Journal of Medicinal Plants Studies

www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
JMPS 2018; 6(5): 07-14

Received: 02-07-2018
Accepted: 03-08-2018

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Male reproductive toxicity studies of *Orthosiphon stamineus* aqueous extract in Sprague Dawley rats

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Abstract
A dose-response study was designed to assess toxicity effects of standardized *O. stamineus* aqueous extract (OSAE) in adult male Sprague Dawley rats via oral gavage for 60 days. Animals were observed for clinical signs. Body weight gain, food and water consumption and reproductive performance were measured. Animals were sacrificed and blood samples were withdrawn for hematological analysis. Vital and reproductive organs weight, sperm concentration and morphology were collected and evaluated. All animals survived and did not show signs of toxicity. No significant changes on food and water uptake, body and organs weight in all groups. The number of implantation, sperm concentrations, morphology, resorptions and lives fetuses was not statistically significant. Levels of red blood cells, haematocrit, haemoglobin and platelet were significantly higher in rats received 2000mg/kg body weight of extract. The OSAE did not cause toxicity effects, rather, it stimulate erythropoiesis in rats and need further investigation.

Keywords: *Orthosiphon stamineus,* reproductive, hematology, Sprague Dawley

Introduction
Malaysia is a country richly blessed with diverse range of medicinal plants, which has the potential to support a wide range of industrial activities that produces traditional and herbal medicines leading to development of pharmaceutical drugs. Along to this development, toxicity evaluation is important in ensuring the safe consumption of these products by the population.

Orthosiphon stamineus (*O. stamineus*), Benth, (Laminaceae) also known as 'Misai kucing' is a popular medicinal plant in Southeast Asia. In Malaysia, the leaf of *O. stamineus* is traditionally prepared as tea to improve health and for treatment of kidney bladder inflammation, epilepsy, gout, reducing blood sugar, eruptive fevers, hepatitis, high blood pressure, syphilis, rheumatism and gonorrhoea (Akowuah *et al*., 2004; Ameer *et al*., 2012) [1, 2]. Phytochemical investigations demonstrated the presence of methoxylated flavones (sinensetin and eupatorin) and phenolic acids (rosmarinic and caffeic acids) in *O. stamineus* leaf extracts (Muhammad *et al*., 2011; Ameer *et al*., 2012) [3, 2]. Few studies have reported that the rosmarinic acid, a major compound of OSAE, exhibit antioxidant, immuno-modulatory and anti-cancer activity (Scheckel *et al*., 2008; Yam *et al*., 2009; Ameer *et al*., 2012) [4, 5, 2].

Although the *O. stamineus* has shown various pharmacological properties, the safety information of this plant and its compounds is not well established. The acute toxicity study of *O.staminues* standardised extract showed no signs of toxicity in Sprague Dawley (SD) rats after orally treated at a single dose of 5000 mg/kg body weight (Abdullah *et al*., 2009) [6]. However, there is no data available on repeated administration of *O. stamineus* for short and long term exposure. Our research group first reported that the OSAE no maternal toxicity effects on pregnant rats after 20 days treatment at 2000 mg/kg body weight/day (Muhammad *et al*., 2013) [7].

Given that there are inadequate studies regarding the toxicological profile on male reproductive system, a repeated dose toxicity study was conducted to evaluate the effect of OSAE in male SD rats by investigating the reproductive systems and performance.

Materials and methods

Plant material

The fresh sample plants of *Orthosiphon stamineus* Benth (Lamiaceae) was purchased from Herbs Bagus (Penang, Malaysia). The whole plants were dried for the preparation of standardized extract and phytochemical analysis.

Preparation of OSAE

Dried aerial parts were ground to form homogeneous powder and left to stand in water at 70 ºC for 30 min. The plant infusion was then filtered, evaporated and concentrated. The resulting concentrated liquid extract was spray-dried at 180 ºC (outlet temperature) and 100ºC (inlet temperature) producing a powder that was further used in the experiments.

Chemicals

Formic acid, methanol and LCMS grade acetonitrile were purchased from MERCK (Malaysia). HPLC grade water was prepared from distilled water using a Milli-Q-system (Millipore, MA) and was used during analytical UHPLC analysis. Rosmarinic acid and kaempferol were purchased from Chromadex (USA) and eupatorine and sinensetin were from (Genay France.) All of other solvents and chemicals used in this study were of analytical grade. Stock and working standards of were prepared by dissolving these analytes in 100% methanol. The standard solutions stored at 4 °C were stable for at least 3 months.

