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Acute Toxicity Studies of Thai Herbal Preparation "Sao Thong Tai" in Animals

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ABSTRACT

The objective of the present study was to investigate acute oral toxicity of the Thai herbal preparation "Sao Thong Tai" in vivo. The study included two experiments: one was twenty healthy albino mice administered with "Sao Thong Tai" extract, each at 2,000 mg/kg body weight; the other was thirty Wistar rats administered with "Sao Thong Tai" extract, each at 2,000 mg/kg and 5,000 mg/kg body weight. The overall observation period was 14 days. During the observation period all mice and rats survived and there was no sign of toxicity. All mice and rats appeared normal and gained weight and no mortality occurred. Necropsy findings at termination did not show any significant gross pathological lesions. In conclusion, the treated mice and rats did not show toxicity of "Sao Thong Tai". The Thai herbal preparation "Sao Thong Tai" extract was recognized as safe and non – toxic, and therefore can be used for applications of traditional medicine in modern complementary and alternative therapeutics in humans.

Keywords: Acute toxicity; Thai herbal preparation; Albino mice; Wistar rat

1. Introduction

A wide variety of human disorders is currently being treated with the use of herbal plants due to their decreased toxicity levels and cost-effectiveness, as well as minimized side effects, in order to avoid drug resistance caused by pharmacological agents. They also provide various nutritional supplements. Among the 250,000 higher plant species on earth, more than 80,000

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plants have medicinal values and about 75-80% of the global population, mainly in developing countries, use herbal medicine for primary health care [1]. Several herbal remedies, plants, animals, minerals and supplements with aphrodisiac potential are used in parts of Asia, Africa, and some regions of Europe, and North and South America for treating ED [2, 3].

Development and assessment of herbal formulations for various beneficial health and or functional effects in animal and human species are increasing in popularity. Despite well-established scientific studies on phytopreparations or herbal ingredients for pharmacological properties, the safety details of herbal substances reported in compliance with internationally accepted guidelines seem to be inadequate [4, 5]. In order to meet the requirement in the increasing use of herbal medicines in the future for various health needs, toxicological investigations are required for evaluation and classification of herbal preparations based on safety data [4, 5].

The investigational substance of the present study,"Sao Thong Tai", is a herbal formulation consisting of four medicinal plants, namely, *Boesenbergia rotunda*(L.)Mansf.(fingerroot), *Sida acuta* Burm.f. (wireweed), *Dactyloctenium aegyptium*(L.) Willd.(crowfoot grass), and *Oryza sativa* L.(rice) , that has the property of aphrodisiac, an agent that arouses sexual desire to treat Erectile Dysfunction [6]. The major chemical constituents in this herbal preparation can also nourish the kidney and strengthen the spleen [6]. Erectile Dysfunction is defined as the inability to achieve and maintain a penile erection adequate for satisfactory sexual intercourse [7]. It is linked to the aphrodisiac [8]. An aphrodisiac is defined as an agent (food or drug) that arouses sexual desire [9]. Sexual desire is controlled and regulated by the central nervous system which integrates tactile, olfactory and mental stimuli [10]. Aphrodisiac potentials inhibit the hydrolyzing action of PDE-5 with the result that active cGMP (cyclic guanosine monophosphate) can accumulate and prolong the erection through increased blood flow [10]. "Sao Thong Tai" has the property of aphrodisiac(Table 1).

The present study was carried out to assess "Sao Thong Tai" for its acute toxic potential by fix dose procedure adopted by the Organization for Economic Co-operation and Development (OECD) [4].

2. Materials and Methods

2.1 Test Substance

"Sao Thong Tai" is a combination of four medicinal herbs developed by Charoensuk Osod Co., Ltd., Nakon Pathom, Thailand. Sao Thong Tai has *B.rotunda*, *S. acuta*, *D.aegyptium*, and *Oryza sativa*. The crude powders obtained from the dried plant materials, after verifying the content of marker compounds, were mixed in equal proportions to prepare spray-dried decoction of the product [11].

The chromatographic apparatus consisted of an HPLC system (Thermo Fischer Scientific Inc., San Jose, CA, USA) with SpectraSYSTEM P4000 Quaternary Gradient Pump and a SpectraSYSTEM AS 3000 Autosampler, SpectraSYSTEM UV-6000LP diode array detector. The chromatograms were recorded and processed with Thermo Scientific ChromQuest Data System. The separation was performed using a VDSpher PUR 100 C18-SE (5 µm, 250mm × 4.6 mm I.D., VDS Optilab Chromatographietechnik GmbH, Berlin, Germany).

