

Reproductive Toxicity of *Orthosiphon stamineus* Benth (Java tea) in Swiss Albino Mice

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Abstract: The java tea is significantly used in traditional medicine for its anti-allergic, antihypertensive, anti-inflammatory and diuretic properties. The present study is designed to assess the effect of java tea after its chronic use on male reproduction, cytological and biochemical changes in Swiss albino mice. Java tea was administered orally at the doses 62.50, 125 and 250 mg/kg/day for 90 days. Higher dose of Java tea caused a significantly reduction in sperm count and motility along with an induction of morphological abnormalities in spermatozoa and also induced aberrations in sperm chromosomes compare to normal mice. Higher dose caused a significant increase in plasma levels of estradiol and decrease in testosterone levels. It also reduced the fertility index in male and female mice and that indicates towards the induction of dominant lethal mutations after sub-chronic administration of Java tea particularly in higher dose treated group. Malondialdehyde (MDA) level was found to be increased along with a concomitant reduction in Non-Protein Sulfhydryl (NP-SH) in testes tissue. In conclusion, Java tea administration at higher dose induces reproductive toxicity in mice and the study paves a path to further investigate its clinical effects on reproductive system.

Keywords: Fertility, java tea, oxidative stress, pituitary gonadal hormones, reproductive toxicity, sperm count

INTRODUCTION

Orthosiphon stamineus Benth [syn: *Orthosiphon aristatus* (B1) Miq., *Orthosiphon grandiflorus* Bold., *Orthosiphon spicatus* (Thumb.) Bak.; Lamiaceae] (Suresh *et al.*, 2003) is one of the popular traditional folk medicines extensively used in Southeast Asia for the treatment of a wide range of diseases. It is used for rheumatism, diabetes, hypertension, tonsillitis, epilepsy, menstrual disorder, gonorrhoea, syphilis, renal calculus and gallstones (Mohamed *et al.*, 2012; Awale *et al.*, 2002; Akowuah *et al.*, 2005; Ameer *et al.*, 2012). There is a paucity of literature on the toxic effects (reproductive and biochemical toxicity) of Java tea or any plant under *Orthosiphon*, except the case reports of the association of acute hepatitis with Java tea (Garcia-Moran *et al.*, 2004).

The main components of Java tea are polyphenols (sinesetone, eupatorine and flavones) and caffeic acid derivatives (rosmarinic acid, cichoric acid, caffeic acid) (Olah *et al.*, 2003; Loon *et al.*, 2005). Tezuka *et al.* (2000) also reported the presence of eupatorin, sinensetin, tetramethoxyflavone, salvigenin, ladanin, vomifoliol, rosmarinic acid, caffeic acid, oleanolic acid, ursolic acid, beta-sitosterol, betulinic acid in java tea. However, the different species of *Orthosiphon* are known to contain several terpenoids (diterpenes and triterpenes) (Awale *et al.*, 2002; Akowuah *et al.*, 2005). The diterpenes isolated from Java tea were found to be of Norstamine-, stamine- and isopimarane-type

(Akowuah *et al.*, 2005; Nguyen *et al.*, 2004). Most of the constituents present in Java tea are reported to be toxic, as revealed by their prooxidant, cytotoxic, genotoxic potentials and have inhibitory effects on proliferation of tumor and embryonic cells and on production of nitric oxide. However, some compounds have antioxidative properties. The cytotoxic and/or antiproliferative nature of the constituents of Java tea is obviously related to their prooxidant properties, since the same compounds (polyphenols, flavonoids, terpenes) could behave as both antioxidants and prooxidants, depending on concentration and free radical source. Sergediene *et al.* (1999) reported polyphenolic antioxidants to exhibit a dose-dependent toxicity against leukemic cells (HL-60) by accelerating the production of malondialdehyde formation. Flavonoids, may act as pro-oxidants that generate free radicals and as inhibitors of key enzymes involved in hormone metabolism. Furthermore, since these compounds cross the placenta, the unborn fetus may be at great risk (Skibola and Smith, 2000).

