

## Protective effect of *Hibiscus sabdariffa* Linn. calyx extract on tetracycline induced testicular toxicity in mice

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**Abstract.** Aqueous *Hibiscus sabdariffa* Linn. (Malvaceae) calyx extract (HSE) was evaluated for the protective effect against testicular toxicity induced by tetracycline dose of 20 mg/100 gBW for 14 days in mice. The extract doses of 20, 50 and 100 mg/100 gBW used in pretreatment by oral administration for 4 days and subsequent co-treatment with tetracycline for 14 days had the protective effect exhibiting significantly increasing quality of seminal fluid including an increase in total sperm count, percentage of mobile sperms and viable sperms when compared to the tetracycline treated group ( $p < 0.05$ ). Furthermore, the seminal analysis in the group treated with highest dose of HSE and subsequently co-treated with tetracycline were recovered and revealed non-significant difference from the control. These occurrences were supported by the concordant results of histological changes in testes sections. They showed that spermatogenic cells in seminiferous tubules were impaired in tetracycline-treated group. Meanwhile, they were attenuated after pretreated and co-treated with HSE. It can be concluded that aqueous *H. Sabdariffa*. calyx extract may be used as protective agent against tetracycline-induced reproductive toxicity in mice.

**Key words:** *Hibiscus sabdariffa*, tetracycline, protective effect, testicular toxicity.

**Introduction.** Antibiotics such as amoxicillin and tetracycline are the most common drugs from drugstores without prescription. Major classes of antibiotics including tetracycline have a significant adverse effect on liver (Schlegel et al 1991; Simmons 2002) and testis (Awobajo et al 2005; Taweebot & Luangpirom 2009), which was mediated by tissue oxidative stress (Farombi et al 2008). Therefore, prevention or reduction of drug toxicity is a crucial and concern topic. The previous studies were reported that many high oxidant fruits and herbs were used to prevent tissue damage by inhibiting lipid peroxidation or decreasing oxidative radical production (Turk et al 2008), such as lycopene (a natural carotenoid in tomatoes) (Atessahim et al 2006; Turk et al 2007) and *Punica granatum* juice (Turk et al 2008). *Hibiscus sabdariffa* is a natural plant whose calyx is used to make cold and hot drink in many countries. Hirunpinich et al (2006) claimed that 5 mg/ml of aqueous *H. sabdariffa* calyx extract showed more antioxidant potency than 10  $\mu$ m of vitamin E *in vitro*. There are many evidences supporting that *H. sabdariffa* calyx extract contained potential antioxidants which have been widely used in alternative therapy such as reducing LDL oxidation (Hirunpinich et al 2006) against hypertension in patients (McKay et al 2008) and enhancing immunostimulatory activity in rats (Fakeye et al 2008). Amin & Hamza (2006) reported that *H. sabdariffa* calyx extract was found to have a protective effect against hepatotoxicity and reproductive toxicity induced by cisplatin (an antineoplastic drug) in rats.

This investigation was to evaluate the protective effect of aqueous *H. sabdariffa* calyx extract against tetracycline-induced testicular toxicity in mice. Testicular toxicity was induced by oral administration with tetracycline dose of 20 mg/100 gBW for 14 days (Taweebot & Luangpirom 2009). The protective effect of the extract was assessed by

seminal analysis and histological changes of spermatogenic cells in seminiferous tubules of testes.

## Material and Method

**Preparation of extract.** The dried calyx of *Hibiscus sabdariffa* Linn. was purchased from grocery in Khon Kaen province, Thailand. They were extracted in boiling water for 1 hour and evaporated in an oven at 50 °C to concentrate at doses of 20, 50 and 100 mg/ml for experiments.

**Animals.** Adult male mice strain ICR (8-week old, 35-40 gram body weight) were obtained from the National Laboratory Animal Center of Mahidol University, Salaya district, Nakornprathom province, Thailand. They were housed under a 12 :12 h of light-day cycle at 25 ± 1 °C and were fed on standard pellet diet with water *ad libitum*. The experiments were performed after the experimental protocols have been approved by the Institutional Animal Ethics Committee, Khon Kaen University, Thailand (Reference No. 0514.1.12.2132).