Identification of phenolic compounds in OSAE using UHPLC–MS-ESI analysis

The UHPLC system was performed on a Dionex 3000 UHPLC system acquired from Thermo Fisher Scientific (USA) that consisted of an autosampler equipped with a column oven, a tray compartment cooler, and a binary pump with built in solvent degasser. The chromatographic separation was performed on a BEH C18 UHPLC column, 100 mm x 2.5 µm, 1.7 µm (WATERS) at a flow rate of 0.2 mL/min. The mobile phases used were (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The gradient started with 15 % mobile phase B, reaching 50% mobile phase B at 20 min, at isocratic elution of 90% B for 3 min. The gradient reached the initial conditions were held for 2 min as a re-equilibration step. The injection volume was 10 µL and the column temperature was maintained at 40 °C.

The UHPLC system was coupled to a linear ion-trap-Orbitrap mass spectrometer Q Exactive from Thermo Fisher Scientific (U.S.A) equipped with an electrospray ionization (ESI) source. The mass detection was performed in a range of 150-1500 m/z. The ESI source was operated in negative ion mode under the following specific conditions: source voltage, 3.2kV; sheath gas, 35 arbitrary units; auxiliary gas, 15 arbitrary unit; sweep gas, 10 arbitrary unit; and capillary temperature, 320°C. Nitrogen (> 99.98%) was employed as sheath gas, auxiliary and sweep gas. Instrument control and data acquisition were performed with Chameleon 6.8 software and Xcalibur 2.2 software (Thermo Fisher Scientific).

Animals

Fifty healthy males and 100 females Sprague Dawley (SD) rats with a body weight ranging from 200-250g and 180-200g respectively were obtained and quarantined in the Animal Resource Unit, Medical Resource Centre, Institute for Medical Research. Animals were housed in individual ventilated (IVC) cages, lined with corn cob and at controlled temperature (20± 2 ºC), with 40-60% humidity under 12 hours of light and dark cycle. The animals were acclimatized for a week prior to start of the study. Commercial rat diet (Specialty Feeds, Australia) and water were available ad libitum. Approval for this study was obtained from the Animal Care and Use Committee, Ministry of Health Malaysia (ACUC No: ACUC/KKM/02(3/2014).

Experimental design

This study was performed according to OECD Guideline for Testing of Chemicals 421, Reproduction/ Developmental Toxicity Screening Test with few adaptations [8]. Animals were randomly assigned into five groups which consist of 10 males each. Four different concentrations of OSAE (250, 500, 1000 and 2000 mg/kg body weight) and a control (distilled water) were used in this study. The chosen dose was based on the acute, prenatal developmental and genotoxicity studies, which were previously conducted, in our laboratory (Muhammad *et al*., 2011, 2013; Abdullah *et al*., 2009) [3,6,7]. The OSAE were freshly prepared and administered daily by oral gavage while control group received distilled water for 60 days.

Clinical observations

Once a day, after treatment, the rats were observed in their cages for 60 min, and behavioural changes and clinical signs of toxicity were recorded.

Body weight, food and water intake

Body weights were measured and recorded daily. Food and water intake (on a weekly basis) were measured and recorded.

The assessment of male reproductive performance

After 60 days of treatments, the treated adult males were mated with untreated females (1:2) for 20 minutes daily for 5 days. The day when sperms were detected in the vaginal smear was considered as day 0 of pregnancy. The pregnant female rats were individually caged. On day 21 of pregnancy, the rats were euthanized and caesarean section was performed. The gravid uterus was removed and its contents were weighed. The implantation sites were determined by the method of Salewski (1964) [9]. Resorptions as well as living and dead foetuses were counted. Foetuses that were removed by cutting the umbilical cord close to their bodies were numbered and examined for externally visible abnormalities under a stereomicroscope. The sex of the foetuses was determined.

Haematological analysis

The male rats that had successfully impregnated the female rats were humanely sacrificed by diethyl ether anaesthetization. Blood collected by cardiac puncture was separated into two different tubes containing EDTA for the haematological analysis and plain tube for whole blood analysis. The measured hematological values were haemataocrit (HCT), haemaglobin concentration (HB), erythrocyte counts (RBC), total and differential leucocyte count (WBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet.

Vital organs and sex accessory organ weights

During necropsy, the liver, kidneys, lungs, adrenals, heart, testes, epididymis, prostate and seminal vesicles were harvested. Fat and connective tissue were removed. The organs were examined for gross pathology abnormalities,

weighed and recorded. The seminal vesicles were weighed without fluid. Organs weights were reported as absolute and relative weights (organ weight/body weight x 100).