Diterpenes are reported to be membrane active, lipolytic agents in scleractinian corals (Aceret *et al.*, 1995). These compounds are also found to possess insecticidal activity against *Macaronesia fortunate*, *Heliothis armigera* and *Manduca sexta* (Herdrickson and Cardellina II, 1986; Coloma *et al.*, 1982). Diterpenes, like taxol (a cytotoxic alkaloid) are reported to cause, myelosuppression, especially neutropenia and other side effects such as sensory neurotoxicity (with

typical numbness, tingling and painful paresthesia in the extremities), mucositis, diarrhea, alopecia and hypersensitivity reactions. Miyata *et al.* (2004) found, diterpenes, in general, to possess inhibitory effects on tumor proliferation, mainly the mammary tumor cells. Okouchi *et al.* (2005) reported diterpenes (Kansuinin and ingenol) to inhibit the proliferation of isolated embryonic cells from *Xenopus* embryos. These compounds were also found to inhibit proliferation of certain mammalian cell lines (MMT and LC540). Parthenin, a sesquiterpene lactone has been found to possess allergenic and irritant action. In a battery of bioassays, it has been reported to induce chromosomal aberrations, mainly chromatid breaks, in blood lymphocytes. It is also reported to cause nuclear alterations, such as pycnosis, micronuclei, karyorrhexis and polyploids (Ramos *et al.*, 2002). Staminane- and isopimarane-type diterpenes from Java tea were found to inhibit the nitric oxide production in lipopolysaccharide-activated macrophages. However, since endogenous nitric oxide is an important functional mediator in several physiological systems, including the reproductive processes (sexual competence in male and ovulation, implantation, pregnancy maintenance in female), the inhibition of nitric oxide production could interfere with these physiological functions (Rosselli *et al.*, 1995; Maul *et al.*, 2003; Ratnasouriya *et al.*, 2004). In a study on growth and fertility in rats, researchers found that prolonged inhibition of nitric oxide exhibited decreased neonatal weight, postnatal growth and fertility (Witlin *et al.*, 2002).

On the basis of the above facts the present study was designed to assess the reproductive toxicity of Java tea in Swiss albino mice, in view of its:

- Immense folkloric relevance
- A paucity of literature on reproductive toxicity of Java tea (as a whole product)
- Reported toxic nature of its constituents

MATERIALS AND METHODS

Test herbal product: *Orthosiphon stamineus* (Java tea) was used as the test herbal product in the present study. It was obtained in form of capsules. Each capsule contained 126 mg of the leaf extract of Java tea, which was titrated and of high quality. It is manufactured by Boiron Laboratories, France and distributed by Dallah Healthcare Company, Riyadh, Saudi Arabia.

Animal stocks: Male and female mice (Swiss albino, bred at Experimental Animal Care Center, College of Pharmacy, King Saud University, Saudi Arabia) aged 6-8 weeks, weighing 25-30 g were used. The animals were assigned to different groups of treatment and were housed in polypropylene cages maintained under standard conditions of temperature (23±1°C), light/dark

cycle (12/12 h) and relative humidity. The animals were given free access to standard laboratory animal feed and water *ad libitum*. All procedures using animals were reviewed and approved by the Institutional Animal Ethical Committee.

Treatment regimen: The experimental groups of mice consisted of the following: group I, control (tap water 0.3 mL/mouse/day); group II, Java tea (62.5 mg/kg/day); group III, Java tea (125 mg/kg/day); group IV, Java tea (250 mg/kg/day). The doses selected to conduct the studies were based on the MTD values and literature reports (Chan *et al.*, 1986). Aqueous suspension of Java tea was administered by gavage (oral) daily for a period of 90 days. The protocol of sub-chronic treatment adopted in reproductive and genetic toxicity was according to methodology of individual parameter. The MTD for Java tea was determined to be 4.03 g/kg. The doses selected for sub-chronic study were 62.50, 125 and 250 mg/kg/day, corresponding to 1/64, 1/32 and 1/16th of MTD.

