45 ± 2.35^{a}	19.69 ± 0.22^{a}	1.13 ± 0.08^a	67 % - ve

Table-2Sperm motility, sperm density and fertility rate of control, S. emarginatus, T. belerica, A. cepa and C. cyminum
treated group of rats.

Values are mean \pm S.E.

a = P < 0.001

		Protein (mg/g)			Acid ;/g)	Fructose (mg/g)	Cholestrol (mg/g)	
Group	Testis	Cauda Epididymis	Ventral Prostate	Testis	Cauda Epididymis	Seminal Vesicle	Adrenal	Testis
Control	86.73 ± 4.34	57.39 ± 2.95	132.03 ± 3.17	1.16 ± 0.02	$\begin{array}{c} 0.95 \\ \pm \ 0.008 \end{array}$	0.24 ± 0.013	$\begin{array}{c} 14.51 \\ \pm \ 0.31 \end{array}$	6.11 ± 0.34
<i>S.emarginatus</i> (50 mg/day/rat for 60 days	76.07 ± 6.09	52.14 ± 5.72	126.49 ± 9.64	0.67 ^a ± 0.02	$\begin{array}{c} 0.58^{a} \\ \pm \ 0.009 \end{array}$	0.11 ^a ± 0.005	25.49 ^a ± 1.61	16.47 ^a ± 1.25
<i>T. bellerica</i> (50 mg/day/rat for 60 days)	34.08 ^a ± 3.52	44.87 ^b ± 1.32	85.01ª ± 2.75	$\begin{array}{c} 0.82^a \\ \pm \ 0.026 \end{array}$	0.82 ^a ± 0.031	0.18 ^a ± 0.004	27.18 ^b ± 3.11	7.74 ± 0.94
A. <i>cepa</i> (50 mg/day/rat for 60 days)	59.88 ^b ± 5.00	38.78 ^a ± 1.93	118.91 ± 5.14	0.74ª ± 0.04	0.69 ^a ± 0.03	0.19 ^c ± 0.01	19.30 ^a ± 0.28	8.43 ^a ± 0.19
<i>C. cyminum</i> (50 mg/day/rat for 60 days)	62.04 ^b ± 3.17	43.95 ^b ± 1.06	126.66 ± 2.58	$0.56^{a} \pm 0.05$	$\begin{array}{c} 0.75^{b} \\ \pm \ 0.06 \end{array}$	0.17 ^c ± 0.02	20.92 ^a ± 0.42	11.52 ^a ± 0.73

Table-3Protein, sialic acid, fructose and cholestrol concentration of control, S. emarginatus, T. belerica, A. cepa and
C. cyminum treated group of rats.

Values are mean \pm S.E.

a = P < 0.001 b = P < 0.01

c = P<0.05

	Glycogen (mg/g)		Ascorbic Acid (mg/g)		Acid Phosphatase (mg ^{pi} /gm/h)	Alkaline Phosphatase (mg ^{pi} /gm/h)
Group	Testis	Liver	Testis	Cauda Epididymis	Ventral Prostate	Ventral Prostate
Control	$\begin{array}{c} 3.62 \\ \pm 0.38 \end{array}$	22.83 ± 0.71	0.17 ± 0.004	$\begin{array}{c} 0.13 \\ \pm \ 0.002 \end{array}$	$5.20 \\ \pm 0.64$	2.18 ± 0.23
<i>S.emarginatus</i> (50 mg/day/rat for 60 days)	3.39 ± 0.22	10.13ª ± 0.44	$0.13^{a} \pm 0.003$	$0.12^{\rm b} \pm 0.003$	$2.06^{b} \pm 0.28$	1.42° ± 0.19
<i>T. belerica</i> (50 mg/day/rat for 60 days)	4.47 ± 0.19	23.99 ± 0.30	$\begin{array}{c} 0.16 \\ \pm \ 0.004 \end{array}$	0.12 ± 0.007	2.71 ^b ± 0.24	1.57° ± 0.13
A. cepa (50 mg/day/rat for 60 days)	3.19 ± 0.32	$\begin{array}{c} 20.04^b \\ \pm \ 0.85 \end{array}$	$\begin{array}{c} 0.16 \\ \pm \ 0.014 \end{array}$	$\begin{array}{c} 0.11^{\mathrm{b}} \\ \pm \ 0.004 \end{array}$	3.58 ± 0.32	1.55° ± 0. 11
C. cyminum (50 mg/day/rat for 60 days)	3.44 ± 0.46	$\begin{array}{c} 21.06 \\ \pm \ 0.47 \end{array}$	0.10 ^a ± 0.005	0.12 ^c ± 0.004	2.51° ± 0.41	$\begin{array}{c} 1.63 \\ \pm \ 0.12 \end{array}$