Sperm evaluation (Sperm concentration, Daily sperm production (DSP), Efficiency of DSP, Epididymis transit time and Sperm morphology)

After removal of the tunica albuginea, the right testis were minced and homogenized in 10 ml of 0.9% NaCl containing 0.5% Triton X-100 at medium speed using homogenizer for 1 minute. After dilution (1/10), the number of homogenization resistant spermatids was counted in a hemocytometer for sperm concentration measurement. The number of homogenization-resistant spermatids obtained was divided by 6.1 to convert them to daily sperm production (DSP) calculation (Robb *et al*., 1978) [10].

The cauda epididymis was cut into small pieces, homogenized and spermatozoa were counted as described above. The epididymal sperm transit rate was calculated for each male rat by dividing the epididymal sperm number by the DSP (Amann *et al*., 1976) [11].

To assess the percentage of morphologically abnormal sperm, the ductus deferens were rinsed with 0.5ml 0.9% NaCl to obtain a sperm suspension. Aliquots of the sperm suspension were stained with Congo red (10 seconds) and Crystal violet (30 seconds). Two-hundred sperms per animal will be analysed microscopically at 400x magnification and sperm with abnormal heads and/or abnormal tails were scored.

Statistical analysis

Data was analysed using SPSS 16.0. Mean between groups were analysed using one way ANOVA. Data were presented in mean and standard deviation (SD). Bonferroni post-hoc test was conducted when p value showed significant findings. Non-parametric data were analysed using Kruskal Wallis analysis. Dunnet T3 test were used when heterogenous data were detected. Data were presented in median and interquartile range (IQR). P value < 0.05 was considered as significant.

Results

Identification of phenolic compounds in OSAE using UHPLC–MS-ESI analysis

OSAE was analysed based on the accurate mass data of the molecular ions, in which ions detected were tentatively identified by their generated molecular formula, through the software Data analysis (Xcalibur) which provided list of possible elemental formulas, together with the use of standard when available and after thorough survey of the literature. The UHPLC-ESI analysis of OSAE revealed the presence of 18 phenolic compounds (Table 1) which list the peak number, retention time, observed m/z, the generated molecular formula and the proposed compound detected. Main compounds detected in OSAE are illustrated in Figure 1.

Table 1: Identification of phenolic compound of *O. stamineus* by UHPLC-MS

Peak No	Retention Time (min)	Compound	[M-H]-	Formula	Error (ppm)
1	0.53	Malic acid	133.01389	$C_4H_5O_5$	5.565
2	0.68	Citric acid	191.0907	$C_6H_7O_7$	2.309
3	1.09	Syringic acid	197.04500	$C_9H_9O_5$	2.792
4	1.14	Protocatechuic acid	153.01926	$C_7H_5O_4$	6.698
5	1.15	Vanilllic acid	167.7101	$C_9H_11O_3$	4.425
6	1.16	Caftaric acid	311.04095	$C_{13}H_{14}O_9$	3.831
7	1.25	Chlorogenic acid	353.0887	$C_{16}H17_7O_9$	6.122
8	1.31	Salvianolic acid C	491.08463	$C_{22}H_{29}O_{15}$	5.321
9	1.07	Dashensu	389.09869	$C_{18}H_{19}O_{10}$	3.586
10	1.41	Kaempferol-3-rutinoside	593.1528	$C_{27}H_{29}O_{15}$	4.608
11	1.50	Caffeic acid	179.03436	$C_9H_7O_4$	2.652
12	2.08	p-coumaric acid	163.04001	$C_9H_7O_3$	6.375
13	2.17	Chicoric acid	473.07370	$C_{22}H17_7O_{12}$	4.751
14	4.03	Rosmarinic acid	359.07782	$C_{18}H_{15}O_8$	4.668
15	4.06	Ferulic acid	193.04993	$C_{10}H_9O_4$	2.045
16	4.38	Salvianolic acid A	493.011539	$C_{19}H_{25}O_{15}$	-6.908
17	5.45	Eupatorin	343.08307	$C_{18}H_{16}O_7$	5.453
18	5.87	Sinensetin	373.09375	$C_{19}H_{17}O_8$	5.243