Mobile phase A was 2%(v/v) acetic acid in water and mobile phase B was 2%(v/v) acetic acid in acetonitrile. The gradient elution program of the mobile phases was as follows: 20%B in 10 min, 20%B for 5 min, 60%B in 15 min, 80%B in 10 min, 100%B in 10 min, 100%B for 10 min.

Table 1. Details of the plants.

Plants	Country of origin / Parts of use	Traditional Indication / Type of Ingredient	Major chemical constituents / Possible mechanism of action	Pre-clinical Study
Boesenbergia rotunda (L.) Mansf.	Southeast Asia/ Rhizome	Aphrodisiac / Main active	Alpinetin, pinocembrin, cardamonin, boesenbergin, pinostrobin,chavicinic acid, 5,7-dihydoxyflavone, 1,8-cineole, panduratin A Pinostrobin, / PDEs 5 inhibitor	Phosphodiasterase inhibitory (compared with 3-isobutyl-1-methylxanthine (IBMX), antimicrobial, antiparasitic, antioxidant, antiulcer,obesity treatment, antimutagenic, antitumor, anticancer, antifungal, antiviral
Sida acuta Burm.f.	Central America, Southeast Asia/ Root	Aphrodisiac, Diuretic, Tonic / Main active, Complementary active	Alkaloids, tannins, sterols, saponins, flavonoids, glycosides, amino acids, carbohydrates, proteins, fats and oils / Aphrodisiac potential	Increase sexual behavior of normal rats, hepatoprotective in Wistar albino male rats
Dactyloetenium aegyptium (L.) P. Beauv.	Africa/ Whole plant	Diuretic/ Main active, Complementary active	Alkaloids, terpenoids, saponin, phenols, tannins, anthraquinones, flavonoids, carbohydrates, proteins, amino acids, / Nourishing kidney	Antimicrobial, anticancer in cells, to treat gastrointestinal ailments
Oryza sativa L.	Asia, Africa/ Seeds	Aphrodisiac, Diuretic, Tonic/ Main active and Complementary active	High protein, lipid, fiber, carbohydrate, ash and highest amount of α-tocopherol, γ-tocopherol, γ-oryzanol, amino acid / Nourishing kidney, Strengthen spleen, antioxidant	Providing fast and instant energy, providing essential source of vitamin B to human body, skin care, resistance to high blood pressure, and dysentery and heart diseases.

*Ref. [3,6,7,8,9,10,14-23]

The mobile phase flow rate was maintained at 1.0 mL/min and the column temperature was 25 °C. The detection was carried out at wavelength 254 nm.

The product and respective grinded reference plant materials weighing 1.0 g were weighed separately into 25.0 mL volumetric flask and extracted by sonication in an ultrasonic bath with 15.0 mL methanol for 30 min and the solution was diluted to volume with the same solvent after cooling and mixed well. The fingerprint of the product sample and reference plant materials were compared with alpinetin, cardamonin, pinocembrin and pinostrobin as chemical markers. Quantification of the chemical markers in the product sample was done using a standard calibration method applicable within the range of 0.05 to 1.0 µg.

A 20 µL portion of each of the final solutions was injected onto the column after filtration through a 0.2 µm cellulose acetate filter (Sartorius, Goettingen, Germany).

2.2 Plant materials

The plant materials were prepared as extract by using decoction and further drying process by spray drying technique. They were purchased from Charoensuk Osod, Nakhon Pathom Province of Thailand. They were rinsed carefully with running water to remove contaminants and

dried at 45-60 °c . The extraction ratio of crude to extract was 3.3:1 with 400 mg. per capsule.

2.3 Determination of fingerprint of Thai herbal preparation "Sao Thong Tai"

The fingerprint of Thai herbal preparation "Sao Thong Tai" was analyzed by high-performance liquid chromatography (HPLC) [13]. The methodology of analysis of quality indicators from chromatographic fingerprint profiles of raw materials and extracts was based on the evaluation of the quality of herbal medicines by the World Health Organization [11] with reversed phase chromatography. Pinocembrin, cardamonin and pinostrobin, which were components of the B. rotunda, were the quality indicators.

