



INVESTIGATION OF ESTROGENIC POTENTIAL OF *SARACA INDICA* LINN. IN BILATERALLY OVARIECTAMISED SWISS ALBINO RATS

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Abstract

The stem bark of *Saraca indica* has stimulating effect on endometrium and the ovarian tissue. It is useful in dyspepsia, fever, biliousness, burning sensation, abnormal enlargement of visceral organs, colic dysentery, internal bleeding, haemorrhoids, ulcers, uterine affections, menorrhagia due to uterine fibroids, meno-metrorrhagia, leucorrhoea and pimples. In this study, we conducted a study of estrogenic activity of alcoholic extract of stem bark of *Saraca indica* with diethylstilbestrol in bilaterally ovariectomized young albino rats. Bilaterally ovariectomized albino rats were divided into five groups (n = 6) receiving different treatments, consisting of vehicle (0.6% w/v sodium carboxy methyl cellulose), ethanolic extract of stem bark of *Saraca indica* at three different doses (*viz.*, 100, 200, 400 mg/kg body weight) and standard drug diethylstilbestrol (DES) at a dose of 2 mg/kg body weight. All these were administered orally daily for 7 days. Estrogenic activity was assessed by taking percentage vaginal cornification, uterine wet weight, uterine glycogen content and uterine histology as parameters of assessment. Alcoholic extract of stem bark of *Saraca indica* showed a significant increase in percentage vaginal cornification, uterine wet weight ($P < 0.001$), uterine glycogen content ($P < 0.001$) and a proliferative changes in uterine endometrium compared to the control.

Key words: *Saraca indica*, diethyl stilbesterol, estrogenic activity.

Introduction

Saraca indica, popularly known as “Ashoka,” is distributed from India to South-West China via Malaysia, Sumatra and east to Celebes. In India, two species have been recorded. It is a small evergreen tree, 6-9 m tall, found wild along streams or in the shade of evergreen forests. It is used as a shade tree for coffee planting. In ancient times, Charaka and Sushruta used flower buds, seeds and bark of *Saraca indica* in internal medicines for haemorrhages, haemorrhages, gynecological disorders; asthma, sciatica, neuralgia and neurological disorders. Milk boiled with Ashoka bark is utilised for menorrhagia. Ashoka seeds are used for the measurement and preservation or removal of urine. It is widely used in the treatment of excessive uterine bleeding, dysmenorrhoea, leucorrhoea and depression in women. Indian medicine use bark and flowers for biliousness,

dyspepsia, colic dysentery, hemorrhagic diarrhea, clusters, ulcers and pimples.

Materials and Methods

Estrogens activate secondary sex structures in mammals (Branham *et al.*, 1993). Bioassays can be carried out using different methods, using different animal species and specific criteria for the efficacy evaluation. Female rats or mice are commonly used. If oestrogens are given to immature rats, a significant rise in uterus size is achieved.

Ovariectomy, results in lack of estrogen leading to uterine atrophy. This is typically quantified using parameters such as increase the weight of the uterus compared to the standard. Estrogens also induce vagina opening, which can be used as a quantum response to a specific dose in a group of immature animals (Williamson *et al.*, 1996).

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Various methods available for testing of estrogenic activity of drugs on the experimental animals are as follows:

Vaginal cornification

Allen and Doisy, (1923) suggested vaginal cornification as a method for estrogen assay. Vaginal smear preparation can reveal vaginal epithelium changes *viz.* thickening and keratinization and shedding of superficial cells. The accuracy of this system depends mainly on five main factors *viz.* total ovariectomy, priming of the animal (Suchowsky, 1964).

