

EVALUATING THE ANTIFERTILITY POTENTIAL OF THE ETHANOLIC EXTRACTS OF *BUPLEURUM SULPHUREUM* AND *CICHORIUM INTYBUS* IN MALE RATS

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ABSTRACT

Objective: In developing countries, population explosion is one of the most important causes of poverty and pollution. Therefore, several approaches have been investigated to decrease fertility, including hormonal, chemical and immunological approaches. However, no suitable method has been found to be effective and free from side effects. Thus, the aim of this study was to investigate the effect of *B. sulphureum* and *C. intybus* extracts on various parameters of male fertility using a rat model.

Methods: Ethanolic extracts of both plants at doses of 125, 250 and 500 mg/kg, were each given orally to male Wistar rats for 7 weeks. Sperm characters and serum levels of sex hormones were assessed. Mating success %, fertility success % and fertility index were also calculated. The testes, liver and kidney were processed for histological examination. The effect on some biochemical and hematological parameters were quantified. **Results:** Results showed significant decrease in the weight of testes, epididymis, seminal vesicle and ventral prostate in the 250 and 500 mg/kg groups after 7 weeks of treatment. Both extracts reduced the motility, count and viability of sperms. Significant reduction in serum levels of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) with hyperprolactinemia were observed. Both extracts did not significantly alter any of the biochemical and hematologic parameters studied.

Conclusion: These data suggest that administration of *B. sulphureum* and *C. intybus* extracts for 7 weeks contribute to reduce testicular and epididymal function in exposed rats.

Keywords: *B. sulphureum*, *C. intybus*, mating trial, fertility index, testosterone, male fertility.

INTRODUCTION

Population growth is a great concern world-wide and most of the developing countries are characterized by rapid population growth (Ezeh *et al.*, 2012). Hence, various methods are being used to reduce the total fertility rate in both men and women. Great attention is being given to many plant species with anti-fertility effects with the aim of screening and were subsequently supported by the international agencies. Though, the search for an orally active, safe and effective plant preparation is yet to be needed for regulation of fertility due to incomplete fertility inhibition or side effects.

B. sulphureum is a member of the *Umbelliferae* (*Apiaceae*) family. *Bupleurum* L. is a genus comprising about 200 species that primarily located in Eurasia, Mediterranean and North Africa (Saracoglu *et al.*, 2012). The genus *Bupleurum* L. includes 49 taxa in Turkey, with 21 endemic taxa. Extracts and essential oils of plants of the genus *Bupleurum* have been largely used in traditional medicine for the treatment of inflammatory disorders and infectious diseases (Ashour and Wink, 2011). Different parts of *B. sulphureum* plant are used in the Turkish folk medicine to treat breast fissures (Ozgen *et al.*, 2012), wounds and hemorrhoids (Sezik *et al.*, 2001).

The genus *Cichorium* consists of six different species with great distribution areas in Europe and Asia. *Cichorium intybus* or chicory is a member of the *Asteraceae* family, with a main phytoconstituent; phenol (Kshirsagar *et al.*, 2011). It is found as a wild plant on roadsides in its native Europe, North America and in Australia, where it has become naturalized. It is known in Turkey as catlangaç, çatlangaç süpürgesi, Talişk Badik out and Çitlak out. *C. intybus* is an erect, somewhat woody, perennial herbaceous plant with bright blue flowers. Various varieties are cultivated for salad leaves, chicons (blanched buds), or for roots (var. *sativum*), which are baked, ground and used as a coffee substitute and additive. Chicory is well

known for its toxicity to internal parasites. Studies showed that ingestion of chicory by farm animals results in worm elimination (Heckendorn *et al.*, 2007) which has encouraged its widespread use as a feed supplement.

The aim of this study was to investigate the effect of *B. sulphureum* and *C. intybus* plants on various male fertility parameters. Since the toxicity of these plants is not yet known, a rat model was used.

MATERIALS AND METHODS

Animal model

Both sexes of adult albino mice (27–30 g b. wt) were used in the acute toxicity test. Adult male (210–230 g) and female (170–190 g) Wistar rats were used in fertility study. Animals were obtained from Lab Animal Care Unit, Pharmacy College, Salman bin Abdulaziz University, Al-Kharj, KSA. All animals were kept under uniform and controlled conditions of temperature and light/dark (12/12 h) cycles, fed with standard rodent diet and given fresh purified potable water *ad libitum*. Commercially obtained sawdust was used as bedding material. The cages were washed once a week. The animals were allowed to acclimatize to the laboratory condition for one week before commencement of the experiment.

Plant material

Fresh flowering aerial parts of *B. sulphureum* Boiss and *C. intybus* L. were collected at summer 2011, from the A4 Ankara: Kızılcahamam, Çeltikçi way and A3 Ankara: Beypazarı-Nallıhan way, respectively. Taxonomic identification was determined by Prof. Dr. Galip Akaydin and a voucher specimen from each plant (Akaydin 13499 & Akaydin 10301, respectively) were deposited at the Herbarium of Faculty of Education (HEF), Hacettepe University, Ankara, Turkey. The

collected plants were shade dried and then grinded to fine powders. One hundred grams of the dried powders of each plant were extracted by percolation in 70% aqueous ethanol with occasional shaking for 48 h. Percolation was repeated three times, and then the ethanolic extracts of each plant were combined and concentrated under vacuum to give the total extracts of *B. sulphureum* and *C. intybus* (31.6 and 15.4 g, respectively).

