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Antiestrogenic and toxicological evaluation of methanolic extract of *Saraca asoca* and *Cynometra travancorica*

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ABSTRACT: *Saraca asoca,* a member of the *Caesalpinaceae* sub-family, is a native plant utilized extensively in ayurvedic medicine. Excessive menstrual bleeding, bleeding hemorrhoids, bleeding ulcers, and hemorrhagic dysentery can all be treated with the tannins found in the bark of *S. asoca*. Due to the scarcity of *S. asoca*, the ayurvedic formulation business was forced to use barks of comparable origin. Until recently, the pharmacological potential of frequently substituted *Cynometra travancorica* was unknown. The purpose of this research is to investigate and evaluate the antiestrogenic and toxicological effects of two *Caesalpiniaceae* members, *S. asoca* and *C. travancorica*, on Wistar female rats. Methanolic bark extract of both plants (600 mg/kg) was shown to be significantly effective in reducing the elevated estrogen (20 µg/animal) levels in Wistar female rats. The *C. travancorica* treated group showed an 85.63 ± 11.38 pg/ml reduction. In the toxicological evaluation, even at high concentrations of 800 mg/kg, neither of the extracts was fatal to Swiss albino mice. Food and water consumption, body weight, and the weight of organs such as the liver, kidney, spleen, heart, and lungs did not change significantly. The hematological parameters also stagnated. The study concluded that *C. travancorica* had shown a substantial and comparable anti-estrogenic effect to *S. asoca* in Wistar female rats and, similarly, the toxicological evaluation of the former plant was analogous to the latter. As a result, *C. travancorica* has a comparable therapeutic and safety profile in a wide range of ayurvedic formulations.
KEYWORDS: *Saraca asoca; Cynometra travancorica; Caesalpinaceae;* anti-estrogenic activity; toxicological evaluation.

1. INTRODUCTION

Asoka (Asokam) has been used in large quantities in ayurvedic medicine [1] from antiquity till date, to treat various diseases. *S. asoca* (Roxb.), W. J. de Wilde, is the commonly used binomial Latin name for the asoka tree [2]. The phytochemical study isolated the bark of *S. asoca* found chemical constituents like glycosides, flavonoids, tannins etc. [3,4]. *S. asoca* dry bark and flowers are used as a tonic for women experiencing uterine problems in India [5,6]. It is a sacred tree in India, famous for usage in the management of gynecological and is particularly useful as an astringent to treat menorrhagia [7,8]. 'Asokarishtam', the fermented formulation of 'Asokam', is used as a tonic for menorrhagia. The bark of asoka finds its use in conditions such as biliousness, indigestion, diarrhea, colitis, hemorrhoids, sores, and acne [8] and claimed that the alcoholic extract has potential antibacterial action against a wide spectrum of microorganisms. It's applied topically to cure bites, ulcers, and discoloration of the skin [9]. This plant's phenolic glycoside has also been found to have oxytocic action [10]. Apart from the major constituents, a class of compounds such as tannin, catechol, ketosterol have also been isolated from the bark. Due to the wide spectrum of properties of *S. asoca*, the plant has become over exploited and the size of natural populations has been declining over the years in the country. The pharmaceutical industry in india requires about 5300 tonnes of bark annually. The annual consumption of 'Asokam' in the ayurvedic drug industry in kerala is about 105 tonnes/year [11]. This tree's native habitat has been nearly destroyed as a result of its widespread usage. Due to the paucity of this tree,

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the bark of other adjacent or unrelated species has been substituted. Other *Caesalpiniaceae* members, particularly *C. travancorica* are commonly used as substitutes. The therapeutic properties of *C. travancorica* aren't well understood. Hence, work was carried out to analyze, compare and assess the pharmacological and toxicological properties of *S. asoca* and its substitute, *C. travancorica*.

2. METHODS

2.1. Collection plant samples

S. asoca, and *C. travancorica* (Figure 1) were collected from different parts of the western ghats like Trissur, Munnar, Wayanadu, and Nelliyampathy areas and authenticated by Mr. Harinarayanan CM, Scientist, Pharmacognosy Division, Arya Vaidyasala, Kottakkal, Malappuram, Kerala, India. A sample of the dried plant were recorded in the Herbarium of the centre for medicinal plants research (CMPR), Arya Vaidyasala, Kottakkal (No.148363).