Uterine Weight

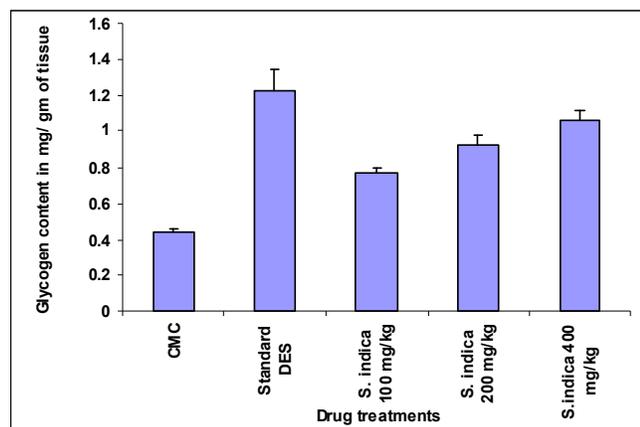
This bioassay is based on endometrial proliferation. If estrogens are administered, the uterine weight can rise within a few hours due to intrauterine fluid accumulation (Suchowsky, 1964). Repeated administration of estrogens causes a dose-dependent rise in uterine weight in castrated female rats (Vogel, 2002).

Vaginal Opening

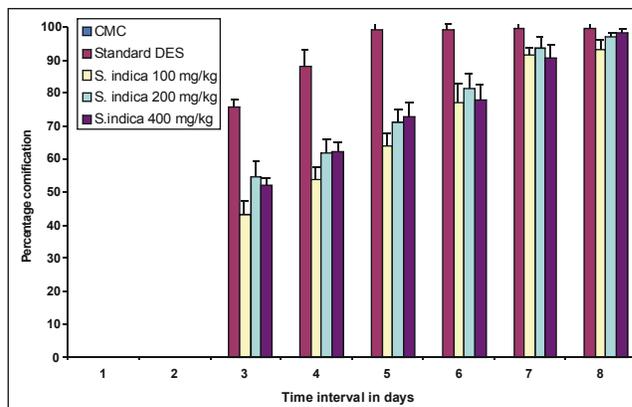
Estrogens also allow the vagina to open. The time of vaginal opening may provide an accurate and reliable index of estrogen production (Suchowsky, 1964). The membrane that covers the vagina sheds as the estrous begins. It regains normalcy after four days. During the opening phase, a pinpoint opening at any point of the crescent, which may increase in size can be observed. There is mucus secretion and vaginal cornification when complete opening occurs. The time of opening is related to the quantity of estrogen administered (Young, 1972).

Uterine Glycogen Content

Estrogens raises uterine glycogen content within six hours of administration and maximum concentrations are reached within 24-48 hours of single treatment (Bitman and Cecil, 1970). Hence used as a functional biochemical marker for the assessment of estrogen production



Graph 1: Showing the effect bark extract of *S. indica* on glycogen content of uterus of rat.



Graph 2: Showing the effect of *Saraca indica* alcoholic extract on the percentage of vaginal cornification in bilateral ovariectomised albino rats.

(Tripathi, 1983).

Methodology

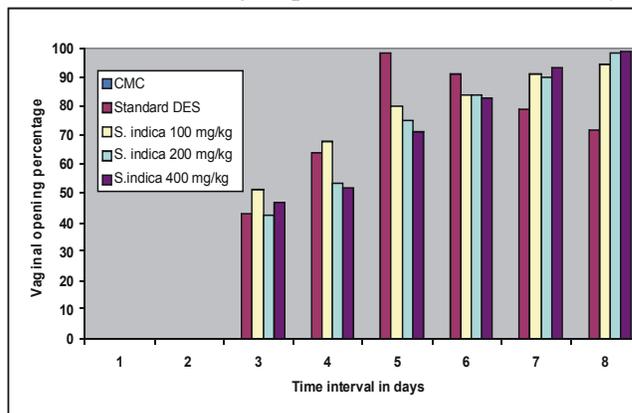
Estrogenic activity of the alcoholic extract of *Saraca indica* bark was evaluated in young albino rats using a standardized procedure with few modifications. (Jonathan *et al.*, 1995).

Chemicals used:- Diethylstilbestrol (0.2 mg/ml suspension).

Anthrone reagent (0.15% w/v in 95% v/v sulfuric acid), Pentobarbitone anesthesia Haematoxylin, eosin and methylene blue stains, Bouin’s fluid fixative, Sodium carboxy methyl cellulose, Ice cold ethyl alcohol, 30% ice cold potassium hydroxide solution, Saturated sodium hydroxide.