Treatment protocol

Acute oral toxicity study: Acute oral toxicity study was performed according to Health Effect Test Guidelines (2002). Two groups of mice (6 animals/ group) were fasted overnight then treated orally with *B. sulphureum* and *C. intybus* extracts, respectively at a dose of 2000 mg/kg. Another control group received the vehicle (3% v/v Tween 80 in distilled water) and kept under the same conditions without any treatment. Animals were observed individually after dosing for 48 h for clinical signs of toxicity and/or mortalities. Since, there was no mortality at this level; the dose of both extracts was increased to 5000 mg/kg and animals were observed for another 48 h.

Justification for dose selection: *B. sulphureum* and *C. intybus* extracts were nontoxic at the dose of 5000 mg/ kg so, 1/40th, 1/20th and 1/10th of this dose (125, 250 and 500 mg/kg, respectively) were selected for the study.

Effect on male fertility: Forty two sexually mature male Wistar rats (210–230 g b. wt) were randomly divided into three groups. The 1st group (6 animals) received the vehicle (5 mL/kg) and kept as control. The 2nd group (18 animals) was divided into 3 equal sub-groups that received *B. sulphureum* extract in doses of 125, 250 and 500 mg/kg, respectively. Rats of the 3rd group (18 animals divided into 3 equal sub-groups) received *C. intybus* extract in doses of 125, 250 and 500 mg/kg, respectively. Both extracts and vehicle were administered to animals by oral intubation for 7 weeks. This administration period is necessary to determine the effect of the extracts on sperm production because rats need a period of 48–52 days for the exact spermatogenic cycle.

Sacrifice schedule: Twenty-four hours after their last dose, the rats were weighed and sacrificed under light ether anesthesia.

Parameters

Estimation of sex hormones: Blood samples were collected from rats for estimations of serum levels of sex hormones. Sera were separated into clean bottles, stored frozen and used within 12 h of preparation for the estimation of testosterone (Chen *et al.*, 1991), prolactin (Tietz, 1995), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Uotila *et al.*, 1981).

Assessment of sperm motility and count: Progressive motility was tested immediately. The right cauda epididymidis was incised and semen was squeezed on a pre-warmed slide. Two drops of warm 2.9% sodium citrate was added to semen and mixed by a cover-slip. The percentage of progressive sperm motility was evaluated visually at 400× magnification (Sönmez *et al.*, 2005). Motility estimates were performed from three different fields in each sample. The mean of the three successive estimations was used as the final motility score.

For sperm count, the left cauda epididymidis was incised and semen that oozed was quickly sucked into a red blood pipette to the 0.5 mark, and then diluted with warm normal saline up to the 101 mark. A drop of the semen mixture was placed on the Neubauer counting chamber and viewed under the magnification of ×40 (Sönmez *et al.*, 2005). The total numbers of sperm cells were counted and expressed as 10⁶/mL.

Assessment of sperm viability and morphology: A viability study (percentage of live spermatozoa) was done using eosin/nigrosin stain. A drop of semen was squeezed onto a microscope slide and two drops of the stain were added. Thin smears were then prepared and observed under a light microscope at ×400 magnification. Viable sperm remained colorless while non-viable sperm stained red. The stained and the unstained sperm cells were counted using ×40

microscope objectives and an average value for each was recorded from which percentage viability was calculated.

To determine the percentage of morphologically abnormal spermatozoa, the slides stained with eosin–nigrosin (5 slides/ rat) viewed under a light microscope at 400× magnifications. A total of 300 sperm cells was examined on each slide (1500 cells for each rat), and the head, tail and total abnormality rates of spermatozoa were expressed as a percent.

Mating trial test: Mating trial test of male rats was done, 5 days before the termination of the experiment. Each male rat was cohabitated overnight with proestrous females in a ratio of 1:2 and housed in a single cage. Positive mating was confirmed by presence of sperm and vaginal plug in the vaginal smear the following morning (Dehghan *et al.*, 2005). Each sperm positive female was kept under observation and the resultant pregnancies were noted, when dam gave birth. The following reproductive parameters were then computed: mating success % = [(number mated/number paired) × 100]; fertility success % = [(number pregnant/number paired) × 100]; Fertility index = [(number pregnant/number mated) × 100].

Body and sex organ weights: The initial and final body weights of the animals were recorded. The testes, epididymides, seminal vesicle and ventral prostate were dissected out, freed from adherent tissues and blood, and weighed to the nearest milligram. Organ weights were reported as relative weights (organ weight/body weight × 100).

Quantification of fructose in seminal vesicle: For fructose quantification, seminal vesicular homogenate was prepared at a tissue concentration of 50 mg/mL. The supernatant (seminal plasma) was deproteinized by adding 50 µL of zinc sulphate and sodium hydroxide to make a total dilution of seminal plasma 1:16, followed by centrifugation at 2500 rpm for 15 min. For fructose measurement, 200 µL of clear seminal plasma was used and the optical density of standard and samples were measured against blank at 470 nm. The concentration of fructose was obtained by plotting the value in standard curve and the value expressed in the unit of µM/ mL of seminal plasma (Lu *et al.*, 2007).