After the last treatment, all the animals were sacrificed. From each animal, blood was aspirated and centrifuged for plasma. Testes were quickly excised and stored at -80°C until analysis. Hormones levels were estimated in plasma. Nucleic acids (RNA and DNA), total proteins, MDA and NP-SH concentrations were estimated in testicular cells.

Evaluation of spermatozoa motility, count and abnormalities: The spermatozoa were obtained by making small cuts in caudae epididymis and vas deferens placed in 1 mL of modified Krebs Ringer-bicarbonate buffer (pH 7.4). The sperm suspension was evaluated for sperm content, percent motility. The percent motility was determined by the progressive and non-progressive movements of sperms observed under a compound microscope (Anderson *et al.*, 1983). The sperm count was determined under a Neubauer haemocytometer (Al-Shabanah, 1997). To evaluate the spermatozoa abnormalities, the sperm suspension was stained with eosin; smears were made on slides, air-dried and made permanent. Coded slides were examined by bright field microscope with an oil immersion lens. The different spermatozoa abnormalities screened were amorphous, banana shaped, swollen achrosome, triangular head, macrocephali and rotated head screened (Al-Majed *et al.*, 2006).

Cytological analysis of germ cells: The protocol described in a study of chromosomal aberrations in the testis (Al-Shabanah, 1997) was followed. The mice were killed after the last day of the treatment, testes were removed in an isotonic sodium citrate solution and the seminiferous tubules were teased to form a cell suspension. The suspension was centrifuged and the pellet re-suspended in the hypotonic citrate solution.

After the second centrifugation the supernatant was discarded and the pellet suspended in a fixative (methanol and acetic acid, 3:1). The chromosomal preparations were made by the air drying technique. The coded slides were stained in Giemsa solution and screened for the aberrations including aneuploids, autosomal univalents, sex-univalents and polyploids.

Studies on rate of pregnancy and mean implants per female mouse: The methods described in male anti-fertility study and dominant lethal assay (Al-majed *et al.*, 2006) were followed to evaluate the:

- Rate of fertility in male mice
- Induction of pregnancy
- Total and pre-implantation loss
- Embryo-toxicity

After the treatment, each male mouse in the treated and control groups was caged with three females, which were allowed to stay with the male for 1 week. The female mice were necropsied 13 days following the mid-week of their caging and presumptive mating and the number of pregnant mice was recorded to determine percent fertility. The pre-implantation loss was calculated by comparing the number of implantations per pregnant female in the treated and control groups. The dead implants per pregnant female were determined to obtain the post-implantation embryonic loss.

Estimation of pituitary gonadal hormones in the plasma: The plasma samples were analyzed to determine the concentrations of human Chorionic Gonadotropin (hCG), Progesterone, Leutenizing Hormone (LH), Follicle Stimulating Hormone (FSH), estradiol, prolactin and testosterone. The analysis was carried out by direct immunoenzymatic colorimetric method based on ELISA. The protocol used for each hormone was according to the methods described for the particular kit (DIA.METRA, Italy).

Determination of MDA concentrations: The method described by Ohkawa *et al.* (1979) was used to determine the MDA concentration in testes tissue. Testes were homogenized in TCA solution and the homogenate suspended in thiobarbituric acid. After centrifugation the optical density of the clear pink supernatant was read at 532 nm. Malondialdehyde b is (dimethyl acetal) was used as an external standard. Results were expressed as nmol MDA/g wet tissue.

Quantification of NP-SH levels: The method described by Sedlak and Lindsay (1968) was used to determine the levels of NP-SH. The testes were homogenized in ice-cold Ethylenediaminetetraacetic Acid disodium (EDTA) (0.02 M) and mixed with TCA. The homogenate was centrifuged at 3000×g. The supernatant was suspended in tris buffer, 5-5'-dithiobis-

(2 nitrobenzoic acid) (DTNB) and read at 412 nm against reagent blank with no homogenate. Results were expressed as nmol NP-SH/100 mg wet tissue.