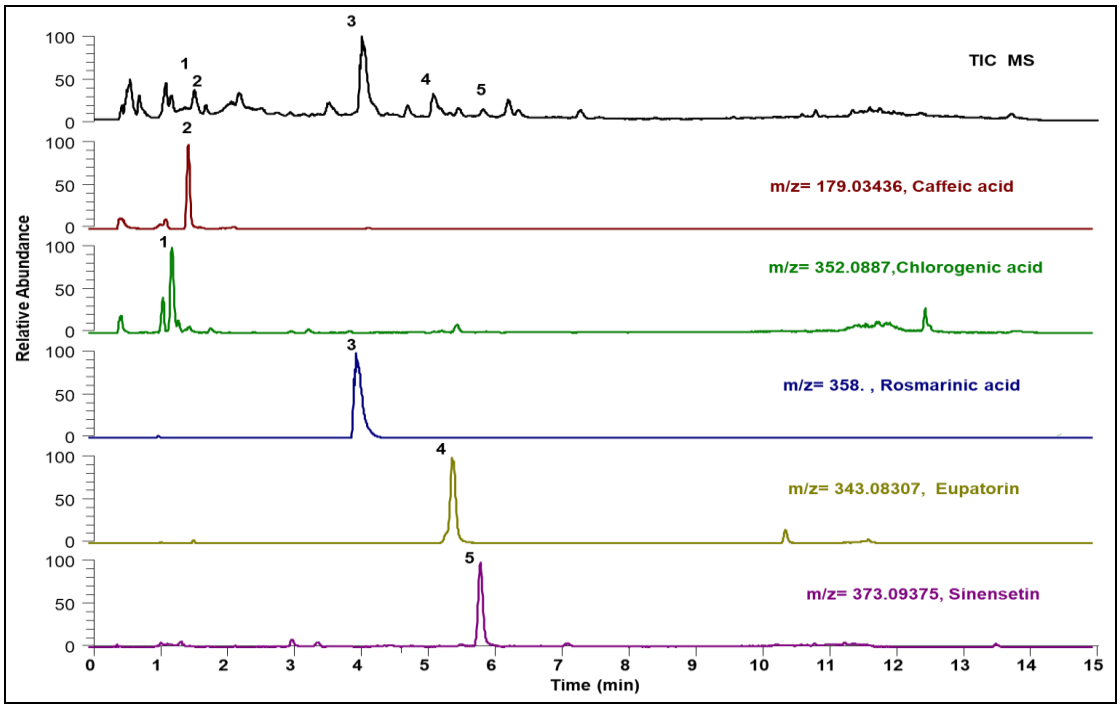


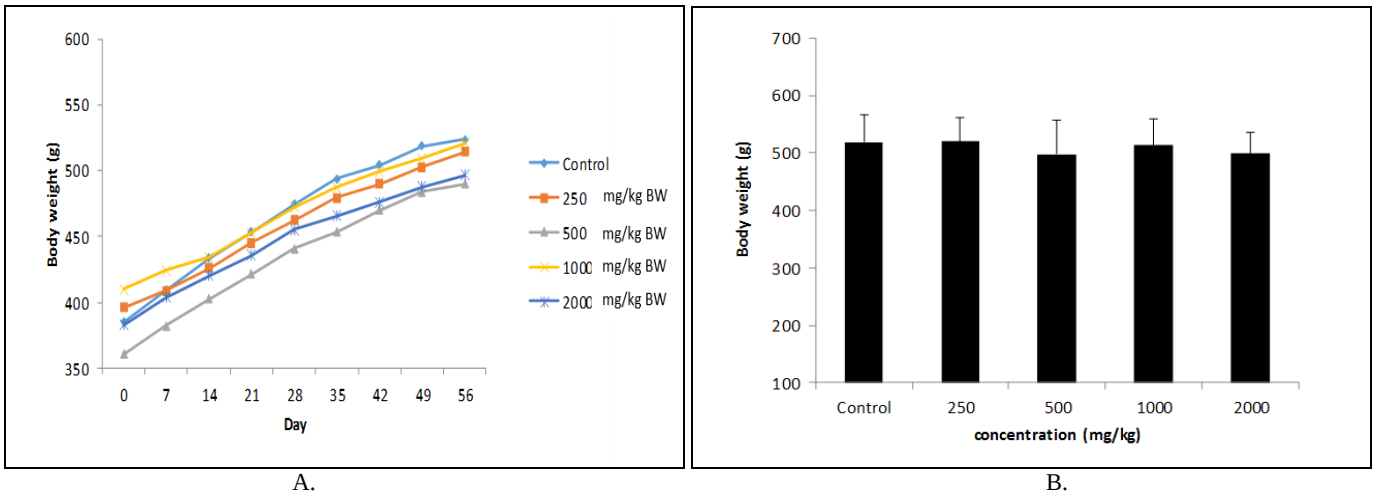
Fig 1: Representative UHPLC-MS traces of OSAE in negative ion mode. Assigned peaks number labelled the chemical marker found in this plant.

Clinical observations

All control and treated male rats survived to the scheduled date of the sacrifice. No behavioural alterations and no clinical signs of toxicity (diarrhea, piloerection, and changes in behaviour) were observed among treated animals.

Body weights, water and food intake

Figure 2 shows body weight of animals following 60 days of administration of OSAE. There were no statistically significant differences in the body weight of the animals between the different concentrations of OSAE-treated and control groups. However, the average body weights of rats in control group were slightly higher than treated groups throughout the study period. No significant changes on the water and food intake throughout the experiments in all groups (Data not shown).