Histological analysis: Testes were carefully dissected out following abdominal incision and fixed in 10% formal-saline and processed routinely for paraffin embedding. Sections of 5 µm were obtained with rotary microtome, stained with Hematoxylin and Eosin Stain (H/E) and observed under a light microscope.

Measurement of some biochemical and blood parameters : Blood samples were collected from the heart of each rat at the time of scarification into non-heparinized and heparinized tubes. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and urea in addition to red blood cell (RBC) count, total leucocytic count (TLC), haemoglobin (Hb) concentration and packed cell volume (PCV) were determined by standard methods.

Ethical aspects: The study was approved by the Institutional animal ethical committee of Pharmacy College, Salman bin Abdulaziz University, Al-Kharj, KSA.

Statistical analysis: The values are expressed as mean ± SEM of six observations in each group. All groups were subjected to one-way analysis of variance (ANOVA), which was followed by Dunnett's post hoc test to determine the intergroup variability by using SPSS ver. 14.0. A comparison was made with the experimental control group. Differences were regarded statistically significant at the P ≤ 0.05 and 0.001 levels.

RESULTS

The obtained results indicated that *B. sulphureum* and *C. intybus* extracts at oral doses up to 5000 mg/kg did not produce any symptom of acute toxicity and none of the mice died during 48 h of observation. All animals did not exhibit diarrhea, haematuria, restlessness, uncoordinated muscle movements, and respiratory distress accordingly; it suggested that oral median lethal dose (LD₅₀) of the tested extracts were higher than 5000 mg/kg b.wt.

Table 1: Effect of oral administration of *Bupleurum sulphureum* and *Cichorium intybus* extracts for 7 weeks on serum levels of reproductive hormones of male rats, (n = 6).

Groups	Doses (mg/kg)	Testosterone (ng/mL)	Prolactin (ng/mL)	FSH* (mIU/mL)	LH* (mIU/mL)
Control	00	4.46±0.33	0.80±0.05	7.80±0.35	0.60±0.03
<i>B. sulphureum</i>	125	4.18±0.27	0.89±0.04	7.26±0.31	0.57±0.03
	250	3.14±0.20 ^a	1.26±0.11 ^a	5.64±0.37 ^a	0.46±0.02 ^a
	500	2.73±0.17 ^b	1.59±0.12 ^b	5.16±0.28 ^b	0.42±0.03 ^a
<i>C. intybus</i>	125	4.01±0.25	0.92±0.06	7.10±0.31	0.56±0.02
	250	2.92±0.13 ^a	1.54±0.14 ^b	5.30±0.45 ^a	0.44±0.03 ^a
	500	2.40±0.19 ^b	1.86±0.13 ^b	4.85±0.26 ^b	0.39±0.02 ^b

Values represent the mean ± S.E. of six rats for each group. ^a P<0.05: Statistically significant from control (Dunnett's test). ^b P<0.001: Statistically significant from control (Dunnett's test).

* FSH: Follicle-stimulating hormone. * LH: Luteinizing hormone.

Table 2: Effect of oral administration of *Bupleurum sulphureum* and *Cichorium intybus* extracts for 7 weeks on semen characteristics and fructose content in seminal vesicle of male rats, (n=6).

Groups	Doses (mg/kg)	Sperm count (X 10 ⁶ /mL)	Sperm motility (%)	Sperm viability (%)	Fructose level (µM/mL)
Control	00	49.7±1.78	83.6±2.0	85.7±3.1	4.24±0.28
<i>B. sulphureum</i>	125	45.9±1.53	79.5±2.7	83.5±2.1	4.15±0.27
	250	40.4±1.75 ^a	74.9±2.4 ^a	70.1±2.9 ^a	3.17±0.17 ^a
	500	38.2±1.26 ^b	71.8±2.7 ^a	64.8±3.2 ^b	2.74±0.15 ^b
<i>C. intybus</i>	125	44.5±1.65	81.2±2.7	83.2±2.1	4.21±0.20
	250	39.9±1.83 ^a	73.1±2.9 ^a	66.1±3.3 ^a	2.95±0.19 ^a
	500	35.3±1.82 ^b	69.2±2.1 ^b	60.0±2.3 ^b	2.70±0.15 ^b

Values represent the mean ± S.E. of six rats for each group. ^a P<0.05: Statistically significant from control (Dunnett's test). ^b P<0.001: Statistically significant from control (Dunnett's test).

Table 3: Effect of oral administration of *Bupleurum sulphureum* and *Cichorium intybus* extracts for 44 days on the mating trial of male rats with normal untreated females (mating ratio = 1 male: 2 females).

Groups	Doses (mg/kg)	No. of females mated ^a	Mating success %	No. of females pregnant	Fertility success %	Male fertility index (%) ^b
Control	00	12/12	100.0	12/12	100.0	100.0
<i>B. sulphureum</i>	125	12/12	100.0	11/12	91.66	91.66
	250	9/12	75.00	8/12	66.66	88.88
	500	7/12	58.33	6/12	50.00	85.71
<i>C. intybus</i>	125	11/12	91.66	10/12	83.33	90.90
	250	7/12	58.33	6/12	50.00	85.71
	500	6/12	50.00	5/12	41.66	83.33

Data are expressed as numbers and % of 6 males and 12 females. ^a Evidenced by vaginal plug and sperm in a vaginal smear. ^b Male fertility index = [(number pregnant/number mated) × 100].