Procedure

Ovariectomized rats were housed in polypropylene cages and fed with normal rat feed and ad libitum water. Animals were allowed a period of 15 days for recovery. Following surgery, vaginal tests were taken daily to screen for residual estrogen production. After 15 days,



Graph 3: Showing the effect of *Saraca indica* alcoholic extract on percentage of vaginal opening in bilateral ovariectomised albino rats.

experimental rats were divided into five treatment groups, each of which consisted of 6 rats to test the estrogenic activity of *Saraca indica* bark alcoholic extract.

Treatments

For the assessment of estrogenic activity, following doses of *Saraca indica* were administered orally for 7 days daily for 5 groups of rats.

Statistical analysis

One way analysis of variance followed by Dunnett's test was employed to analyze the difference in percentage of uterine wet weight, uterine glycogen content between different treatment groups of rats.

Results and Discussion

Uterine wet weight

The *S. indica* bark extract showed a significant increase in uterine wet weight compared to control rats.

Table 1: Treatment groups.

Experimental animals	Treatment
Group 1	sodium CMC 0.6% w/v
Group 2	Diethylstilbestrol (2mg/kg in 0.6% CMC)
Group 3	extract of <i>S. indica</i> at a dose of 100 mg/kg,
Group 4	extract of <i>S. indica</i> (200 mg/kg)
Group 5	extract of <i>S. indica</i> (400 mg/kg)

Table 2: Effect of different doses of *S. indica* bark extract on uterine wet weight, glycogen content and histological observations of rat uterus.

Group	Treatment (p.o.)	Dose (mg/kg)	Uterine wet weight (mg)	Uterine glycogen content ($\mu\text{g}/\text{mg}$ of uterus)	Uterine histology
1	Control 0.6% w/v Sod.CMC	-	93.85 \pm 1.79	0.439 \pm 0.021	uterine endometrium disintegrated.
2	Standard DES	2	210.7 \pm 2.47*	1.226 \pm 0.125*	Height of luminal epithelium increased and number of glands increased.
3	<i>Saraca indica</i> extract	100	157.3 \pm 1.09*	0.768 \pm 0.028*	
4		200	181.8 \pm 2.06	0.9280 \pm 0.048*	
5		400	204.3 \pm 2.11*	1.058 \pm 0.063*	

*p<0.01 compared to control group

There was 1.68, 1.94 and 2.18 fold increase in uterine wet weight at doses of 100, 200, 400 mg extract per kg weight, respectively. On the other hand, DES demonstrated a statistically significant, 2.25 fold rise in uterine wet weight as opposed to control at a dose of 3 mg / kg. (Table 1).

Uterine histology

Saraca indica's bark extract induced proliferative changes in rat's uterine endometrium as evidenced by increased luminal epithelium height, with loose stroma and increased gland count (Fig. 6), compared to control. The uterine endometrium had been disintegrated in the control rats (Fig. 4). Specific proliferative changes were also caused in DES (Fig. 5).

Uterine glycogen content

A statistically significant dose-dependent increase in uterine glycogen after administration of *Saraca indica* extract was observed. A dosage of 100, 200 and 400 mg / kg showed statistically significant rises in uterine glycogen content compared to control by 1.74, 2.11 and 2.41 fold, respectively. Treatment with DES also showed a statistically significant 2.79 fold increase compared to rat control (Table 2).