Figure 1. Saraca asoca and Cynometra travancorica

2.1. Preparation of plant sample

S. asoca and *C. trvancorica* barks were properly collected, cut into tiny pieces, carefully cleaned with distilled water, shade dried, then air dried for 7 days at room temperature. The dried materials were ground in an electric blender (Cookman pulverizer 0.2hp), and the powdered sample of 150g was extracted using 500ml of methanol in a Soxhlet apparatus for 48 hours at 50°C. The yield of extract was found to be 13.56±0.75% and was stored at 4°C. The extracts were agitated and filtered on a periodic basis. After solvent evaporation, the filtrate yielded an active solid residue that was kept in a desiccator (Borosil 100mm Flenge 3082041) for later use.

2.3. Selection of Animals

The Wistar female rats and Swiss albino mice (aged 7 weeks) were bought from the Small Animal Breeding Station (SABS), College of Veterinary, Agricultural University, Thrissur, Mannuthi, Kerala, and were housed in a controlled settings (22-28^{0}C temperature, 60-70% relative humidity, 12 h dark/light cycle) in the animal house of Al Shifa College of Pharmacy. The rats and mice were provided normal rat and mouse diet (Sai Durga Feeds and Foods, Bangalore, India) as well as free access to water. All animal experiments in this study were approved by the Institutional Animal Ethics Committee (IAEC) and carried out strictly according

to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) established by the animal welfare division of the Indian government.

2.4. Toxicity study

The acute oral toxicity study was carried out as per OECD guidelines 423. Swiss albino mice were divided into three groups, each with six animals. Animals were sacrificed 21 days after treatment with each extract dose of 600 and 800 mg/kg including normal (excluding lethality test animals at doses of 800 mg/kg, Table. 1) and organs were collected for hematological and histopathological evaluation.

2.5. Anti-estrogenic activity

Wistar female rats were used to test the anti-estrogenic action of methanolic extracts produced using *S. asoca* and its replacement plant, *C. travancorica*. The animals were split into four groups, each with six animals. The first group was kept as control (vehicle only), second group was administered alone with estrogen (i.p). The third and fourth groups received 400mg/kg of each extract dissolved in DMSO (orally). After 10 days of the treatment, blood was collected from all animals and serum was separated. The amount of estrogen was analyzed by radioimmunoassay (Gamma Counter WIZARD2).

2.6. Method of anesthesia

For acute oral toxicological evaluation, the Swiss albino mice (except lethality test animals) undergo euthanasia (OECD Guideline 420) for post-operational studies. (Pentobarbital sodium 25mg/kg i.p Mfg. by Vysali Pharmaceuticals, Kochi, Kerala, India).

3. RESULTS

3.1. Anti-estrogenic activity

Administration of extract prepared with *S. asoca* and its substitute *C. travancorica* were found to be effective in preventing increased estrogen levels in Wistar female rats (Figure 2). On the 10^{th} day of the treatment blood was collected from each Animals and serum was separated. The level of estrogen in the normal rat was 55.88±8.23 pg/ml. When administered estradiol, the level was increased to 256.5±17.66 pg/ml. Estrogen levels were significantly lower in both extract-treated groups. The *C. travancorica* extract-treated group showed the greatest decrease (85.63 ± 11.38 pg/ml).