Determination of nucleic acids: The method described by Bregman (1983) was used to determine the levels of nucleic acids. Tissues were homogenized and the homogenate was suspended in ice-cold Trichloroacetic Acid (TCA). After centrifugation, the pellet was extracted with ethanol. DNA was determined by treating the nucleic acid extract with diphenylamine reagent and reading the intensity of blue color at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol and the green color was read at 660 nm. Standard curves were used to determine the amounts of nucleic acids present.

Statistical analysis: The different studies undertaken were statistically analyzed by One way ANOVA and post hoc Tukey-Kramer multiple comparisons. Some parameters in studies on reproductive toxicity were analyzed using a Chi-square (χ^2) test.

RESULTS

Effect of Java tea on sperm motility and count: The sub-chronic treatment with Java tea caused a significant ($p < 0.05$) reduction in the sperm count and their motility at the higher dose as compared to control animals. Significant ($p < 0.05$) increase was found in screened (more than 5,000) sperms abnormalities in mice treated with Java tea for three months compared to control group or mice. Banana shaped and swollen achro some sperms were screened significantly ($p < 0.05$) higher in mice treated with higher dose (250 mg/kg/day) of Java tea for 90 days (Table 1).

Effect of java tea on testes chromosomes: The sub-chronic treatment with Java tea induced a significant increase in the frequency of aneuploids ($p < 0.05$), autosomal univalent ($p < 0.05$), sex-univalents ($p < 0.05$), polyploids ($p < 0.05$) at the higher dose (250 mg/kg/day). In total of around 5,000 screened chromosomal stages, more than 20% of them had abnormalities and that found significantly ($p < 0.01$) higher as compared to control group (Table 2).

Effect of java tea on pituitary-gonadal hormones: Mean plasma hormones, estradiol and testosterone levels were significantly ($p < 0.05$) increased and decreased after the higher dose (250 mg/kg/day) treatment of Java tea for 90 days respectively. However, the other estimated hormone levels in plasma: Human-Chorionic Gonadotropin, Leutenizing hormone, Follicle-Stimulating Hormone and Prolactin were not significantly ($p > 0.05$) altered in male mice treated with different doses of Java tea for 90 days (Table 3).

Table 1: Effect of java tea on sperm motility, count and abnormality in swiss albino mice after sub-chronic treatment

Parameters	Control (tap water, 0.3 mL/mouse/day)	Java tea (62.50)	Java tea (125.00)	Java tea (250)
Percent sperm motility	90.00±2.24	86.80±2.59	85.00±2.54	80.00±3.16*
Sperm count (log N/mm ²)	4.878±0.02	4.876±0.06	4.819±0.03	4.778±0.04*
Percent sperm abnormalities				
Amorphous	0.45±0.07	0.40±0.09	0.68±0.11	0.68±0.13
Banana shaped	0.51±0.09	0.55±0.09	0.72±0.15	0.80±0.09*
Swollen achrosome	0.37±0.08	0.56±0.16	0.57±0.11	0.70±0.10*
Triangular head	0.35±0.05	0.41±0.07	0.49±0.09	0.42±0.42
Macrocephali	0.35±0.09	0.44±0.12	0.45±0.05	0.63±0.17
Rotated head	0.15±0.02	0.17±0.04	0.21±0.07	0.22±0.05
Total abnormalities	2.21±0.36	2.50±0.52	3.20±0.46	3.90±0.54*
Total sperms screened	5108	5455	5252	5488

Values are means±S.E. (n = 6); Significant differences are indicated by *: p<0.05, **: p<0.01 and ***: p<0.001 when compared with control animals (one-way ANOVA and post hoc Tukey-Kramer multiple comparison test was done individually for different parameters)