2.4 Experimental animals

Twenty albino mice (ten of each sex) weighing from 35-41 g for males and 27-33 g for females were used in the first study. Thirty white Wistar rats (fifteen of each sex) weighing from 230-255 g for males and 170-190 g for females were used in the second study. All were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhonpathom Province. The animal acute oral toxicity test for mice was conducted according to the Test Guideline (TG) No.420: Acute Oral Toxicity-Fixed Dose Method (Limit test) of the OECD Guidelines for Testing of Chemicals (2001). The animal acute oral toxicity test for Wistar rats was conducted according to the Test Guideline (TG) No.423: Acute Oral Toxicity-Acute Toxic Class Method of the OECD Guidelines for Testing of Chemicals (2001). They were acclimatized to the laboratory environment for 1 week prior to experimentation with room temperature of 24±1 °C and relative humidity of 50-70 % in order for them to adapt to the environment. All mice were fasted for 8 hrs prior to the dosing with test materials while drinking water was available ad libitum; while all rats were fasted for 16 hrs prior to the dosing with test materials while drinking water was available ad libitum.

2.5 Study design

For the first test, twenty healthy mice were assigned as control and treatment groups. Each group consisted of five male and five female mice. The mice were randomly chosen using the simple random sampling method and identified with numbers by tail labeling. On experimentation day, the test material "Sao Thong Tai" extract was orally dosed to the mice in the treatment group at the dose of 2,000 mg/kg body weight calculated based on their body weights after fasting. The mice in the control group were dosed with 0.5% (w/v) CMC at the equivolume as the treated mice. After dosing, all mice were further fasted for 4 hrs but water was provided ad libitum. Any toxic signs were immediately observed at 1/2, 1 and 3 hrs and once daily for 14 days. For the second test, thirty healthy white Wistar rats were assigned as one control group and two treatment groups. Each group consisted of five male and five female rats. On experimentation day, the test material "Sao Thong Tai" extract was orally dosed to the rats in the two treatment groups at the dose of 2,000 mg/kg and 5,000 mg/kg body weight calculated based on their body weights respectively after fasting. The rats in the control group were dosed with distilled water at the equivolume as the treated rats. After dosing, all rats were further fasted for 4 hrs but water was provided ad libitum. The observation of toxic signs and the record of the body weights were conducted as those on the albino mice in the first test. Acute toxicity was observed on the tested animals during the 14-day observation period. On day 14, all survived animals were euthanized by CO_2 asphyxiation and gross pathology was performed.

2.6 Body weight

Body weight data of individual animals were recorded on day 1 (prior to dosing), day 7 and day 14 (at termination) or after death during experimental period. The mean of body weight gain of the animals of the treatment group was calculated and compared to that of the control group.

2.7 Gross pathology and histopathology

During the observation period in the study, any animal dying, sacrificed moribund for humane reasons or sacrificed terminally subjected to a complete necropsy and the gross pathological changing was recorded. Histopathology examination of organs and tissues was considered in case of evidence of any gross pathology findings [4].

3. Results and Discussion

3.1 Acute Toxicity test of animals

The toxicity of the Thai preparation "Sao Thong Tai" extract when given as 14-day daily doses in animals was investigated. Results indicated virtually no toxicity of "Sao Thong Tai" at a maximum oral dose of 2,000 mg/kg bodyweight of mice. The results of the study did not show any major adverse effect on the body weight gain throughout the treatment period. The body weights (gms) on day 1, day 7 and day 14 were 34.4, 37.6 and 39.5 in the control group; while in the "Sao Thong Tai" group, they were 33.7, 36.0 and 37.5, respectively (Fig.1). The other study group with daily oral doses of 2,000 mg/kg and 5,000 mg/kg bodyweight of rats was found to have mean weight (gms) of 211, 244 and 257 in the "water group"; 210, 244 and 256 in the Sao Thong Tai group taking doses of 2,000 mg and 214, 243 and 261 in the "Sao Thong Tai" group taking doses of 5,000 mg respectively (Fig. 2). All animals in this study survived and appeared normal. Overall, the percent body weight increased during the 14-day observation period. No mortality occurred in any of the groups. Necropsy findings at termination did not show any significant gross pathological lesions. Based on the results, the oral LD_{50} of "Sao Thong Tai" in animals of both sexes was greater than 2,000 and 5,000 mg/kg body weight.

3.2 14-Day dose range finding study

All the male and female animals from control and different dose levels of "Sao Thong Tai" survived till the terminal sacrifice and no abnormal clinical science was noticed throughout the study period. There were no statistical differences in body weight gain between the control and the "Sao Thong Tai" groups of mice and rats. Necropsy examination did not show any treatment-related evidence of toxicity.