**Experiment.** Male mice were divided into 6 groups, 6 mice for each. Group I received distilled water dose of 1 ml/100 gBW for 14 days as a negative control, Group II received *H. sabdariffa* calyx extract (HSE) dose of 100 mg/100 gBW for 18 days as extract toxicity test, Group III received tetracycline dose of 20 mg/100 gBW for 14 days as a positive control, Groups IV, V and VI were pretreated with HSE doses of 20, 50 and 100 mg/100 gBW, respectively for 4 days and then were co-treated with tetracycline for 14 days as treated groups.

**Seminal evaluation.** At the end of experimental period, epididymis and vas deferens of all groups were excised and tore with syringe needle (No.25) in 2 ml of 0.9% NaCl and incubated at 35 °C for seminal evaluation. Total sperm counts and viable sperms were determined by modified method of Yokoi et al (2003), mobile sperms were evaluated by the method described by Sonmez et al (2005) and abnormal sperms were investigated by the method described by Atessahim et al (2006).

**Testicular histological studies.** After the seminal sampling, testes were removed and weighed, then fixed in Bouin's solution, and subjected to a paraffin method process. They were sectioned at the thickness of 5 µm and the sections were stained with Haematoxylin and Eosin (H&E). The histological changes of spermatogenic cells in seminiferous tubules of testes were observed under a light microscope.

**Statistical analysis.** All results were expressed as mean ± standard deviation ( $\bar{x} \pm SD$ ). Data were analyzed by one-way analysis of variance (ANOVA) and Duncan's test for multiple comparison using SPSS software version 11.0. The ( $p < 0.05$ ) will be accepted as significant.

## Results

**Seminal evaluation.** The results of seminal analysis of the control and treated groups were presented in Table 1. HSE-treated group (100 mg/100 gBW) showed non-toxicity on spermatogenesis. Tetracycline (20 mg/100 gBW) caused adverse effect on spermatogenesis showing a significant decrease in total sperm count, percentage of mobile sperms, viable sperms ( $p < 0.05$ ) and a significant increase in abnormal sperms ( $p < 0.05$ ) compared to the control. Morphological abnormalities in sperms were found in many forms including medial protoplasmic droplet, coiled-tail and detached-head sperms. Interesting results were found in all groups co-treated with HSE (20, 50 and 100 mg/100 gBW) which revealed a significant increase in quality of seminal fluid when compared to the tetracycline-treated group as a dose-dependent manner of HSE administration ( $p < 0.05$ ). Seminal evaluation in the highest dose of HSE co-treated group showed non-significantly different results ( $p > 0.05$ ) when compared to the control as shown in Figure 1.

**Histological studies.** The layer numbers of spermatogenic cells in seminiferous tubules of control and treated groups were presented in Table 2. Testicular histological changes in HSE-treated group at dose of 100 mg/100 gBW were not found. Tetracycline (20 mg/100 gBW) treated group caused severe testicular toxicity showing a significant

decrease of spermatocyte and spermatid layers in seminiferous tubules when compared to the control ( $p < 0.05$ ). All groups co-treated with HSE (20, 50 and 100 mg/100 gBW) showed protective effect against tetracycline toxicity as a dose-dependent manner by revealing a recovery of spermatid layers. Meanwhile, normal histology of seminiferous tubules was found in the group co-treated with the highest dose of HSE and tetracycline as presented in Figure 2.

Table 1

$\bar{x} \pm SD$  of total sperm count, percentage of mobile sperms, abnormal sperms and viable sperms in the groups treated with tetracycline (T) and co-treated with *H. sabdariffa* calyx extract (HSE) and control group