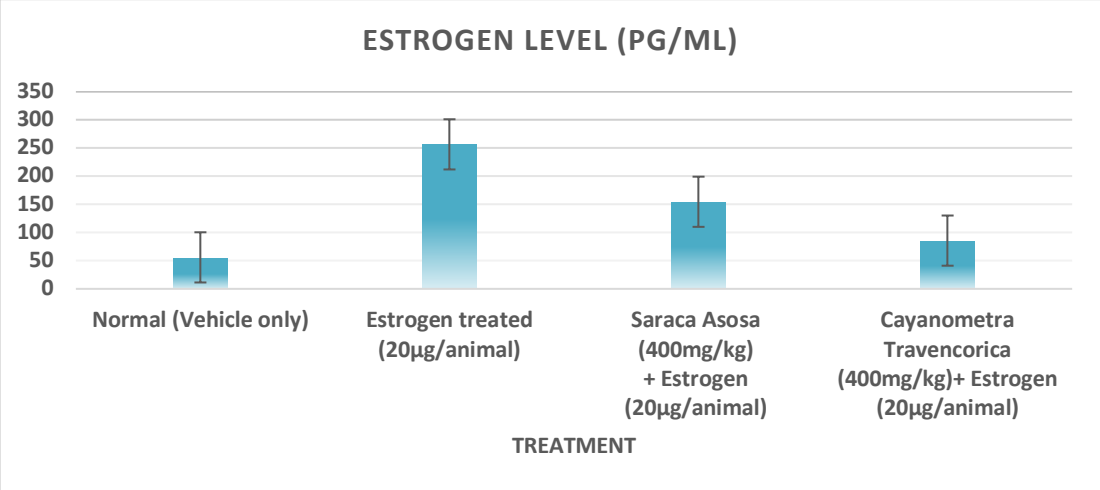

Figure 2. Effect of each extract in anti-estrogenic activity. Values are mean ± SD; for six animals in each group. **P value < 0.001.

3.2. Acute Toxicity study

There were no signs of toxicity or deaths observed in mice. Animals given each extract at an 800 mg/kg dosage for 14 days had no mortality (Table 1). This indicated that extracts prepared with *S. asoca*, and *C. travancorica,* even at a very high concentration, were not lethal to mice. Acute administration of each extract did not result in a substantial reduction in body weight, food and water consumption (Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, and Figure 7). As evidenced by weight changes, concentrations far higher than the therapeutic dosage in humans did not cause toxicity in mice. The weight of organs such as the liver, kidney, spleen, heart, and lungs (Table 2) and the hepatic parameters such as SGOT, SGPT, Bilirubin, Albumin & Globulin (Table 3) did not change significantly after administration of each extract. Renal function and hematological parameters were all within normal limits (Table 4, Table 5 and Table 6).

Table 1. The mortality rate of each extract during the acute toxicity.

Group	**Dose** (mg/kg)	**Mortality**
S. asoca	800 (mg/kg)	Nil
C. travancorica	800 (mg/kg)	Nil

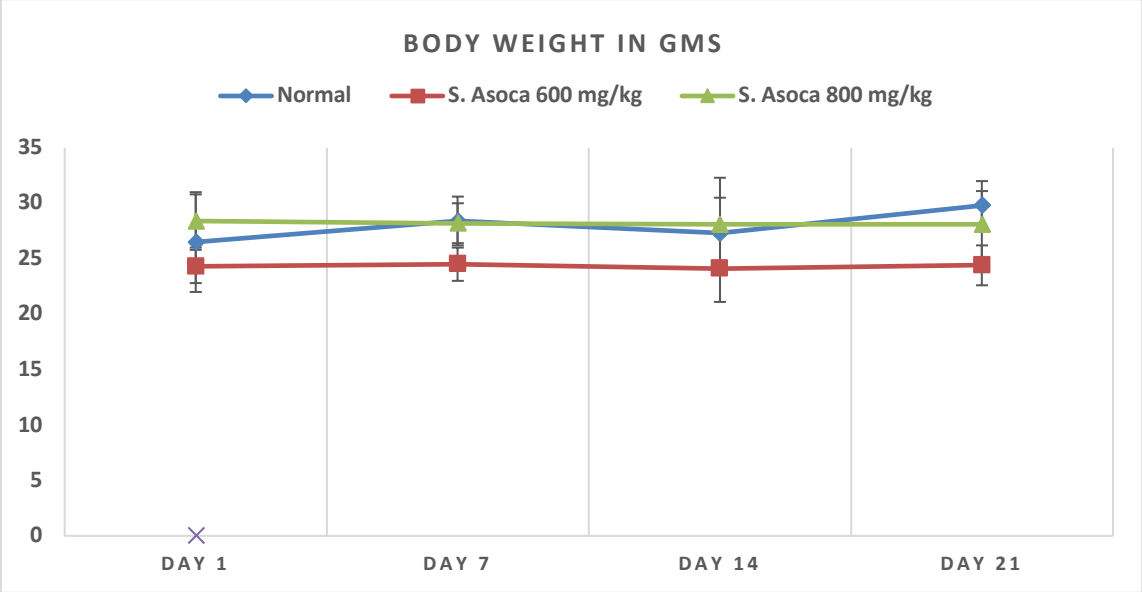

Figure 3. The effect of *S. asoca* extract on the body weight of animals. Values are mean ± SD of 6 animals / group.