Table 4: Effect of oral administration of *Bupleurum sulphureum* and *Cichorium intybus* extracts for 7 weeks on body and sexual organs weights of male rats, (n=6).

Groups	Doses (mg/kg)	Final body weight (g)	Relative weight of reproductive organs (g/100 g b.wt)			
			Testes (Pair)	Cauda epididymis	Seminal vesicles	Ventral prostate
Control	00	259.99±7.34	1.76±0.12	0.65±0.03	0.66±0.03	0.47±0.02
<i>B. sulphureum</i>	125	245.72±8.53	1.70±0.14	0.62±0.04	0.62±0.04	0.44±0.02
	250	248.79±7.65	1.12±0.10 ^a	0.51±0.03 ^a	0.54±0.03 ^a	0.35±0.03 ^a
	500	252.71±7.55	1.03±0.10 ^b	0.47±0.02 ^b	0.47±0.02 ^b	0.31±0.03 ^a
<i>C. intybus</i>	125	246.87±7.38	1.64±0.14	0.60±0.04	0.61±0.04	0.43±0.03
	250	253.94±8.43	1.00±0.13 ^a	0.50±0.03 ^a	0.50±0.02 ^a	0.32±0.03 ^a
	500	249.40±6.80	0.92±0.08 ^b	0.44±0.02 ^b	0.41±0.03 ^b	0.29±0.02 ^b

Values represent the mean ± S.E. of six rats for each group. ^a P<0.05: Statistically significant from control (Dunnett's test). ^b P<0.001: Statistically significant from control (Dunnett's test).

Table 5: Effect of oral administration of *Bupleurum sulphureum* and *Cichorium intybus* extracts for 7 weeks on serum levels of AST,ALT, urea and creatinine of male rats, (n = 6).

Groups	Doses (mg/kg)	AST* (U/L)	ALT* (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	00	63.24±3.22	136.57±5.39	38.56±1.42	0.36±0.03
<i>B. sulphureum</i>	125	65.64±2.74	134.24±5.22	38.83±1.73	0.36±0.03
	250	66.77±3.16	133.73±6.84	37.66±1.44	0.37±0.02
	500	67.15±3.50	138.06±6.39	41.39±1.78	0.35±0.03
<i>C. intybus</i>	125	64.68±2.74	134.87±5.18	36.59±1.82	0.36±0.02
	250	67.32±3.16	138.73±6.52	37.35±1.53	0.37±0.03
	500	68.85±3.50	136.25±6.55	37.74±1.26	0.37±0.03

Values represent the mean ± S.E. of six rats for each group. No statistical significance from control (LSD test).

*AST: Aspartate Aminotransferase. *ALT: Alanine Aminotransferase.

Table 6: Hematological analysis of male rats after treatment with *Bupleurum sulphureum* and *Cichorium intybus* extracts for 7 weeks (n=6).

Groups	Doses (mg/kg)	RBCs (x 10 ⁶ / mL)	Hb (g%)	PCV* (%)	TLC* (x 10 ³ / mL)
Control	00	8.8±0.28	10.57±0.36	30.56±1.14	11.7±0.87
	125	8.9±0.27	10.24±0.40	31.83±1.33	12.1±0.73
<i>B. sulphureum</i>	250	8.5±0.24	9.73±0.36	29.66±1.07	11.2±0.64
	500	8.9±0.29	10.06±0.32	30.39±1.25	11.5±0.72
<i>C. intybus</i>	125	8.2±0.27	9.87±0.30	28.59±1.30	10.3±0.55
	250	8.5±0.25	9.73±0.38	29.35±1.24	11.3±0.38
	500	8.6±0.30	10.25±0.36	29.74±1.18	11.5±0.48

Values represent the mean ± S.E. of six rats for each group. No statistical significance from control (LSD test).

*PCV: packed cell volume. *TLC: total leucocyte count.