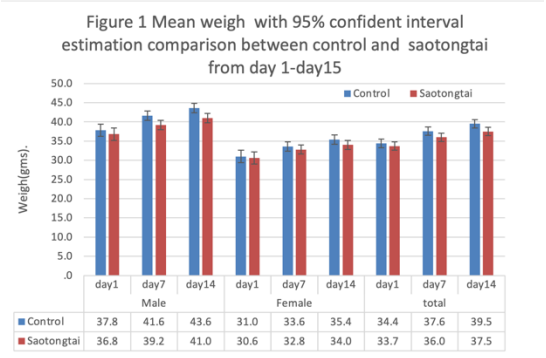

Fig. 1. Mean weight with 95 % confident interval estimation comparison between control and "Sao Thong Tai" 2,000 mg from day 1 - day 14.

This Thai traditional preparation "Sao Thong Tai" from Thai Pharmacopeia has properties of treating erectile dysfunction, diuretic, nourishing kidney and strengthening the body. This preparation has conformed to the standardization and fingerprint of the high-performance liquid chromatography (HPLC).

Before the treatment study three ingredients, *S.acuta, B.rotunda* and *D. aegyptium,* had passed the pre-clinical study. *S. acuta* has the most effective aphrodisiac potential [14,15]. Based on the study of Alok S. and M.Senthil K. the

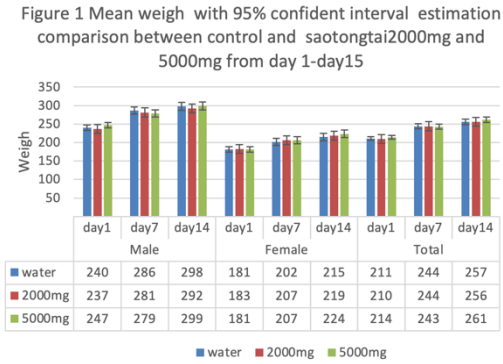

Fig. 2. Mean weight with 95 % confident interval estimation comparison between control and "Sao Thong Tai" 2,000 mg and 5,000 mg from day 1 - day 14.

screening of the leaves extract of *Sida acuta* [16] contains such constituents as alkaloids, carbohydrates, glycosides, proteins, tannins, sterols, saponins, amino acids, flavonoids, fat and oils. A 14-day acute toxicity study was performed on male mice [16]. All extracts were preventing all behavioral attention and toxicity indication, so it was concluded that LD_{50} (50% Lethal dose) is more than 2000 mg/kg body weight. They also used female and male rats divided into five groups for a 28-day sexual behavior study with the dose of 200mg/kg and have observed that mount frequency and intromission frequency increased, which suggested the enhancing of libido. The effect of leaves extract on mount latency and intromission latency were inversely proportional to sexual motivation which led to enhancing sexual behavior; ejaculation latency and post ejaculatory phase was shortened. Therefore, it is effective as aphrodisiac through mechanisms such as vasodilation, generation of nitric oxide, elevation of androgens and gonadotropins. The extracts of *S. acuta* leaves showed significant increase in aphrodisiac activity [16].

The second ingredient in "Sao Thong Tai" is *B.rotunda*. *B.rotunda*, which belongs to Zingiberaceae family [17]. Prapapan T. et al. used nineteen Thai medical plants to search for sources of PDE inhibitors. But the result shows that among the seven plants *B. rotunda* extracted from rhizome has the highest IC_{50} values against phosphodiesterases (PDEs) (16.42±0.96 µg/ml.) in comparison to the standard PDE inhibitor by the scintillation proximity assay (SPA) radio assay [10]. Therefore, *B. rotunda* extract has aphrodisiac and neurotonic properties [19]. A toxicity test was made by Saraithong P. et al. Extracts at doses of 60, 120 and 240 mg/kgbw/day were orally administered to 27 male rats for 60 days, while a control group received distilled water [19]. The results showed that the extracts had no effect on the body weight, regardless of the dosage used. The kidney and liver functions, including blood urea nitrogen (BUN), creatinine (Crea), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were within the normal range [19]. It can be concluded that ethanol extracts of *B. rotunda* at all doses used in the present study have no toxic effect on male rats [19]. Charoensin S. et al also reported that two bioactive compounds from *B. rotunda*, pinostrobin (5-hydroxy-7-methoxy flavanone) and pinocembrin (5, 7-dihydroxy flavanone), indicated no mutagenic effect or toxicity towards Wister rats, further confirming the safety of its consumption [20]. Rats were randomly divided into three groups, 5 rats per group. The treatment groups were given a single dose of 500 mg/kgbw/day of pinocembrin or pinostrobin which was derived from air-dried fingerroot, about 1.4 kg for pinocembrin and 1.0 kg for pinostrobin. The control group received 5% Tween-80 (CAS no.9005-65-6). Rats were observed individually after dosing periodically during the first 24 hr, with special attention given during the first 4 hr, and daily thereafter for 14 days. The result showed that body weights of rats administered a single 500 mg/kg dose of pinocembrin or pinostrobin increased throughout the study period. Water intake and food consumption did not show any