A. B.

Fig 2: The effects of OSAE at different concentrations on the body weight of rats after 60 days oral administration A) The growth curve of body weights; B) The body weights of rats at necropsy. Values are expressed as mean ± SD.

The assessment of male reproductive performance

All sperm positive females were pregnant as all treated dams have implantation sites in their uteri at the caesarean section. The results in Table 2 showed that the administration of OSAE, in the dosage up to 2000 mg/kg body weight, has not significantly changed the mean numbers of implantation sites per litter as compared to controls groups. The occurrence of early and late resorptions was low in control and treated groups and the median percentage of resorptions per litter was not altered by treatment with OSAE. The average number of live fetuses per litter was similar in controls and treated groups. Taken together, data on resorption rates and mean number of live fetuses at term consistently showed that administration of OSAE to male rats before mating did not cause any increase of post-implantation losses over the incidence recorded in the control group.

Table 2: The effect of OSAE on reproductive performance in male Sprague Dawley rats

Parameters measured	OSAE (mg/kg body weight)				
	Control	250	500	1000	2000
Implantation/litter	12.75(2.38)	12.25(1.25)	12.00(1.25)	11.00(3.75)	9.78(6.75)
Live fetuses/litter	11.75(3.38)	11.50(2.25)	11.75(3.15)	10.00(3.15)	10.00(6.75)
Early resorption/litter	0.0(1)	0.75(1)	0.0(0.38)	1.0(0.75)	0.50(0.75)
Late resorption/litter	0(0)	0(-0.13-33)	0(-0.7-0.69)	0(-0.88-2.28)	0(-0.15-0.93)
Gross abnormalities on fetuses	NSF	NSF	NSF	NSF	NSF

Values are expressed as median (inter quartile range).n=10 animals/group.
NSF: No significant finding

Hematological analysis

As shown in Figure 3, there was no significant difference in the hematological parameters compared to the control group except a significant increase of the total red blood cell (RBC), hematocrit (HCT), hemoglobin (HB) and platelet (PLT) in male rats of 2000 mg/kg group ($p < 0.05$).