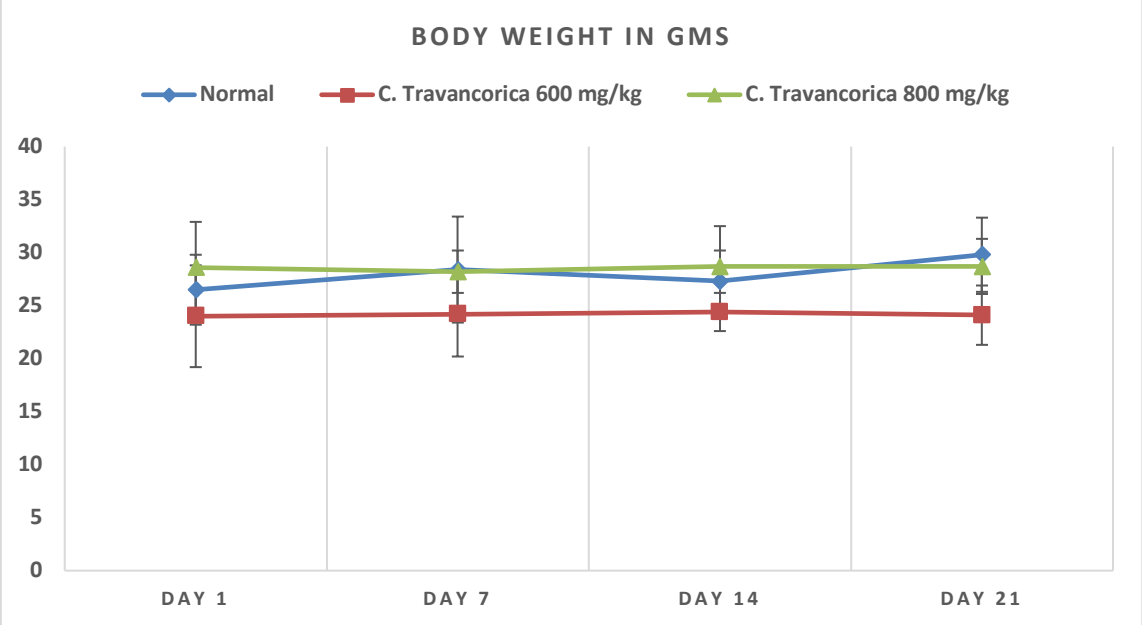

Figure 4. The effect of a *C. travancorica* extract on the body weight of animals. Values are mean ± SD of 6 animals / group.