 Table-4
 Concentration of glycogen, ascorbic acid and enzyme activity of acid phosphatase and alkaline phosphatase of control, S. emarginatus, T. belerica, A. cepa and C. cyminum treated group of rats.

Effect of Sapindus	Terminalia.	Cuminum	and Allium	on Reproductive	Organs
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Broup of russ										
	Blo	od	Serum							
Group	Haemoglobin (%)	Haematocrit (%)	Protein (G/dl)	Cholestrol (mg/dl)	VLDL (mg/dl)	Tg (mg/dl)	Albumin (G/dl)	A/G ratio	Globulin	
Control	$\begin{array}{c} 14.80 \\ \pm \ 0.04 \end{array}$	47.01 ± 0.37	$\begin{array}{c} 6.71 \\ \pm \ 0.08 \end{array}$	50.00 ± 0.70	22.50 ± 1.06	112.00 ± 4.95	3.27 ± 0.01	$\begin{array}{c} 0.95 \\ \pm \ 0.03 \end{array}$	3.45 ± 0.10	
S. emarginatus (50 gm/day/rat for 60 days)	$\begin{array}{c} 14.32 \\ \pm \ 0.15 \end{array}$	$\begin{array}{c} 44.95 \\ \pm \ 0.85 \end{array}$	7.92 ± 0.11	$63.50^{b} \pm 0.35$	$\begin{array}{c} 22.00 \\ \pm \ 0.70 \end{array}$	108.50 ± 3.89	3.29 ± 0.06	$\begin{array}{c} 0.60 \\ \pm \ 0.07 \end{array}$	4.65 ± 0.03	
<i>T. belerica</i> (50 gm/day/rat for 60 days)	$\begin{array}{c} 14.55 \\ \pm \ 0.30 \end{array}$	$\begin{array}{c} 46.22 \\ \pm \ 0.17 \end{array}$	9.58 ± 0.36	$64.50^{b} \pm 0.35$	$\begin{array}{c} 27.50^{c} \\ \pm \ 0.35 \end{array}$	135.50° ± 1.76	3.66 ± 0.06	$\begin{array}{c} 0.55 \\ \pm \ 0.03 \end{array}$	5.90 ^c ± 0.28	
A.cepa (50 gm/day/rat for 60 days)	$\begin{array}{c} 14.22 \\ \pm \ 0.15 \end{array}$	$\begin{array}{c} 45.35 \\ \pm \ 0.85 \end{array}$	7.17 ± 0.231	77.00 ^b ± 1.41	$\begin{array}{c} 10.74^b \\ \pm \ 0.35 \end{array}$	$47.75^{b} \pm 0.18$	2.70° ± 0.07	0.55 ^c ± 0.03	4.25 ± 0.18	
<i>C.cyminum</i> (50 gm/day/rat for 60 days)	$\begin{array}{c} 14.28 \\ \pm \ 0.02 \end{array}$	$\begin{array}{c} 44.60 \\ \pm \ 0.40 \end{array}$	7.26 ± 0.18	$99.25^{a} \pm 0.53$	15.50 ^c ± 0.35	80.50° ± 0.35	2.83 ^b ± 0.03	0.65 ^c ± 0.03	$\begin{array}{c} 4.60 \\ \pm \ 0.07 \end{array}$	
Values are mean \pm S.E.VLDL=Very low density lipoproteins= $P < 0.001$ Tg=Triglycerides= $P < 0.01$ A/G ratio=Albumin/Globulin ratio										

 Table-5 : Haematology and serum biochemistry of control, S. emarginatus, T. belerica, A. cepa and C. cyminum treated group of rats.

c = P<0.05