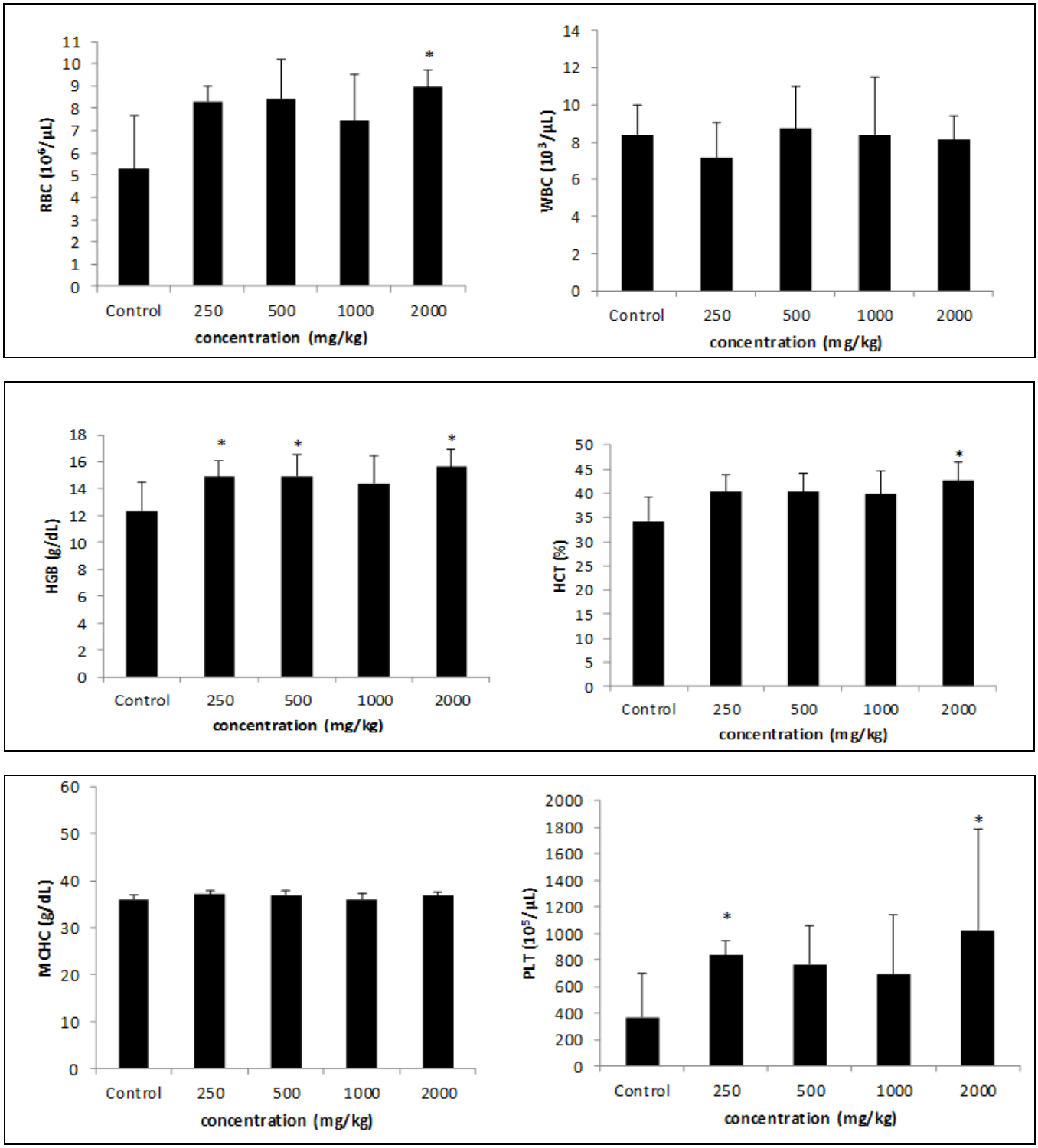


Fig 3: The effects of OSAE at different concentrations on the level of (a)RBC; (b)WBC; (c)HGB; (d)HCT;(e) MCHC;(f)PLT in male SD rats after 60 days oral administration. Values are expressed as mean ± SD. *Significant difference $p < 0.05$ as compared with control group

Vital organs and sex accessory organ weights

At the necropsy, no gross pathology alterations were found in OSAE treated males. Table 3 shows the absolute and relative weight of the vital organs (liver, heart, lungs, kidneys, adrenals, testes, prostate gland and seminal vesicles) and did not differ between treated and control rats. Reproductive organs weight is presented in Table 4. There were no significant changes of both absolute and relative organ weight in testes, seminal vesicles and prostate in all groups.

Table 3: The effect of OSAE on vital organs weight in male Sprague Dawley rats

Organs	OSAE (mg/kg body weight)				
	Control	250	500	1000	2000
Absolute organ weights (g)					
Liver	17.26 ± 0.32	16.63 ± 1.88	16.38 ± 2.33	15.69 ± 0.20	16.29 ± 1.92
Lung	1.95 ± 0.32	1.90 ± 0.17	1.88 ± 0.21	1.78 ± 0.20	1.82 ± 0.18
Heart	1.40 ± 0.19	1.35 ± 0.11	1.37 ± 0.13	1.40 ± 0.12	1.41 ± 0.18
Kidney (right)	1.55 ± 0.18	1.60 ± 0.12	1.48 ± 0.16	1.58 ± 0.15	1.51 ± 0.10
Kidney (left)	1.52 ± 0.20	1.55 ± 0.15	1.44 ± 0.15	1.59 ± 0.16	1.51 ± 0.11
Adrenal (right)	0.02 ± 0.01	0.02 ± 0.04	0.02 ± 0.004	0.02 ± 0.005	0.02 ± 0.003
Adrenal (left)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.002	0.02 ± 0.005	0.02 ± 0.006
Relative organ weights (g/100g BW)					
Liver	3.31 ± 0.36	3.20 ± 0.27	3.29 ± 0.33	3.09 ± 0.33	3.27 ± 0.33
Lung	0.37 ± 0.48	0.37 ± 0.05	0.38 ± 0.04	0.35 ± 0.05	0.37 ± 0.02
Heart	0.27 ± 0.40	0.26 ± 0.26	0.28 ±0.22	0.27 ± 0.26	0.28 ± 0.48
Kidney (right)	0.30 ± 0.37	0.31 ± 0.29	0.30 ± 0.27	0.31 ± 0.32	0.30 ± 0.28
Kidney (left)	0.29 ± 0.41	0.30 ± 0.35	0.29 ± 0.25	0.31 ± 0.35	0.30 ± 0.30
Adrenal (right)	0.004 ± 0.01	0.004 ± 0.01	0.004 ± 0.01	0.004 ± 0.01	0.004 ± 0.01
Adrenal (left)	0.004 ± 0.01	0.005 ± 0.02	0.005 ± 0.01	0.004 ± 0.01	0.004 ± 0.02

The absolute and relative organ weight of male rats treated with different concentrations of OSAE for 60 days. Values are expressed as mean ± SD. n=10 animals/group.