Vaginal cytology

During the treatment time, vaginal smear of

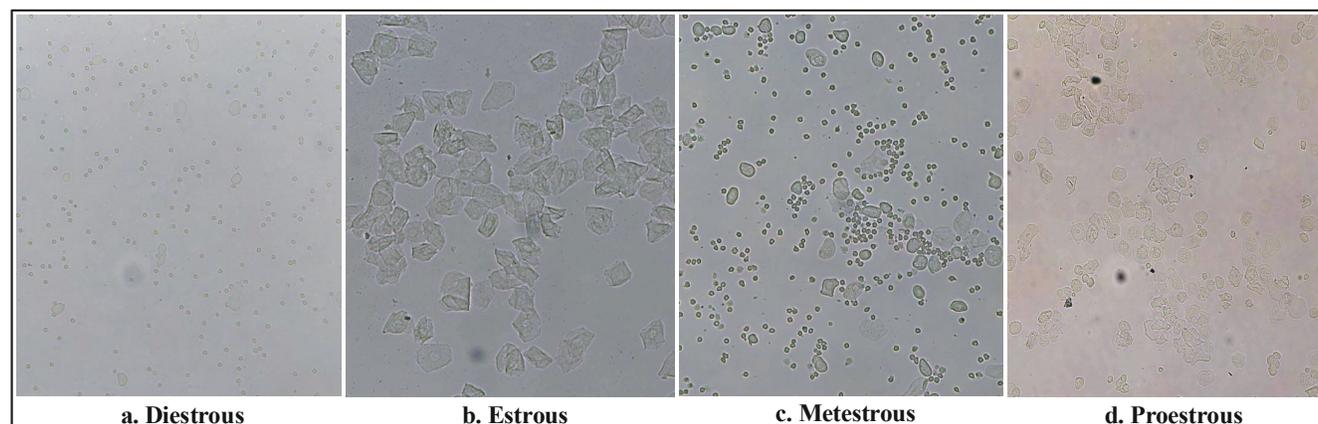


Fig. 1: Photomicrograph showing different stages of the albino rat estrous cycle.

ovarectomized control rats showed no vaginal cornification (Fig. 1), where as an alcoholic bark extract

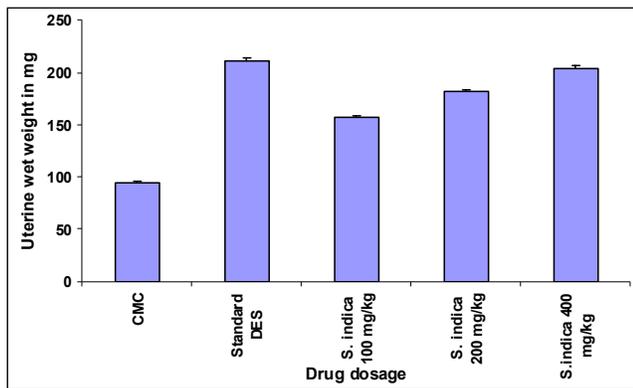


Fig. 1: Effect of different doses of *S. indica* bark extract on rat uterine wet weight.

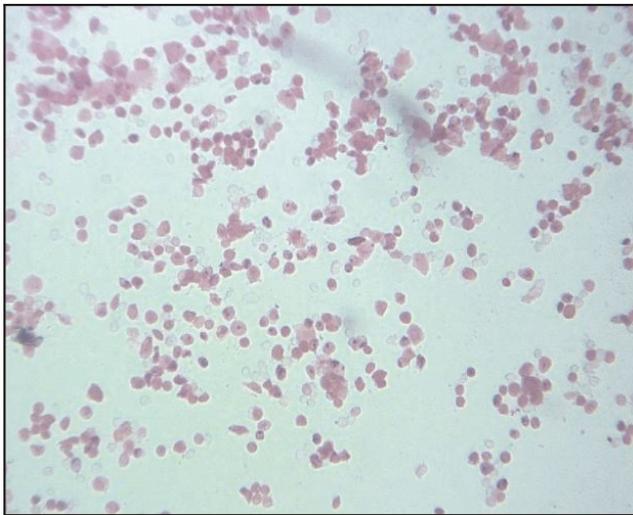


Fig. 2: Displaying control rat photomicrograph ($\times 100$) vaginal smear, displaying only leukocytes (*i.e.*, diestrous).