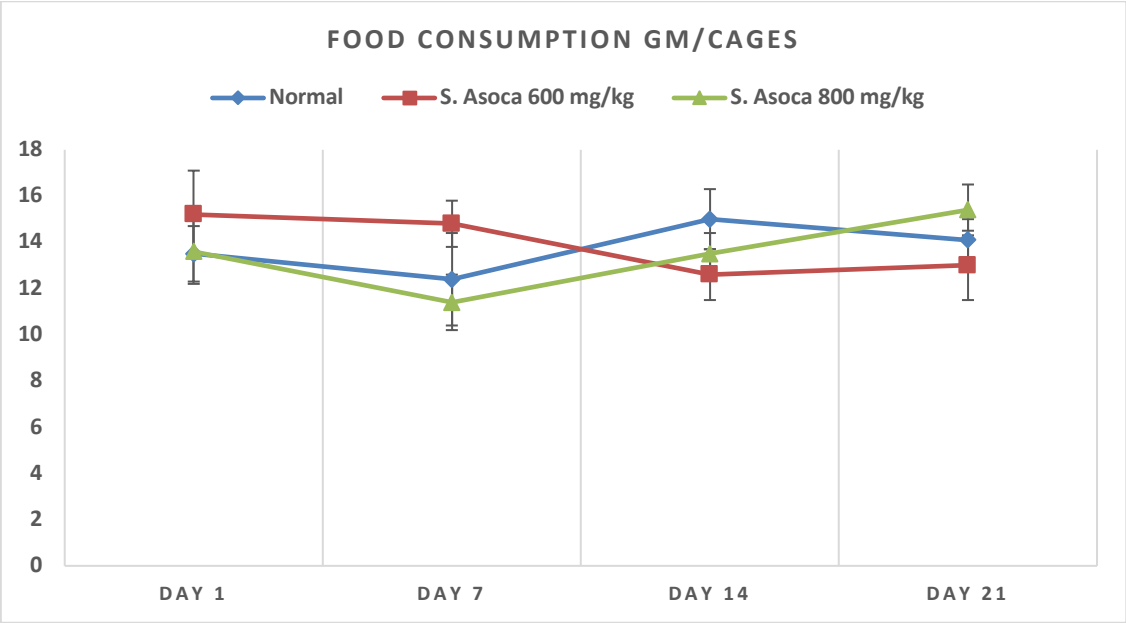

Figure 5. Effect of *S. asoca* extract on food consumption of animals. Values are mean ± SD of 6 animals / group.

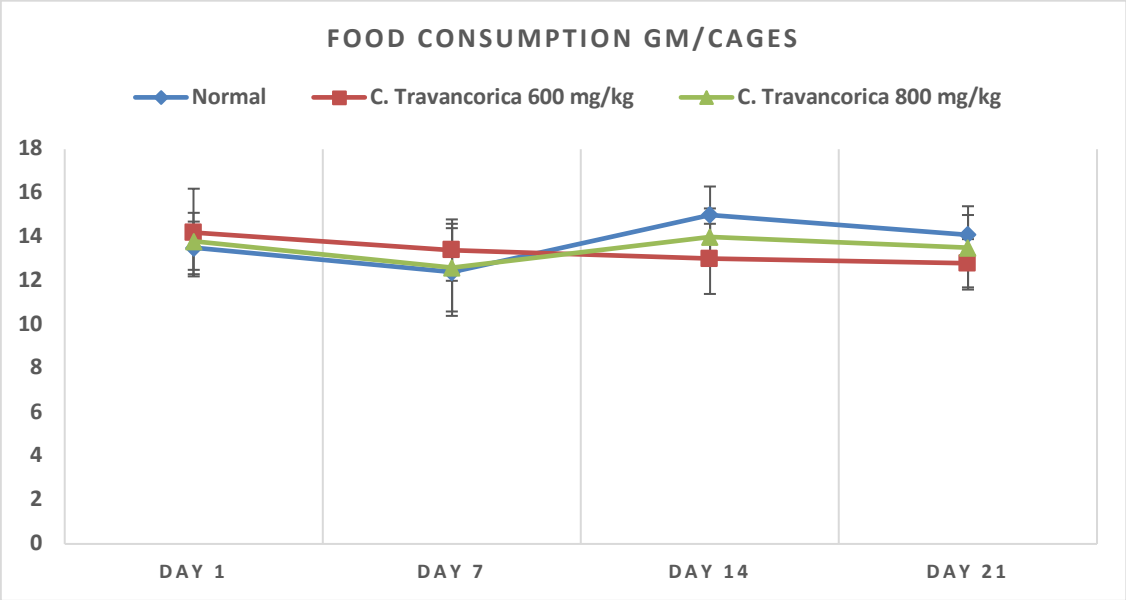

Figure 6. Effect of *C. travancorica* extract on food consumption of animals. Values are mean ± SD of 6 animals / group.