Table 2: Effect of java tea on testis chromosomes in swiss albino mice after sub-chronic treatment

Different chromosomal abnormalities screened/total	Control (tap water, 0.3 mL/mouse/day)	Java tea (62.50)	Java tea (125.00)	Java tea (250)
Aneuploids	3.54±0.50	4.37±0.75	5.34±0.83	7.44±1.20*
Autosomal univalents	1.39±0.25	2.15±0.66	1.87±0.43	4.08±0.86*
Sex-univalents	1.38±0.27	1.82±0.49	2.52±0.62	4.23±0.92*
Polyploids	4.36±0.56	5.54±1.10	5.90±1.12	7.61±1.00*
Total-percent aberrations	10.68±1.33	13.89±2.15	17.63±3.02	22.24±2.50**
Total stages screened	510	520	520	480

Values are means±S.E. (n = 6); Significant differences are indicated by *: p<0.05, **: p<0.01 and ***: p<0.001 when compared with control animals (one-way ANOVA and post hoc Tukey-Kramer multiple comparison test was done individually for different parameters)

Table 3: Effect of java tea on certain pituitary-gonadal hormones in plasma of male swiss albino mice after sub-chronic treatment

Parameters	Control (tap water)	Java tea (62.5 mg/kg)	Java tea (125 mg/kg)	Java tea (250 mg/kg)
Human-chorionic gonadotropin	1.25±0.26	0.99±0.36	1.08±0.31	1.45±0.30
Leutenizing hormone	1.83±0.12	1.78±0.10	1.68±0.08	2.10±0.14
Follicle-stimulating hormone	1.65±0.27	1.90±0.10	1.29±0.06	2.00±0.15
Estradiol	0.30±0.04	0.27±0.06	0.31±0.09	0.48±0.05*
Prolactin	0.50±0.17	0.83±0.20	0.74±0.30	0.86±0.09
Testosterone	12.83±1.13	15.90±2.60	11.00±1.13	8.75±1.00*

Values are means±S.E. (n = 6); Significant differences are indicated by *: p<0.05, **: p<0.01 and ***: p<0.001 when compared with control animals (one-way ANOVA and post hoc Tukey-Kramer multiple comparison test was done individually for different parameters)

Table 4: Effect of java tea on fertility index in male and female mice after sub-chronic treatment in male swiss albino mice

Treatment and dose (mg/kg)	Fertility index in male mice			Fertility index in female mice		
	Mated mice	Fertile mice	Fertility %	Mated mice	Fertile mice	Fertility %
Control (0.3 mL tap water/mouse)	10	10	100	10	9	90
Java tea (62.50 mg/kg)	10	9	90	10	8	80
Java tea (125 mg/kg)	10	9	90	10	7	70
Java tea (250 mg/kg)	10	7	70*	10	6	60*

Values are means±S.E. (n = 6); Significant differences are indicated by *: p<0.05, **: p<0.01 and ***: p<0.001 when compared with control animals (one-way ANOVA and post hoc Tukey-Kramer multiple comparison test was done individually for different parameters)

Table 5: Effect of java tea on the induction of dominant lethal mutations after sub-chronic treatment in male swiss albino mice

Treatment and dose (mg/kg, body weight/ day)	Mating week 1				Mating week 2				
	Pregnant females	Mean implants/pregnant female±S.E.			Pregnant females	Mean implants/pregnant female±S.E.			
		Total	Live	Dead embryos		Total	Live	Dead embryos	
Control (tap water)	22/29 (75.86)	8.21±0.75	7.93±0.82	0.28±0.11	8/232 (3.45)	24/30 (80.00)	8.73±0.83	8.43±0.81	0.30±0.12
Java tea (62.50)	23/28 (82.14)	7.39±0.72	7.07±0.69	0.32±0.09	9/207 (4.35)	25/30 (83.33)	7.50±0.69	7.10±0.65	0.40±0.13
Java tea (125.00)	24/30 (80)	7.07±0.69	6.73±0.67	0.33±0.14	10/212 (4.72)	21/30 (70.00)	6.80±0.88	6.27±0.79	0.53±0.14
Java tea (250.00)	22/30 (73.33)	5.90±0.68*	5.56±0.65*	0.40±0.12	12/179 (6.70)	18/30 (60.00) ^	6.00±0.86*	5.17±0.84*	0.83±0.19*