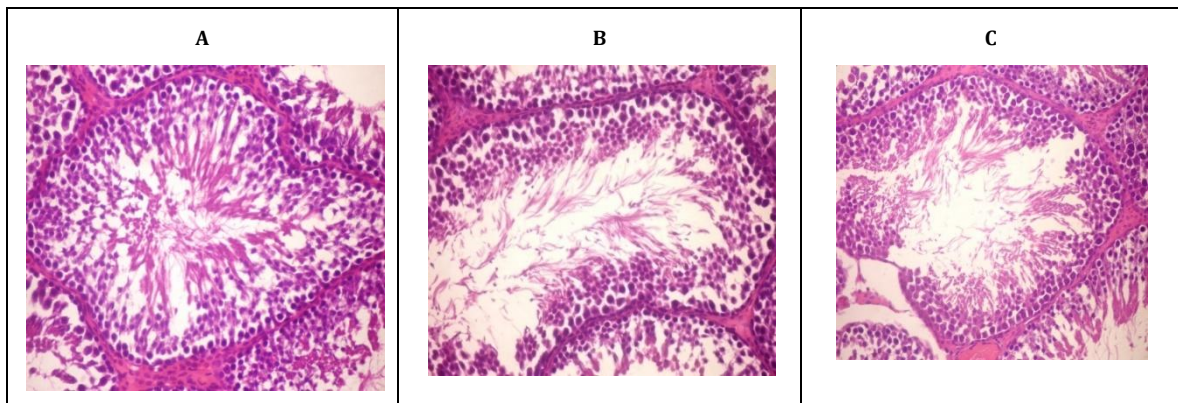


Fig. 1: Photomicrographs of rat testis (H&E X400), (A) Normal control group showing normal seminiferous tubules with complete spermatogenesis; (B) *B. sulphureum* (500 mg/kg) showing degeneration in spermatogonia cells lining the seminiferous tubules associated with incomplete spermatogenesis and sloughing of degenerated germ cells; (C) *C. intybus* (500 mg/kg) showing degeneration in spermatogonia cells lining the seminiferous tubules evidenced by sloughing of germ cells in addition to few scanty spermatozoa in seminiferous tubules of rats.

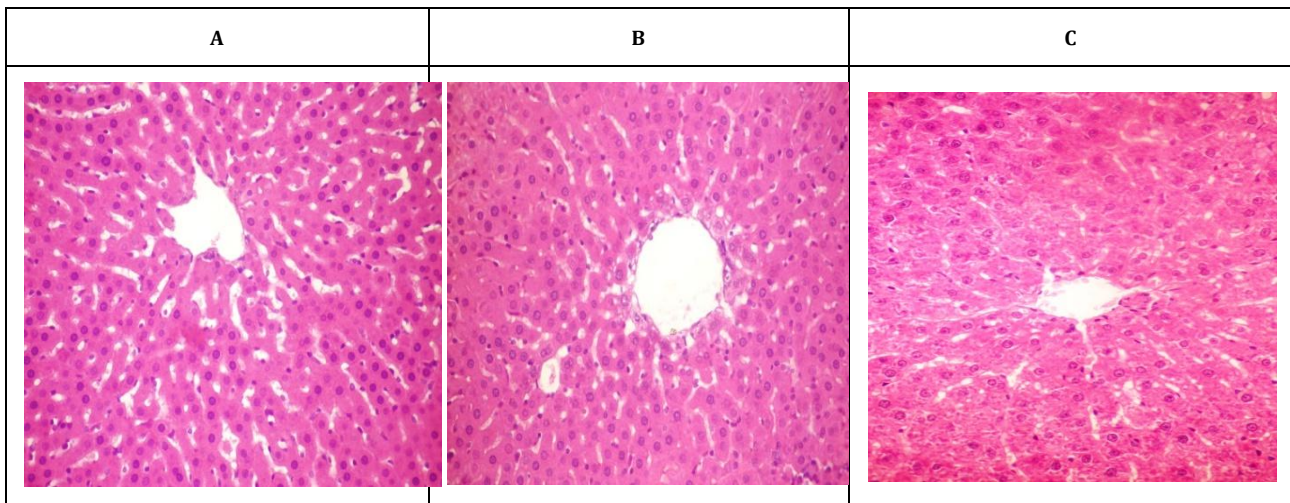


Fig. 2: Photomicrographs of a section of rat liver (H&E X400), (A) Normal control group; (B) *B. sulphureum* (500 mg/kg) and (C) *C. intybus* (500 mg/kg) showing normal structure.

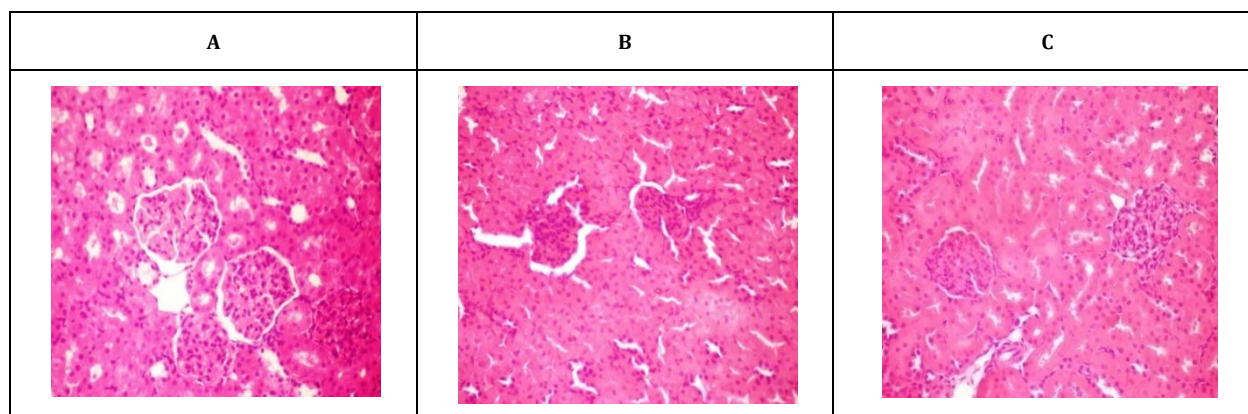


Fig.3: Photomicrographs of a section of rat kidney (H&E X400), (A) Normal control group; (B) *B. sulphureum* (500 mg/kg) and (C) *C. intybus* (500 mg/kg) showing normal structure.

Oral administration of *B. sulphureum* and *C. intybus* extracts at a dose of 125 mg/kg for 7 weeks did not affect any of male fertility parameters (Tables 1-4).