Treated group (mg/100gBW) N = 6	Total sperm counts $\bar{x} \pm SD$ ( $\times 10^6$ ) cells/ml	Motile sperms ( $\bar{x} \pm SD$ , %)	Abnormal sperms ( $\bar{x} \pm SD$ , %)	Viable sperms ( $\bar{x} \pm SD$ , %)
0	24.70 $\pm$ 0.38 <sup>a</sup>	88.11 $\pm$ 0.27 <sup>a</sup>	11.39 $\pm$ 0.97 <sup>a</sup>	95.54 $\pm$ 0.89 <sup>a</sup>
HSE 100	24.47 $\pm$ 0.67 <sup>a</sup>	89.83 $\pm$ 1.99 <sup>a</sup>	14.33 $\pm$ 1.50 <sup>a</sup>	94.95 $\pm$ 0.64 <sup>a</sup>
T 20	20.43 $\pm$ 0.34 <sup>b</sup>	72.72 $\pm$ 5.14 <sup>c</sup>	25.56 $\pm$ 3.88 <sup>c</sup>	87.44 $\pm$ 1.09 <sup>c</sup>
HSE 20 + T 20	23.20 $\pm$ 2.43 <sup>a</sup>	69.72 $\pm$ 2.77 <sup>c</sup>	24.33 $\pm$ 1.05 <sup>c</sup>	91.68 $\pm$ 3.81 <sup>b</sup>
HSE 50 + T 20	24.03 $\pm$ 1.35 <sup>a</sup>	80.61 $\pm$ 7.99 <sup>b</sup>	19.05 $\pm$ 2.56 <sup>b</sup>	96.21 $\pm$ 1.49 <sup>a</sup>
HSE 100 + T 20	25.40 $\pm$ 1.92 <sup>a</sup>	87.55 $\pm$ 1.92 <sup>a</sup>	13.06 $\pm$ 1.18 <sup>a</sup>	96.45 $\pm$ 0.83 <sup>a</sup>

N - number of experimental animals, same alphabet - non-significant different ( $p > 0.05$ ), different alphabet - significant different ( $p < 0.05$ )

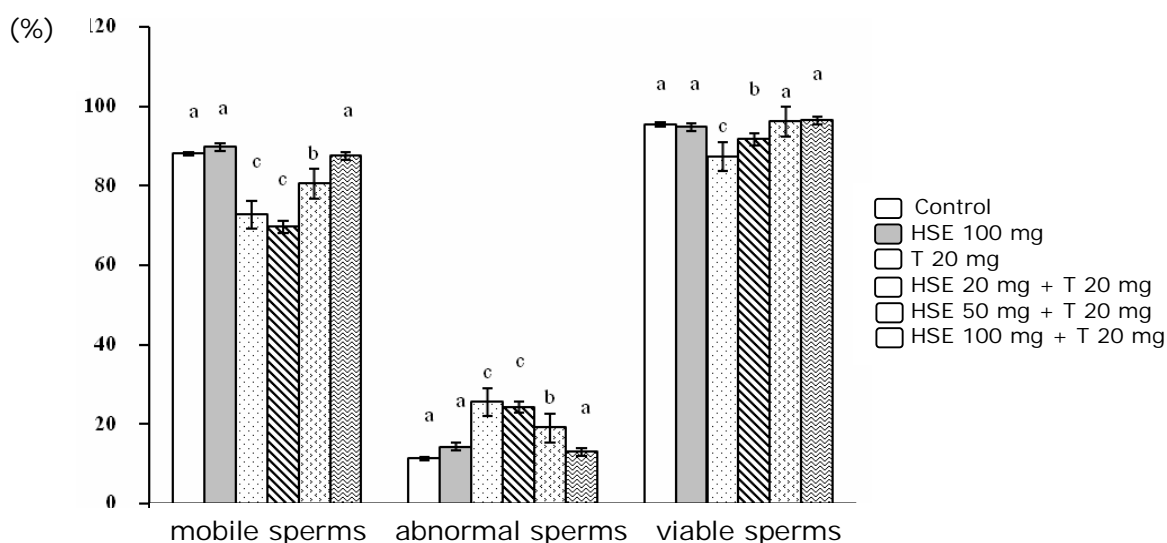


Figure 1.  $\bar{x} \pm SD$  of percentage of moving sperms, abnormal sperms and viable sperms in the groups treated with tetracycline (T) and aqueous *H. sabdariffa* calyx extract (HSE) and control group