significant change as compared to the control group in the period of time studied [20]. There were no adverse effects or behavioral changes observed between control and treated rats. There were no deaths, and the LD_{50} of both phytochemicals is more than 500 mg/kg for the Wistar strain. The relative organ weights, including liver, lungs, kidneys, spleen, heart, testis, and thymus gland of the treated rats showed no significant change compared with control rats [20].

The third ingredient in "Sao Thong Tai" is *D. aegyptium* belonging to Gramineae family. Ethanolic extract revealed the presence of carbohydrates, proteins, amino acids, saponins, flavonoids, tannins, terpenoids and alkaloids [21]. To find out the effect of ethanol extract of *D. aegyptium*, Sreedhar Naik et al. equally divided the experimental rats weighing 200-250 g into four groups of six each [21]. They were given ethanolic extracts of *D. aegyptium* at 200, 400 and 600 mg/kgbw/day orally for 30 days. The result showed significant increase in serum estrogen levels. As to the acute toxicity study of the extract, the test was performed according to OECD guidelines 423 with the mice fed with ethanolic extract up to 2,000 mg/kg for 14 days. No toxicity was found [21].

The last ingredient in "Sao Thong Tai" is *Oryza sativa* belonging to the Gramineae family. Since ancient time, rice has been consumed as the main staple food for more than half of the world's population [22]. Rice is very nutritious. Some scientists believe that whole grains (such as brown rice) contain high amounts of insoluble fiber which may help prevent a variety of cancers. In Ayurveda and Thai folklore the medicinal values of rice have been described: rice is considered to be tonic, aphrodisiac, antioxidant, oleaginous, fattening, diuretic and useful in biliousness [23]. It offers iron to enrich the bloodstream and phosphorus and potassium to maintain internal water balance along with other nutrients.

As mentioned above, apart from the ingredient of rice in "Sao Thong Tai", which is well known for its safety and tonic for consumption, the other three have separately passed the acute toxicity tests by several researchers and physicians in vivo study on rats or mice weighing from 60 mg to 2,000 mg, given 500 mg or up to 2,000 mg for 14 days, 28 days or 30 days as the studies required, and proved to be safe. However, "Sao Thong Tai", being an herbal preparation including the above mentioned ingredients, needed to be further investigated for its acute toxicity and safety. In the present study 20 mice and 30 rats were used for the test. On administration of "Sao Thong Tai" at 2,000 and 5,000 mg/kg for a period of 14 days, no death, treatment-related abnormal clinical or behavioral signs, and gross pathological observation were recorded till the end of the treatment. But there was a slight change in body weight gain.

4. Conclusion

In conclusion, the ingredients in the Thai preparation "Sao Thong Tai" have aphrodisiac property in treating erectile dysfunction and form a tonic without side effect in animals. Especially, *B.rotunda* has the highest IC_{50} values against phosphodiesterases (PDEs) in comparison to the standard PED inhibitor. Nevertheless, this preparation requires more evidence to support the efficacy and safety on patients with ED by clinical study.

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References

[1] Ernst E, Pittler MH. Yohimbine for erectile dysfunction: a systematic review and meta-analysis of randomized clinical trials. J Urol 1998;159(2):433-36.

[2] Muneer A, Kalsi J, Nazareth I, Arya M. Erectile dysfunction. BMJ 2014; 348:g129. doi: 10.1136/bmj.g129

[3] Tan EC, Lee YK, Chee CF, Heh CH, Wong SM, Li-Ping CT, et al. *Boesenbergia rotunda*: from ethnomedicine to drug discovery. Evid Based Complement Alternat Med; Epub 2012;25 pages. doi:10.1155/2012/473637

[4] Joshua AJ, Goudar KS, Sameera N, Pavan KG, Murali B, Dinakar N, et al. Safety assessment of herbal formulations, Rumbion™ and Tyrel™ in Albino Wistar rats. Am J Pharmacol Toxicol 2010;5(1):42-7.