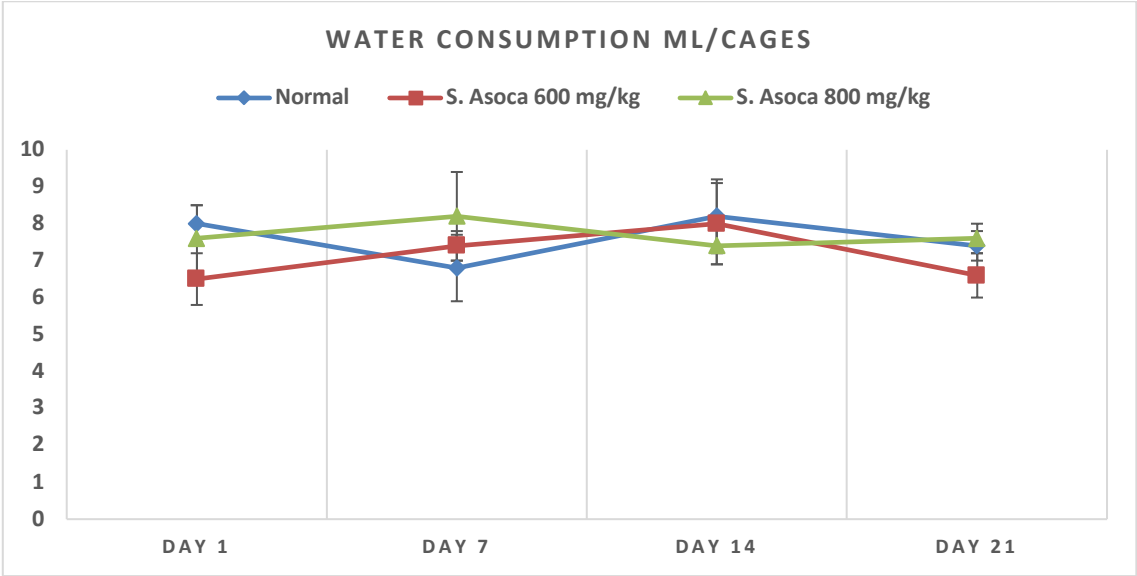

Figure 7. Effect of *S. asoca* extract on water consumption of animals. Values are mean ± SD of 6 animals / group.

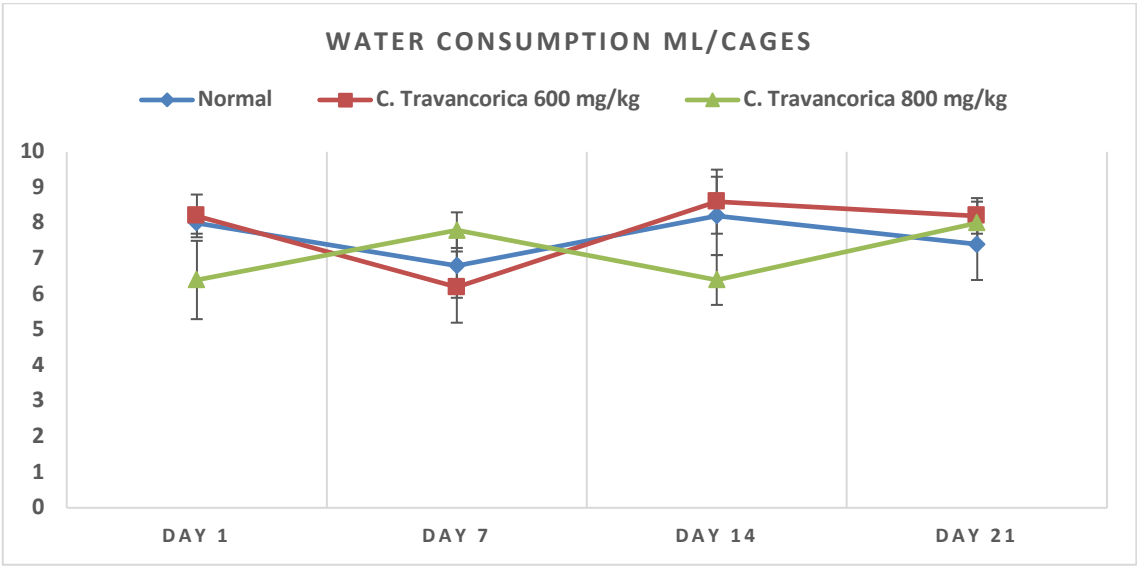

Figure 8. Effect *C. travancorica* extract on water consumption of animals. Values are mean ± SD of 6 animals / group.

Table 2. Effect of acute administration of each extract on organ weight. Values are mean ± SD of 6 animals / group and expressed as the organ weight / 100g of body weight.