Oral administration of *B. sulphureum* and *C. intybus* extracts to male rats for 7 weeks caused a dose related decrease in the serum level of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Table 1). The level of decrease was statistically significant. For instance, serum testosterone level was 3.14 and 2.92 ng/mL, respectively in 250 mg/kg groups while in 500 mg/kg groups was 2.73 and 2.40 ng/mL, respectively compared to 4.46 ng/mL in the control group. While FSH level was 5.64 and 5.30 mIU/mL, respectively in 250 mg/kg groups while in 500 mg/kg groups was 5.16 and 4.85 mIU/mL, respectively compared to 7.80 mIU/mL in the control group. Moreover, serum level of LH was recorded to be 0.46 and 0.44 mIU/mL, respectively in 250 mg/kg groups while in 500 mg/kg groups was 0.42 and 0.39 mIU/mL, respectively compared to 0.60 mIU/mL in the control group. Both extracts caused a significant increase in the serum level of prolactin in males compared to control (Table 1). In this concern, serum level of prolactin was 1.26 and 1.54 ng/mL, respectively in 250 mg/kg groups while was 1.59 and 1.86 respectively in 500 mg/kg groups compared to 0.80 ng/mL in the control group.

The motility of caudal epididymal spermatozoa was reduced significantly following administration of 250 and 500 mg/kg of *B. sulphureum* and *C. intybus* in a dose-dependent manner as compared to control rats. Sperm motilities in 250 mg/kg groups were 74.9% and 73.1%, respectively and in 500 mg/kg groups were 71.8% and 69.2%, respectively compared to 83.6% in the control group (Table 2).

Epididymal spermatozoa of control rats exhibited normal sperm count. The epididymal spermatozoa of *B. sulphureum*-treated rats at doses of 250 and 500 mg/kg exhibited gradual decline in sperm count in a dose-dependent manner showing 18.7% decline at 250 mg/kg and 23.1% decline at 500 mg/kg dose. On the contrary, the epididymal spermatozoa of *C. intybus*-treated rats seemed to be more adversely affected showing 19.7% decline in sperm count at 250 mg/kg dose and 28.9% decline at 500 mg/kg dose (Table 2) which were highly significant.

Please insert tables 1,2 here.

There was a significant decrease in the percentage of viability (Table 2) but no significant change in morphology of sperms of rats treated with *B. sulphureum* and *C. intybus* extracts compared with their control group.

The fructose concentration of the seminal fluid of the animals treated with both extracts (250 and 500 mg/kg) showed significant decrease when compared with that of the control (Table 2).

Mating trial during 44-49 days of treatment schedule culminated in normal pregnancy outcome in control animals. The fertility success and fertility index of treated male rats were declined in a dose-dependent pattern following *B. sulphureum* and *C. intybus*

medication (Table 3). The ability of males to mate was also reduced following 250 and 500 mg/kg doses as evidenced by the number of mated females in *B. sulphureum* group (9/12 & 7/12, respectively) and in *C. intybus* group (7/12 & 6/12, respectively) as compared to 12/12 mated females in control group.

There were no significant changes in the final body weight of all medicated rats compared with the controls after 7 weeks of medication. A significant decline in the relative weights of testes, epididymides, seminal vesicle and ventral prostate (expressed in mg/100 g of body weight) were observed in *B. sulphureum* and *C. intybus*-treated rats (250 and 500 mg/kg) when compared with the controls (Table 4).

Please insert tables 3,4 here.

Histopathology of testes of the control rats showed normal round or oval seminiferous tubules with normal germ cells at various stages covering complete spermatogenic cycle (Figure 1-A). Histological examination of the testes of rats treated with *B. sulphureum* extract (250 and 500 mg/kg) revealed degeneration in spermatogonia cells lining the seminiferous tubules (Figure 1-B), associated with incomplete spermatogenesis and sloughing of degenerated germ cells. The disruption in the seminiferous epithelium of *C. intybus*-medicated rats (250 and 500 mg/kg) was evidenced by degeneration in spermatogonia cells lining the seminiferous tubules evidenced by sloughing of germ cells in addition to few scanty spermatozoa in seminiferous tubules of rats (Figure 1-C).

Histopathology of liver and kidney of *B. sulphureum* and *C. intybus*-medicated rats did not reveal any treatment related changes (Figures 2&3).

Please insert figures 1,2,3 here.

No significant changes were detected in the serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), of rats after 7 weeks of medication (Table 5). Similarly, levels of urea and creatinine were unchanged in serum of *B. sulphureum* and *C. intybus*-treated groups compared to those of control rats. Red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV) and total leucocytic count (TLC) in *B. sulphureum* and *C. intybus* groups did not show any alteration and were comparable to control group (Table 6).

Please insert tables 5,6 here.

DISCUSSION

In the current study, oral administration of *B. sulphureum* and *C. intybus* extracts at doses up to 5000 mg/kg did not produce any symptom of acute toxicity and none of mice died during 48 h of observation. Accordingly, it suggested that oral median lethal dose (LD₅₀) of the tested extracts was higher than 5000 mg/kg. Therefore, *B. sulphureum* and *C. intybus* plants can be categorized as quietly safe since substances possessing LD₅₀ higher than 50 mg/kg are non-toxic (Buck et al., 1976).