Table 4: The effects of OSAE on sex organs weight in male Sprague Dawley rats

Organs	OSAE (mg/kg body weight)				
	Control	250	500	1000	2000
Absolute organ weights (g)					
Testis (right)	1.80 ± 0.26	1.86 ± 0.12	1.80 ± 0.10	1.82 ± 0.16	1.86 ± 0.19
Testis (left)	1.80 ± 0.19	1.78 ± 0.16	1.80 ± 0.10	1.79 ± 0.16	1.86 ± 0.19
Cauda epididymis (right)	0.68 ± 0.08	0.67 ± 0.08	0.70 ± 0.07	0.66 ± 0.07	0.65 ± 0.08
Cauda epididymis (left)	0.67 ± 0.07	0.64 ± 0.07	0.70 ± 0.09	0.65 ± 0.07	0.65 ± 0.09
Seminal vesicle	1.29 ± 0.25	1.07 ± 0.23	1.15 ± 0.23	1.15 ± 0.11	1.10 ± 0.16
Prostate	0.88 ± 0.22	0.83 ± 0.22	0.87 ± 0.16	0.72 ± 0.11	0.73 ± 0.24
Relative organ weights (g/100g BW)					
Testis (right)	0.35 ± 0.56	0.36 ± 0.28	0.36 ± 0.17	0.35 ± 0.35	0.37 ± 0.52
Testis (left)	0.35 ± 0.41	0.34 ± 0.38	0.36 ± 0.17	0.35 ± 0.35	0.37 ± 0.44
Cauda epididymis (right)	0.13 ± 0.18	0.13 ± 0.20	0.14 ± 0.12	0.13 ± 0.15	0.13 ± 0.22
Cauda epididymis (left)	0.13 ± 0.15	0.12 ± 0.16	0.14 ± 0.15	0.13 ± 0.13	0.13 ± 0.23
Seminal vesicle	0.25 ± 0.53	0.21 ± 0.54	0.23 ± 0.39	0.23 ± 0.22	0.22 ± 0.44
Prostate	0.17 ± 0.46	0.16 ± 0.51	0.17 ± 0.28	0.14 ± 0.26	0.15 ± 0.64

The absolute and relative sex organ weight of male rats treated with different concentrations of OSAE for 60 days. Values are expressed as mean ± SD. n=10 animals/group.

Sperm evaluation (Sperm concentration, Daily sperm production (DSP), Efficiency of DSP, Epididymis transit time and Sperm morphology)

As shown in Table 5, there was no significant changes ($p>0.05$) observed in the sperm concentration of rats in all groups. Morphological analysis of sperm extracted from the vas deferens showed that the percentages of normal and abnormal forms were similar among the groups. The most common abnormality of the sperm was headless and bent tail and these defects were observed in both control and OSAE groups.

Table 5: The effect of OSAE on sperm parameters in male Sprague Dawley rats Values are expressed as mean ± SD. n=10 animals/group.

Parameters measured	OSAE (mg/kg body weight)				
	Control	250	500	1000	2000
Morphology Normal sperm	45±32.4	73.10±39.67	25.78±13.82	59.90±46.4	30.11±27.38
Abnormal sperm	155±32.4	126.9±39.37	173.89±13.97	140.55±45.67	169.78±27.45
Epididymal sperm count *(x10^6/sperm/ml)*	2.63±1.37	2.29±1.28	2.47±1.29	3.05±9.00	2.58±1.82
Spermatid count (testes) *(x10^6/spermatid/ml)*	6.72±3.10	7.23±3.00	7.65±3.76	9.87±5.48	7.48±3.42
Daily sperm production (*10^6/testis/day*)	1.10±5.08	1.19±4.91	1.25±6.16	1.62±8.99	1.23±5.61
Epididymal sperm transit rate	23.92±13.72	19.30±26.10	19.68±20.90	18.86±10.00	21.05±32.34

Discussion

The administration of OSAE also did not affect the weight of vital and reproductive organs in all rats. In general, no significant changes on the body weight gain as well as the absence of clinical signs of toxicity were observed throughout the course of administration in all groups. This observation may indicate that the OSAE did not induce any systemic toxicity and metabolic processes which may subsequently affect the body weight. A significant higher level of RBC, HGB, HCT and PLT was demonstrated in the animals from 2000 mg/kg body weight group. No significant difference in abnormal sperm morphology observed in control and treated rats, however, these changes did not significantly affect their reproductive performance as the number of fetuses without abnormalities and implantations after copulation were almost similar in all groups. Similar findings were observed on prenatal developmental studies where no embryolethality and growth retardation were observed on fetuses with no-observed adverse effect levels (NOAEL) at 1000 mg/kg body weight OSAE (Muhammad *et al*., 2013) [7].