Group	Liver (g)	Kidney (g)	Spleen (g)	Heart (g)	Lungs (g)
Normal	1.425±0.47	0.442 ± 0.21	0.103 ± 0.018	0.131±0.03	0.257±0.052
S. asoca 600 mg/kg	1.42±0.19	0.415±0.036	0.097 ±0.016	0.145±0.021	0.289±.05
S. asoca 800 mg/kg	1.54±0.19	0.415±0.036	0.097± 0.02	0.151±0.017	0.305±0.071
C. travancorica 600 mg/kg	1.359±0.15	0.361±0.029	0.107±0.021	0.135±0.011	0.243±0.008
C. travancorica 800 mg/kg	1.328±0.16	0.367±0.028	0.112±0.032	0.138±0.005	0.277±0.06

Table 3. Effect of acute administration of each extract on Liver Function test. Values are mean ± SD of 6 animals / group.

Group	SGOT (U/L)	SGPT (U/L)	Bilirubin (mg/dL)	Total Protein(g /dL)	Albumin (g/dL)	Globulin (g/dL)
Normal	154.2± 29.63	88.42± 22.15	0.2±0.11	6.28±0.5	2.82±0.53	2.1±0.24
S. asoca 600 mg/kg	174.8± 41.12	70.5±12.63	0.16± 0.09	6.92±0. 58	3.25± 0.17	1.8±0.14
S. asoca 800 mg/kg	166.1±39.6	65.4±30.52	0.22±0.17	7.77±0.29	3.55±0.49	2.4±0.56
C. travancorica 600 mg/kg	149.5± 50.53	82.9±19.4	0.20.88	7.48±1.2	3.54±0.19	2.5±0.34
C. travancorica 800 mg/kg	162.2± 42.43	76.2±27.83	0.17±0.13	8.34±0.98	3.23±0.65	3.2±0.29

Table 4. Effect of acute administration of each extract on Renal Function test. Values are mean ± SD of 6 animals / group.

Group	Creatinine (mg/dL)
Normal	0.53 ± 0.09
S. asoca 600 mg/kg	0.62 ± 0.11
S. asoca 800 mg/kg	0.73 ± 0.88
C. travancorica 600 mg/kg	0.63 ± 0.08
C. travancorica 800 mg/kg	0.52 ± 0.17

Table 5. Effect of acute administration of each extract on Haematological parameters. Values are mean ± SD of 6 animals / group.

Group	Hb (g/dL)	WBC (mm^3)	RBC (10^6/cmm)	Platelet (10^5/cmm)
Normal	12.37 ± 0.697	9170±149.88	7.86±0.6	6.48±1.84
S. asoca 600 mg/kg	13.18 ± 1.09	8450 ±528.98	6.89±0.81	5.89±2.19
S. asoca 800 mg/kg	13.64 ± 0.687	7445±527.89	7.01±0.98	6.68±1.15
C. travancorica 600 mg/kg	13.88 ± 1.28	7960±484.8	6.4±0.59	6.19±1.25
C. travancorica 800 mg/kg	12.73 ± 0.75	7450±511.9	7.5±0.69	5.79±1.08

Table 6. Effect of acute administration of each extract on Haematological parameters on differential counts. Values are mean ± SD of 6 animals / group.

Group	Lymphocyte (mm^3)	Eosinophils (mm^3)	Basophils (mm^3)	Neutrophils (mm^3)	Monocytes (mm^3)
Normal	6450.4±324.52	256±40.83	227±52.27	1264±70.5	225.4±64.78
S. asoca 600 mg/kg	5974.8±412.25	196.2±40.31	204±44.2	1064±126.5	210±72.1
S. asoca 800 mg/kg	5780.6±246.85	224±74.81	196±33.8	1664±39.1	273±19.4
C. travancorica 600 mg/kg	5935±386.62	268.6±164.5	188.5±57.6	1702±105.6	205.7±69.5
C. travancorica 800 mg/kg	6056±340.6	21304±94.6	198.3±86.63	1468.5±128.5	176.4±26.98