The non-toxic nature of both extracts in acute toxicity study in mice is well supported in rats following medication for 7 weeks. Treatments with different doses of *B. sulphureum* and *C. intybus* extracts were well tolerated by all the animals, as no toxic effects were recorded by direct visual observation of the animals throughout the experiment. No diarrhea, haematuria, restlessness, uncoordinated muscle movements, and respiratory distress were appeared. There was no death and apparent behavioral changes recorded during the course of the experiment in all treatment groups as compared to the control animals. These observations might suggest the non-toxic effect of both extracts.

Gonadotrophins and testosterone are the leading regulators of germ cell development. Testosterone was reported to act on the seminiferous tubules contributing to in initiation and maintenance of spermatogenesis (Iranloye and Bolarinwa, 2009). Moreover, the efficiency of spermatogenesis and fertility are increased in males as a result of the synergistic effect of follicle-stimulating hormone (FSH) and testosterone (McLachlan et al. 1996). On the other hand, luteinizing hormone (LH) stimulates spermatogenesis through the stimulation of testosterone production in Leydig cells, which sequentially may act on the Sertoli and peritubular cells of the seminiferous tubules (O'Donnell et al., 1999). In the current study, rats treated with *B. sulphureum* and *C. intybus* extracts at doses of 250 and 500 mg/kg exhibited a significant decrease in serum levels of testosterone, FSH and LH compared to animals in the control group. The decrease in testosterone level by both extracts may be added to reduction of the hormone synthesis by the Leydig cells, as the cells are the main source of testosterone. Moreover, hyperprolactinemia observed in the present study following *B. sulphureum* and *C. intybus* medication at doses of 250 and 500 mg/kg is known to suppress testosterone synthesis and male fertility; since prolactin increases the secretion of corticosteroids by the adrenal cortex or by the suppressive effects of prolactin on gonadotropin-releasing hormone (GnRH) through the action on prolactin receptors on the hypothalamic dopaminergic neurons (Albertson et al., 1987).

Sperm motility and their count are considered as the important factors that affect the process of spermatogenesis and fertility. Testosterone promotes differentiation of spermatozoa during the process of spermatogenesis so; a lack of testosterone level would have direct effects on the process of spermatogenesis. In this investigation, the epididymal sperm motility and count of the rats treated with *B. sulphureum* and *C. intybus* extracts at doses of 250 and 500 mg/kg were significantly reduced when compared with their control counterparts. This decrease in epididymal sperm motility and count could be connected to the reduction in the serum testosterone level reported in this study. Gong and Han (2006) confirmed this explanation as they stated that lowering of epididymal sperm motility and count suggested an undersupply of testosterone to the epididymis. It has also been demonstrated that the low levels of FSH and LH prevents the gonads from either producing sperms or insufficient amount of testosterone production (Nieschlag, 2000). Further, it is well established that FSH and testosterone are both required by Sertoli cells/germ cells to support the process of spermatogenesis (Gelain et al., 2005). Depletion in the biosynthesis of any one of these hormones, therefore, could block formation of spermatozoa.

Low fructose concentration in the seminal fluid of *B. sulphureum* and *C. intybus*- medicated rats may be another cause of low sperm motility. Chinoy and Bhattacharya (1997) reported reduced sperm motility after aluminum chloride administration in mice with low levels of seminal vesicular fructose, as fructose supplies energy for sperm motility. The impaired sperm motility and viability may also be due to the reduced activity of the testes, which affects the normal passage of testicular fluid into the epididymis. Moreover, decrease in sperm count is correlated with decrease in the testicular weight indicating that the germ cell death or cell loss from the epithelium may be due to tubular atrophy which is a main reason for decreased testis weight (Narayana, 2008).

Alterations in the sperm viability are indications of a disturbed epididymal microenvironment. The results of this study have shown

that exposure of rats to *B. sulphureum* and *C. intybus* at doses of 250 and 500 mg/kg caused a decrease in sperm viability. The decrease in sperm viability agreed with the reduction in the progressive motility of sperms, as immobile sperms were considered dead since they stained by the Eosin/Nigrosin dye when the smear was examined. The significant reduction of sperm viability, motility and count shows that both extracts has the potential to penetrate the blood-testis barriers and suggests alteration of sperm maturation in the epididymides (Sathiyraj et al., 2010).

Sperm function is determined by the composition of seminal fluid. Fructose concentration has been noted to be essential for spermatozoal metabolism and motility as an energy source. In this connection, Montagnon et al., (1990) reported that fructose content in seminal fluid is an important marker of seminal vesicular function and thus sperm motility and viability are affected; when seminal vesicular function is decreased. Baldessarini (1980) reported that chemical agents have the ability of to penetrate the blood-testis barriers which in turn results in changing sperm composition. Accordingly, the significant depletion of fructose content in the seminal fluid and subsequent decrease in sperm motility, count and viability in *B. sulphureum* and *C. intybus*- medicated rats shows that both extracts has the potential to penetrate these barriers. This depletion of seminal fructose in *B. sulphureum* and *C. intybus* treated groups invariably affects the sperm motility and viability since fructose serves as the most important source of energy for sperms and since fructose is androgen-dependent; this may be a sign of reduction in circulating levels of androgen. As well, the decrease in sperm qualities referred to reduction in the circulating androgen level.