**Discussion.** Tetracycline dose of 20 mg/100 gBW caused adverse effect on seminal quality after 14 day treatment, including a reduction in total sperm count, percentage of viable sperms and mobile sperms. Like wise, testicular histological studies showed the impairment of spermatogenic cells in seminiferous tubules. It has been suggested that tetracycline caused plasma testosterone depletion (Awobajo et al 2005), elevated lipid peroxidation in testis and epididymis (Farombi et al 2008) and high free radical production of sperms (Sanocka & Kurpisz 2004; Vernet et al 2004), thereby, affecting the spermatogenesis and physiological maturation of sperms (Chinoy et al 1995). Sperms are especially susceptible to peroxidative damage because of their high concentration of polyunsaturated fatty acids which are involved in regulation of sperm maturation and spermatogenesis. Additionally, peroxidation of sperm lipid destroys the structure of lipid

matrix in the membrane of sperms and it is capable of inhibiting spermatogenesis in extreme cases (Sanocka & Kurpisz 2004; Sikka 1995; Vernet et al 2004). In the present study, it was found that pretreatment with HSE at doses of 20, 50 and 100 mg, and followed by co-treatment with tetracycline were able to protect tetracycline toxicity. In addition, the groups co-treated with the highest dose of HSE presented normal seminal quality and spermatogenic cell arrangement in seminiferous tubules as found in control. The previous studies were reported that HSE contained numerous natural phenolic compounds such as anthocyanin and protocatechuric acid (Tsai et al 2002).