Figures between parentheses denote percent; A total of 10 male and 30 females were used in each group; *: p<0.05 (one way ANOVA and post hoc Tukey-Kramer multiple comparison test); ^: p<0.05; ^^: p<0.001 (chi square test)

Effect of java tea on fertility index in male and female rats: Fertility index of male and female mice after sub-chronic treatment of Java tea by different doses was estimated and showed in Table 4: Three months treatment with Java tea (250 mg/kg/day) to male Swiss albino mice caused 30% decreased in fertility rate, that found significantly (p<0.05) less

compared to control group of mice. Higher than male mice (40%) infertility was seen in females after Java tea treatment for 90 days and that also found significantly (p<0.05) higher compared to control mice.

Effect of java tea on rate of pregnancy and mean implants per female: Effect of Java tea on the

Table 6: Effect of java tea on MDA, NP-SH, total protein, RNA and DAN levels in testes of mice after sub-chronic

Treatment and dose (mg/kg, body weight)	MDA (nmol/g tissue)	NP-SH (nmol/100 mg tissue)	Total proteins (mg/100 mg tissue)	RNA (μ g/100 mg tissue)	DNA (μ g/100 mg tissue)
Control (tap water)	195.00 \pm 7.30	127.59 \pm 4.55	10.98 \pm 0.27	347.08 \pm 10.34	231.91 \pm 8.23
Java tea (62.5)	191.83 \pm 8.80	119.70 \pm 3.74	11.08 \pm 0.31	343.98 \pm 8.01	226.27 \pm 6.14
Java tea (125)	213.53 \pm 9.46	120.42 \pm 4.26	10.56 \pm 0.32	331.58 \pm 6.96	220.68 \pm 3.44
Java tea (250)	225.19 \pm 8.05*	112.52 \pm 3.27*	10.16 \pm 0.17	319.72 \pm 3.69*	205.59 \pm 6.93*

Values are means \pm S.E. ($n = 6$); Significant differences are indicated by *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$ when compared with control animals (one-way ANOVA and post hoc Tukey-Kramer multiple comparison test was done individually for different parameters)

induction of dominant lethal mutations after sub-chronic treatment in male Swiss albino mice is depicted in Table 5. The sub-chronic treatment of male mice with Java tea significantly ($p < 0.05$) decreased the percent pregnant female mice at a dose of 250 mg/kg/day. At the same dose, there was a significant reduction ($p < 0.05$) in the mean total implants per pregnant female mice and mean live implants per pregnant female mice. A significant increase was found in the mean dead implants per pregnant female mice ($p < 0.05$) and the percent dead embryos ($p < 0.05$), as compared to the values obtained in the control mice.

Effect of java tea on MDA, NP-SH, nucleic acids and total proteins levels in testes: The sub-chronic treatment with Java tea (250 mg/kg/day) caused a significant ($p < 0.05$) increase in the testicle concentrations of MDA and decrease ($p < 0.05$) in NP-SH in mice compared to control group (Table 6). Nucleic acids (RNA and DNA) levels in testicular cells decreased significantly ($p < 0.05$) of mice treated with Java tea for 90 days. The above biochemical changes were also seen in lower doses (62.50 and 125 mg/kg/day) treated groups compared to controls.