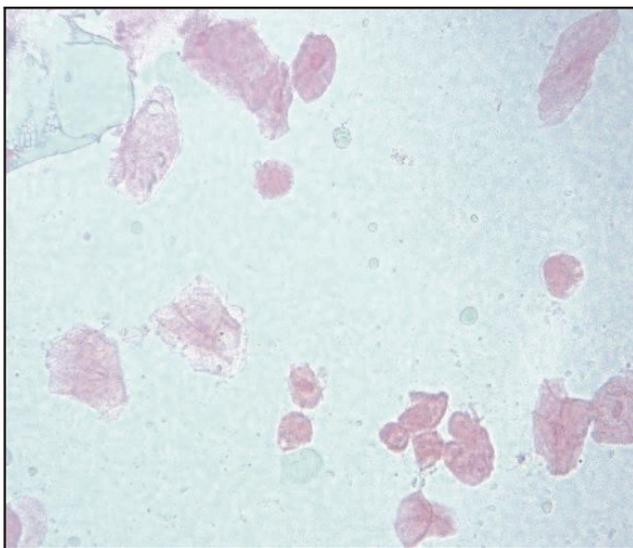


Fig. 3: Vaginal smear of diethyl stilbestrol (2 mg / kg, p.o.) treated rat showing only cornified epithelial cells (*i.e.*, in estrous stage) with photomicrograph ($\times 100$).

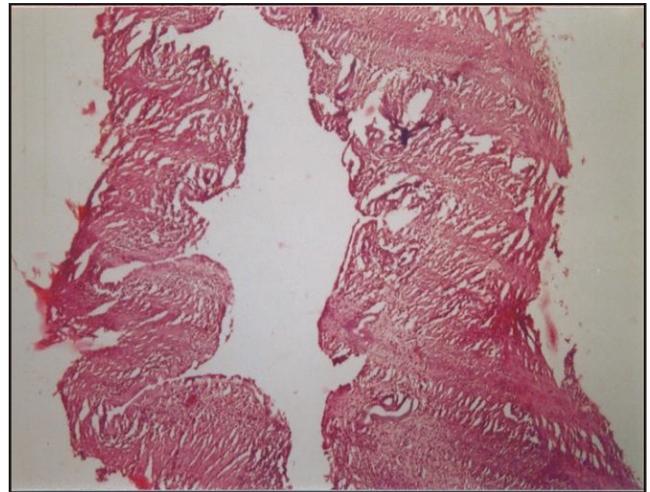


Fig. 4: Displaying photomicrograph ($\times 100$) transverse portion of control rat uterus displaying a disintegrated endometrium.

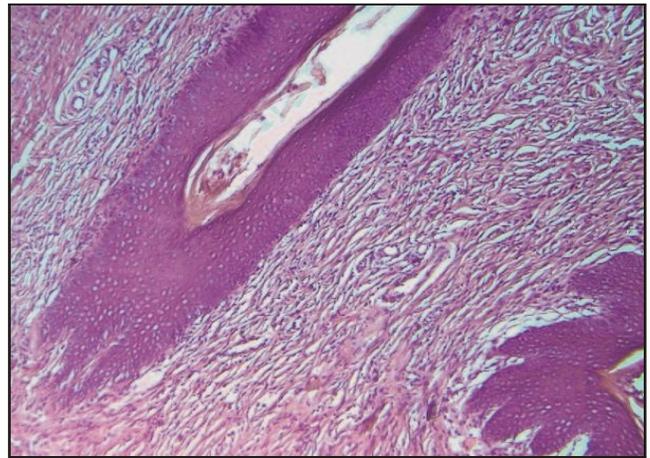


Fig. 5: Displaying transverse uterus photomicrograph ($\times 100$) of diethyl stilbestrol (2 mg / kg, p.o.) treated rat, displaying proliferative stage (*i.e.*, stimulated endometrium with loose stroma and glands).