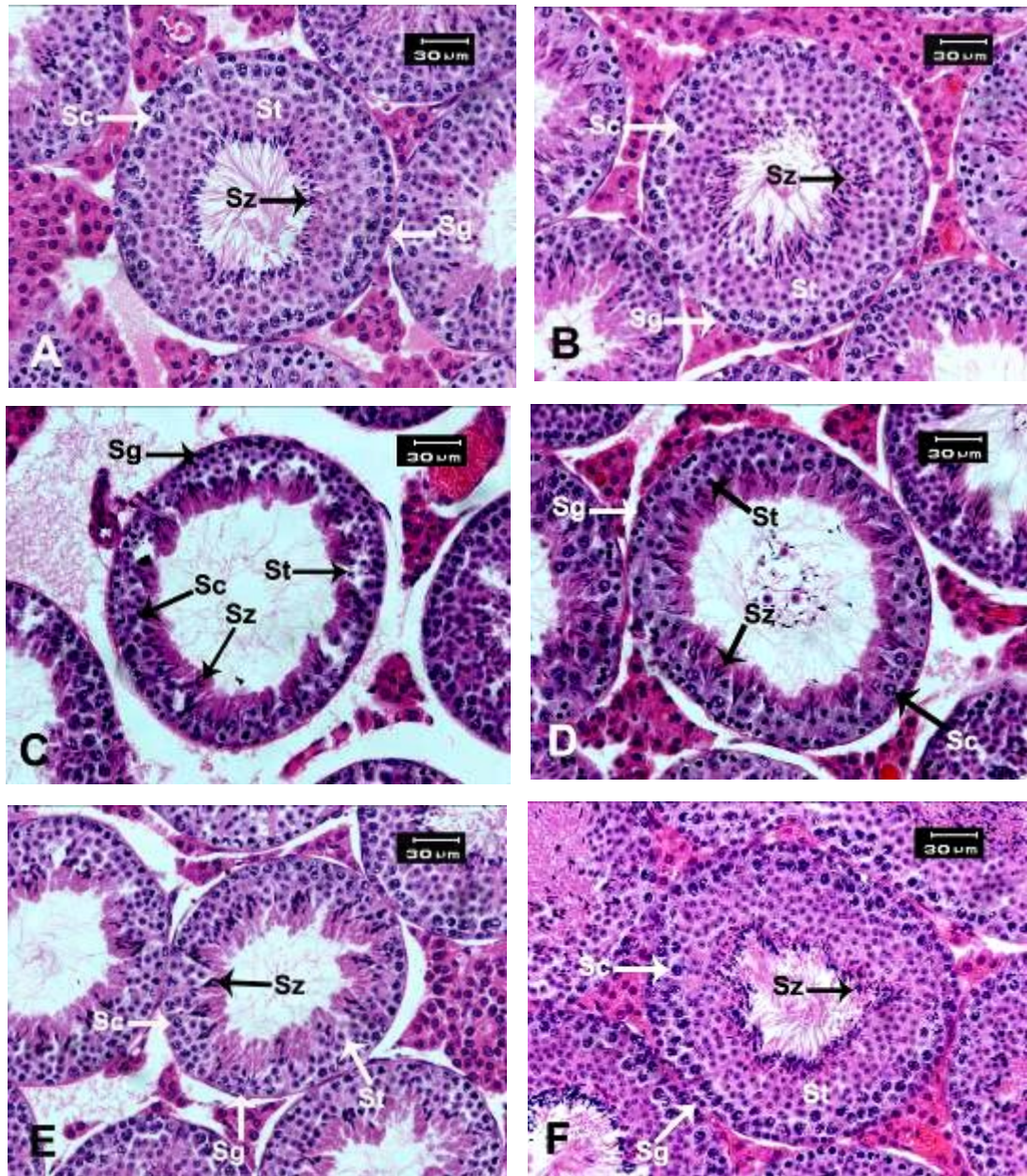


Figure 2. X-section of testes (H & E, bar = 30 µm), showing histological changes of seminiferous tubules; **A.** Control group and **B.** Group received *H. sabdariffa* calyx extract (HSE) 100 mg presented normal arrangement of spermatogenic cells ranging from spermatogonia (Sg), spermatocytes (Sc), spermatid (St) to spermatozoa (Sz); **C.** Group received tetracycline (T) 20 mg caused decreasing layers of spermatocyte and spermatid; **D.** Group received T + HSE 20 mg and **E.** Group received T + HSE 50 mg/100 gBW showed recovery of spermatogenic cells as dose dependent manner of HSE administration and **D.** Group received T+ HSE 100 mg/100 gBW presented normal architecture as control.

Table 2

$\bar{x} \pm$  SD of testicular histological examination of the groups treated with tetracycline (T) and co-treated with *H. sabdariffa* calyx extract (HSE) and control group

Treated group (mg/100gBW) N = 6	spermatocyte layer ( $\bar{x} \pm$ SD)	spermatid layer ( $\bar{x} \pm$ SD)
0	2.41 + 0.09 <sup>a</sup>	3.69 + 0.09 <sup>a</sup>
HSE 100	2.43 + 0.06 <sup>a</sup>	3.57 + 0.09 <sup>a</sup>
T 20	1.25 + 0.33 <sup>c</sup>	1.17 + 0.33 <sup>c</sup>
HSE 20 + T 20	1.42 + 0.66 <sup>c</sup>	2.17 + 0.66 <sup>b</sup>
HSE 50 + T 20	1.41 + 0.47 <sup>c</sup>	2.49 + 0.47 <sup>b</sup>
HSE 100 + T 20	2.10 + 0.26 <sup>b</sup>	3.29 + 0.26 <sup>a</sup>

N - number of experimental animals, same alphabet - non-significant different ( $p > 0.05$ ), different alphabet - significant different ( $p < 0.05$ )

With their free radicals scavenging capacity and antioxidant activity, it was considered that they had protective effect against oxidative stress (Liu et al 2002). Similar protective effect of HSE against drug toxicity had been documented using cisplatin-induced testicular toxicity in rats (Amin & Hamza 2006). However, Hirunpinich et al (2006) claimed that 5 mg/ml of HSE showed more antioxidant potency than 10  $\mu$ m of vitamin E *in vitro*. There are some evidences supporting that tetracycline-induced testicular toxicity was protected by vitamin E (Raji et al 2007) and vitamin C (Farombi et al 2008). Futhermore, our work showed that the group treated with 100 mg/100 gBW HSE did not showed any signs of testicular toxicity when compared to the control. Ali et al (2005) reported that HSE had a low toxicity and its median lethal dose (LD<sub>50</sub>) in rats were above 5,000 mg/kgBW. In Thailand, HSE is consumed as local and commercial beverage.

**Conclusions.** Tetracycline treatment at dose of 20 mg/100 gBW for 14 days caused adverse effect on reproduction of male mice. The pretreatment with HSE at least 4 days before co-treated with tetracycline may be used as a protective agent against tetracycline toxicity.

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