DISCUSSION

During the last two decades lot of research work has been done on the medicinal effects of plants and their constituents. Several reports indicated towards various kinds of toxic effects of these plants (Ateyyat *et al.*, 2009; Bussmann *et al.*, 2011). Java tea is a widely used medicinal herb in the treatment of arteriosclerosis, kidney stones, nephritis, gout, diabetes and rheumatism (Mohamed *et al.*, 2012; Awale *et al.*, 2002; Akowuah *et al.*, 2005; Ameer *et al.*, 2012). Till date, chronic treatment of java tea has not been studied on male reproductive system. The general toxicity studies revealed its nontoxic effects and declared therapeutically safe (Han *et al.*, 2008; Muhammad *et al.*, 2011). However, in the present study the highest (250 mg/kg/day) treatment for 90 days found its toxic effects on reproductive system in mice.

Present data revealed the increase of estradiol and decrease of testosterone in the plasma of male mice indicates towards the endocrine logical effects of Java tea in mice model. These results support our finding on the fertility index of male and female mice, which showed that the fertility in male and female mice was significantly reduced. Java tea was found to reduce

sperm motility and count along with significant increase of abnormal sperms morphology and it further confirmed by reduction in fertility in high dose treatment (250 mg/kg/day) of Java tea. The reduction in fertility index in the male mice may be related with the increased number of abnormal shapes of the spermatozoa. These effects are probably due to pro-oxidant nature of Java tea and the results are confirmed by our observation of the biochemical changes in testes, which showed an increase of the levels of MDA and depletion of NP-SH, RNA and DNA. Earlier studies also found oxidative stress to be linked with DNA damage in the testis, induction of abnormal sperms and effect on fertility in mice (Rajesh *et al.*, 2002).

Analysis in testes tissue showed significant increase of MDA and decrease of NP-SH after the sub-chronic treatment with Java tea. Increase in MDA level and reduction in NP-SH level in testes tissue after sub-chronic administration of higher dose of Java tea (250 mg/kg/day) indicate towards induction of oxidative stress and consequent damages. The reduction observed in the fertility of male mice and the abnormal shapes of the sperms observed might be related with the increased accumulation of free radicals. Previous studies, Habior (1992) and Suzuki *et al.* (1993) also showed that the depletion of glutathione cause spermatotoxicity and produce abnormal shapes of the sperms (Watanabe and Endo, 1991). Our results of dominant lethal assay showed decrease in the percent female mice mated to treated males, after sub-chronic treatment with Java tea. The total and live implants per pregnant female mice were significantly reduced, while the dead implants per pregnant female mice and the percent dead embryos were increased significantly. These results clearly showed the induction of pre- and post-implantation loss and reduction in fertility of male mice.

The toxic symptoms observed after the treatment of Java tea are attributed to the toxic constituents, including diterpenes of Java tea. Recent studies found diterpenes to cause, myelosuppression and other side effects such as sensory neurotoxicity (with typical numbness, tingling and painful paraesthesiae in the extremities), mucositis, diarrhea, alopecia and hypersensitivity reactions. Okouchi *et al.* (2005) reported that, diterpenes to inhibit the proliferation of isolated embryonic cells from *Xenopus* embryos. The exact mechanism of the Java tea-induced toxicity is not known, however, these changes might be related with the genesis of free radicals by the prooxidant constituents of Java tea, such as terpenes and

diterpenes. These constituents have been found to induce chromosomal aberrations in blood lymphocytes. They also caused nuclear alterations, such as pycnosis, micronuclei, karyorrhexis and polyploids (Ramos *et al.*, 2002). The treatment with Java tea was also found to induce significant changes in the chromosomal aberrations after sub-chronic treatment. The induction of chromosomal aberrations might be related to the oxidant constituents present in Java tea, such as terpenes and diterpenes (Galati and O'Brien, 2004). These constituents have been found to induce chromosomal aberrations in blood lymphocytes.

In conclusion, the results of the present study revealed that, Java tea has toxic effects on reproductive system of male mice which affect the fertility after long term administration at high doses. The study paves a path to further investigate the role of Java tea in toxic clinical manifestations.

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