Testosterone is required for the maintenance of normal sexual desire and penile erection in males. In the current study, the fertilizing ability of male rats was significantly reduced by the administration of *B. sulphureum* and *C. intybus* extracts at doses of 250 and 500 mg/kg. The reduction in mating and fertility success of male rats following *B. sulphureum* and *C. intybus* medication could be due to decreased testosterone level that reduces androgen-dependent parameters like mating behavior, libido and penile erection (Gauthaman et al., 2002). It is well known that low sexual desire is related to low serum testosterone level (Jannini et al. 1999). Another hormone related to sexual function is prolactin which facilitates sexual response in men. However, hyperprolactinemia that induced by both extracts at doses of 250 and 500 mg/kg is related to low libido (Schwartz et al. 1982); in addition to the reduction of testosterone synthesis and suppression of male fertility subsequently (Albertson et al., 1987). Pregnancy rates of the untreated female rats were reduced following mating with *B. sulphureum* and *C. intybus*-medicated males (250 and 500 mg/ kg). The decrease in the pregnancy rate might be due to the effects of both extracts on the progressive epididymal sperm motility as sperm motility is positively correlated with fertilization of oocytes and pregnancy rates (Donnelly et al., 1998). Consequently, the decline of pregnancy rate is an indicator for the onset of male infertility and is one of male reproductive toxicity markers (Sally, 1997). Moreover, sperm motility is an important functional measurement to predict the fertilizing capacity of sperms, so the negative impact of both extracts at doses of 250 and 500 mg/kg on sperm motility would seriously affect the fertilizing ability (Mali et al., 2002).

Monitoring body weight provides information on the general health level of animals, which can be important to the interpretation of reproductive effects. In this study, the body weight of rats treated with *B. sulphureum* and *C. intybus* extracts were not altered indicating that the general metabolic condition of the animals was within normal range. Creasy (2003) has reported that weights of testes and accessory organs are sensitive end points that can be used in evaluation of deleterious effect on male reproduction. In the present study, weights of testes, epididymis, and accessory organs obtained from rats exposed to *B. sulphureum* and *C. intybus* at doses of 250 and 500 mg/kg were significantly reduced following 7 weeks-medication. The decreasing weight of testes and the accessory reproductive glands clearly indicated that both extracts caused structural alteration in the reproductive organs of male rats. It has

also been demonstrated testosterone level determines the maintenance of the weights of accessory reproductive glands (Jana *et al.*, 2003). Accordingly, the significant reduction in the reproductive organ weights of male rats in this study may be attributed to the decrease in testosterone levels and inhibition of spermatogenesis. In addition, Zitzmann (2008) have demonstrated that physiologic concentrations of testosterone, LH and FSH play an important role in spermatogenesis. Thus, in the current study, the significant decrease in testosterone, LH and FSH levels could decrease the number and function of somatic and germinal cells of testis followed by decrease in testicular weight.

The deleterious effects of *B. sulphureum* and *C. intybus* extracts at doses of 250 and 500 mg/kg on male fertility were supported by the histopathological findings in the testes of treated rats. Both extracts provoked some histopathological changes in the testes such as disorganized seminiferous tubules with incomplete spermatogenesis and sloughing of degenerated germ cells. One possible explanation for the incomplete spermatogenesis is the reduction in testosterone level. Moreover, sloughing of germ cells was observed in the lumen of some seminiferous tubules of *C. intybus* medicated rats at doses of 250 and 500 mg/kg indicating testicular dysfunction (Narayana *et al.*, 2006).

Since the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are specific assayable liver enzymes, their normal levels in the serum of experimental groups of rats treated with both extracts for 7 weeks indicated that the tested extracts are not hepatotoxic. Serum creatinine and urea levels are sensitive and reliable biochemical parameters for the evaluation of kidney function in animals. Since the activity of ALT and AST are specific assayable liver enzymes, their normal levels in the serum of experimental groups of rats treated with both extracts for 7 weeks indicated that the tested extracts are not hepatotoxic. Serum creatinine and urea levels are sensitive and reliable biochemical indices for evaluation of renal function in animal models. In the present study, the mean values of urea and creatinine in serum of rats were not affected by treatment with both extracts for up to 7 weeks. In kidney damage, there will be retention of urea and creatinine in the blood; therefore marked increases in serum urea and creatinine are indications of functional damage to the kidney (Panda, 1999). By these indicators, ethanol extracts of *B. sulphureum* and *C. intybus* are therefore, not nephrotoxic in rats.

The assessment of haematological parameters could be used to reveal the deleterious effect of plant extracts on the physiological and pathological status of man and animal (Magalhaes *et al.*, 2008). The normal range of red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV) and total leucocytic count (TLC) in any of the treatment groups suggest that *B. sulphureum* and *C. intybus* extracts did not cause any adverse effects on the general health of rats. The biochemical and haematological data were supported by the normal histopathological findings in the livers and kidneys of treated rats.

It is concluded that the ethanolic extracts of *B. sulphureum* and *C. intybus* at doses of 250 and 500 mg/kg are capable to suppress male fertility without altering general body metabolism. This is demonstrated by the decrease in the fertility parameters (motility, count and viability of sperms, serum level of sex hormones, and fertility rate) in treated male rats. However, further studies are required for better understanding of the mechanism of reproductive toxicity induced by both plants.

Conflict of Interest

We have no conflict of interest in the work.

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