4. DISCUSSION

The existence of numerous biologically active elements known as secondary metabolites that generate a defined pharmacological action on the human body may explain the therapeutic capabilities of medicinal plants [12,13]. Increased usage of synthetic fertilizers and pesticides may result in a decrease in secondary metabolite production and a build-up of hazardous compounds in plant components [14]. As a result, in the phytochemical industry, plant identification, chemical composition analysis, and biological property analysis are all critical. Some laboratories have already begun analytical parameter investigations to evaluate active component concentrations, pesticide contamination, fertilizer contamination, hormone contamination, mycotoxins contamination, and overall efficacy of medicinal plants. Herb substitution accomplished a number of objectives, the most important of which was to generate therapeutic effects that were similar to those of the original medication [15]. The ayurvedic preparation of the plant, *S. asoca,* is widely recommended for excessive menstrual bleeding, bleeding hemorrhoids, bleeding ulcers, and hemorrhagic dysentery etc. [6]. The anti-

estrogenic activity of extract prepared with *S. asoca* and its substitute was measured and the result showed a significant decrease in the increased estrogen level in Wistar female rats. The gonads release estrogens and androgens, which are essential for embryonic maturation and the development of main and secondary sexual characteristics. At puberty, the hormones from the hypothalamus and anterior pituitary (FSH & LH) stimulate the ovary (granulosa cells) to secrete estrogen. Thus, secreted estrogen will stimulate the lactiferous duct and, after parturition, estrogen, along with prolactin, is responsible for stimulating and maintaining lactation. The starting substance for estrogen synthesis is cholesterol. The three mains endogenous estrogens are estrone, estriol and estradiol. Estradiol, also known as 17 estradiol, is the most potent estrogen generated and released by the ovary and the key estrogen in menopausal women. Estriol is the primary estrogen generated by the placenta, while estrone is a by-product of estradiol. The level of estrogen in the blood will rise in cases of breast and ovarian cancer [16]. In our study, the increased level of serum estrogen in Wistar female rats (by the administration of estradiol) reached a near-normal level by the treatment of extract prepared with *C. travancorica*. So, this antiestrogenic property exhibited by this extract points out its therapeutic efficacy against estrogen-sensitive breast cancer. Most of the patients reported with breast cancer are estrogen sensitive and show a higher level of serum estrogen level. The study for the estrogen reductive property mechanism has not yet been conducted. So, here the results show a preliminary response of each extract towards estrogen sensitive breast cancer. More research is necessary to confirm the whole mechanism of action of this extract at the clinical level, as well as whether it may be used in place of *S. asoca*.

5. CONCLUSIONS

The current study's findings clearly show that extracts made with *S. Asoca* and its replacement *C. Travancorica* reduce elevated oestrogen levels by a substantial amount. The administration of each extract did not result in any fatality in the toxicity trial, even at higher dosages of 600 and 800 mg/kg. The animals' body weight and food consumption did not change significantly, showing that the extracts had no harmful effects even at greater doses. The anti-estrogenic activity results demonstrate that the extract made using *S. Asoca* and its replacement plant, *C. Travancorica*, has a strong anti-estrogenic effect. The elevated level of oestrogen in serum was considerably reduced by treating with each extract, and the preparation of *C. Travancorica* exhibited a percentage decrease in the oestrogen level approaching normal, according to the results collected. The study's findings offered a preliminary biological foundation for using *C. Travancorica* as a replacement for *S. Asoca* in medicinal formulations. Almost all ayurvedic medications are now tainted. Because of the adulterations, there is a broad range of quality control. As a result, ayurveda has been unable to leverage on its richness by spreading its use. The majority of ayurvedic formulations using *S. Asoca* are contaminated with *C. Travancorica* and other *Caesalpinaceae* species. As a result, quality assurance is a vital aspect of all medical systems today in order to provide high-quality medicine.

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Author contributions

Concept – Suhail PT and Sheron Joseph; Supervision – Sheron Joseph; Resources and Materials– Suhail PT and Ajeesh V; Data Collection and Processing – Suhail PT and Sreelakshmi SS; Analysis and Literature Search – Suhail PT, Sreelakshmi SS and Krishnapriya Anil.

Conflict of interest statement: The authors declare no conflict of interest in the manuscript.

Ethics Committee Approval: All the animal experiments related to the work is carried out from Al Shifa College of Pharmacy, approved by CPCSEA, Registration No. 1195/PO/Re/S/08/CPCSEA.

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