A reduction in the weight of internal organs is a simple and sensitive indicator of toxicity after the exposure to toxic substances (Raza *et al*., 2002) [12]. The alteration in sperm parameters could be attributed to direct effect on testicular tissue which leads to reproductive dysfunction such as reduced sperm count, motility and morphology (de Souza Predes *et al*., 2010) [13]. In this study, none of the male rats treated with OSAE at 250, 500, 1000 and 2000 mg/kg body weight showed significant changes to both organ and sex organ weights suggesting that the extract has no toxicity effects on the internal organs. OSAE also did not disrupt the sperm production as is evident in the present study from observations of similar testicular weights in all groups. One of the possible reasons is because of cinnamics such as rosmarinic acid and caffeic acids present in the extract. This is keeping with the findings by Abd El Tawab (2014) [14] that rosmarinic and caffeic acids from *Satureja montana* increased the testicular weight of cyclophosphamide-induced testicular injury in rats.

The sperm count and percentage of sperm mortality are the most commonly assessed sperm parameters used in infertility evaluation (Venkatesh *et al*., 2009) [15]. However, the fertility of animals is still related to the morphological features of its spermatozoa (Ozturkler *et al*., 2001) [16]. There were no significant changes on the percentage of morphologically normal sperm although it was lowered predominantly due to higher number of sperm with abnormal tail morphology in both control and OSAE treated animals. The abnormalities on sperm morphology in this study may be caused by various possible sources associated with abnormal spermatozoa production. Consequently, these abnormalities did not affect the reproductive performance of male rats in copulating with healthy female rats even at the higher concentration (2000 mg/kg body weight) of OSAE. In contrast, findings by Abdulaziz A (2013) [17] demonstrated that the 90 days treatment of *O. stamineus* leaves extract in a capsule form at 250 mg/kg body weight to male Swiss albino mice has increased the abnormal sperm morphology and decreased in the sperm count hence reduced the total number and live implantation of pregnant female rats. The exact mechanism contributed to the occurrence was not fully understood. However, there is possibility that the chemicals compounds or any other adulterants presence in the capsules might be responsible for the toxicity effects. Therefore, it is crucial to conduct chemical profiling prior to toxicity evaluation to justify the relationship between the compounds and its bioactivity. In addition, although the reduction of sperm production reaches to 90% or more, this does not affect the fertility on some strains of animal models like rats and mice (Meistrich, 1982; Robaire *et al*., 1984; Working, 1988) [18-20]. This finding is in agreement with the previous research reported that the sperm morphology is the poor indicator of male infertility (Nallella *et al*., 2006) [21].

The hematopoietic system is one of the targets to toxic substances and haematological analysis is an important parameter for assessing the physiological and pathological status in humans and animals (Li *et al*., 2010) [22]. Few studies have reported the erythropoietic effect of plants from Lamiaceae family. Repeated administration of *Hyptis martiusii* and *Ocimum suave* in rodents did not significantly alter the blood parameters (Germana *et al*., 2013, Tan *et al*., 2008) [23, 24]. Interestingly, in this study, the mean RBC, HCT, HB and PLT concentration of rats were increased, especially for rats administered with higher dose of OSAE (>2000 mg/kg body weight). The increase in circulating RBC occurs in response to a stimulation of the erythropoietic system. One of the possible reasons for this finding is could be due to antioxidant activity of the flavonoids and phenolic acids in this plant. As previously reported, administration of rosmarinic acid up to 200 mg/kg body weight effectively restored the PLT, HB and WBC levels as well as decreased the damage on bone marrow of gamma ray-irradiated group of mice (Wenqing.Xu *et al*., 2016) [25]. In steady state inside human body, the RBC antioxidant system can cope with the reactive oxygen species threat to avoid the preliminary RBC aging that leads to anemia like in certain blood pathological conditions (Van Zwieten *et al*., 2014) [26]. Therefore, further study need to be conducted to explore the mechanism and therapeutic potential of *O. stamineus* in relation to blood disorders.

Conclusion

In conclusion, this study showed that the administration of standardized OSAE in oral doses up to 2000mg/kg/body weight/day to male Sprague-Dawley rats for 60 days, has not stimulated general toxicity over the reproductive system. High level of RBC, HCT, HB and PLT at the highest dose tested as compared to that in control rats was the most conspicuous effect of OSAE found in this study. The study-derived NOAELs for male toxicity was set at >2000 mg per kg body weight per day by the oral route.

Acknowledgement

We would like to thank the Director General of Health Malaysia for his permission to publish this article. This study was supported by the Ministry of Health, Malaysia. (NMRR ID: 14-537-21257)

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