[5] Kouitcheu Mabeku LB, Penlap Beng V, Kouam J, Essame O, Etoa FX. Toxicological evaluation of ethyl acetate extract of *Cylicodiscus gabunensis* stem bark (Mimosaceae). J Ethnopharmacol 2007; 111(3):598-606.

[6] The Phaetthayasatsongkhro chabap luang, Book 1 and 2 (Wat Po). Bangkok: Pratapjai Service Center Limited Partnership; 1986.

[7] Singh R, Ali A, Jeyabalan G, Semwal A. Current status of Indian medicinal plants with aphrodisiac potential. J Acute Dis 2013;2(1)13-21.

[8] Sumalatha K, Kumar SA, Lakshmi SM. Review on natural aphrodisiac potentials to treat sexual dysfunction. Inter J Phar Ther 2010;1(1):6-14.

[9] Aboua G, Manirafasha C, Boitumelo R.Mosito, van der Linde M, du Plessis SS. Potentials of phytotherapeutic treatment of erectile dysfunction, antioxidant-antidiabetic agents and human health. In: Oguntibeju O, Editor. IntechOpen; 2014;p.279-95. doi:10.5772/57174

[10] Temkitthawon P, Viyoch J, Limpeanchob N, Pongamornkul W, Sirikul C, Kumpila A, Suwanborirux K, Ingkaninan K. Screening for phosphodiesterase inhibitory activity of Thai medicinal plants. J Ethnopharmacol 2008;119(2): 214-7.

[11] General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine,WHO/EDM/TRM/2000.1, World Health Organization, Geneva; 2000.

[12] Rasheed A, Sravya RB, Roja C. A review on standardization of herbal formulation. Inter J Phytotherapy 2012;2(2):74-88.

[13] Kunle, OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines - A review. Inter J Biodiver Conserv 2012;4(3):101-112.

[14] Karou SD, Nadembega WMC, Ilboudo D P, Ouermi D, Gbeassor M, De-Souza C, Souza CD, Simpore J. *Sida acuta* Burm. f.: a medicinal plant with numerous potencies. Afr J Biotechnol 2007;6(25):2953-9.

[15] Malviya N, Jain S, Gupta VB, Vyas S. Indigenous herbal remedies used by tribals of Madhya Pradesh for improving their sexual performance and problem associated with sexuality. Int J Res Ayurveda Pharm 2011; 2(2):399-402.

[16] Semwal A, Kumar MS. Influence of chloroform extract of Sida acuta Burm.f. leaves on the sexual behavior of normal rats. Asian Pac J Health Sci 2014;1(4S):84-93.

[17] Sudwan P, Saenphet K, Aritajat S, Sitasuwan N. Effects of Boesenbergia rotunda (L.) Mansf. on sexual behaviour of male rats. Asian J Andro 2007;9(6): 849-855.

[18] Bella AJ, Shamloul R. Traditional plant aphrodisiacs and male sexual dysfunction. Phytother Res 2014;28(6): 831–5.

[19] Saraithong P, Saenphet S, Saenphet K. Safety evaluation of ethanol extracts from *Boesenbergia rotunda* (L.) Mansf. in male rats. Trends Res Sci Technol 2010:2(1):19-22.

[20] Charoensin S, Punvittayagul C, Pompimon W, Mevatee U, Wongpoomchai R. Toxicological and clastogenic evaluation of pinocembrin and pinostrobin isolated from *Boesenbergia pandurata* in Wistar rats, Thai J Toxicology 2010;25(1):29-40.

[21] Naik BS, Dangi NB, Sapkota HP, Wagle N, Nagarjuna S, Sankaranand R, Kumara BA. Phytochemical screening and evaluation of anti-fertility activity of Dactyloctenium aegyptium in male albino rats. Asian Pac J Reprod 2016; 5(1):51-7.

[22] Umadevi M, Pushpa R, Sampathkumar KP, Bhowmik D. Rice-traditional medicinal plant in India. J Pharmacogn Phytochem 2012;1(1):6-12.

[23] Das GK, Oudhia P. Rice as a medicinal plant in Chhattisgarh (India). Agric Sci Digest 2001;21(3):204-5.