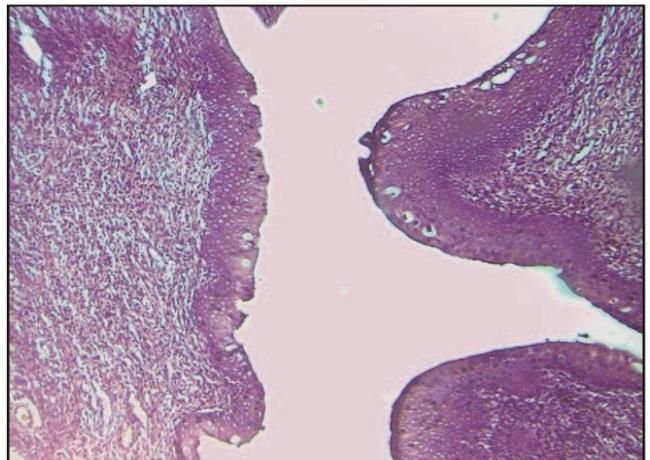


Fig. 6: Showing transverse portion of *Saraca indica* extract (400 mg / kg, p.o.) treated rat photomicrograph ($\times 100$), showing proliferative stage (*i.e.* stimulated endometrium with loose stroma).

Table 3: Effect of alcoholic extract of *S. indica* on vaginal cornification percentage in bilaterally Ovariectomized albino rats.

Treatment group	Dose (mg/kg) (p.o.)	VAGINALCORNIFICATION (%)							
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Control, 0.6% w/v Sod.CMC		0	0	0	0	0	0	0	0
Standard, DES	2	0	0	75.48±2.23	88.35±4.58	98.98±2.78	99.01±1.91	99.57±3.05	99.8±2.73
<i>Saraca indica</i> extract	100	0	0	43.4±3.96	53.9±3.74	63.8±3.95	77.2±5.65	91.3±2.44	93.5±2.61
	200	0	0	54.7±4.45	61.89±4.39	71.2±3.83	81.4±4.79	93.8±3.41	96.8±1.73
	400	0	0	52.1±2.4	62.2±2.85	72.9±3.89	77.7±4.98	90.8±3.7	98.5±1.07

Table 4: Effect of *Saraca indica* alcoholic extract on vaginal opening in bilateral ovariectomised albino rats.

Treatment (p.o.)	Dose (mg/kg)	VAGINAL OPENING (%)							
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Control, 0.6% w/v Sod.CMC		0	0	0	0	0	0	0	0
Standard, DES	2	0	0	43	64	98	91	79	72
<i>Saraca indica</i> extract	100	0	0	51	68	80	84	91	94
	200	0	0	42	53	75	84	90	98
	400	0	0	47	52	71	83	93	99

Table 5: Effect of alcoholic extract of *Saraca indica* on vaginal opening in bilaterally ovariectomized albino rats.

Treatment (p.o.)	Dose (mg/kg)	VAGINAL OPENING (%)							
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Control, 0.6% w/v Sod.CMC		0	0	0	0	0	0	0	0
Standard, DES	2	0	0	43	64	98	91	79	72
<i>Saraca indica</i> extract	100	0	0	51	68	80	84	91	94
	200	0	0	42	53	75	84	90	98
	400	0	0	47	52	71	83	93	99

of *Saraca indica* administration showed a dose-dependent increase in vaginal cornification percentage from day 3 onwards. It was found that the proportion of vaginal cornification at a dose of 400 mg / kg was equal to that of DES (Fig. 3 and Table 3).

Vaginal opening

From day 3 onwards, the alcoholic extract showed a dose-dependent increase in vaginal opening compared to control rats, in which it remained close. Treatment with DES also demonstrates a rise in rat vaginal opening (Table 3).

Under the influence of ovarian hormones such as estrogen, uterus and female reproductive tract undergo countless physiological and biochemical changes (Prakash and Mathur, 1979). If female rats are ovariectomised, the resulting lack of estrogen causes uterine atrophy and reproductive tract. The administration of ovariectomized rat oestrogenic substances contributes to uterotrophic effects, vaginal cornification, vagina opening, increased uterine glycogen content and proliferative changes in uterine endometrium (Williamson *et al.*, 1996).

From this research, it has been found that the administration of *Saraca indica* alcoholic extract at

different doses produced a significant increase in uterine weight; it also caused an increased percentage of vaginal opening and a percentage of cornification. The rise in wet uterine weight was incremental and progressive with dose increase. Histological analysis of the uterus of rats treated with extract revealed estrogenic effect as demonstrated by increased luminal epithelium height with loose stroma and increased gland count.

Alcoholic bark extract administration increased the amount of uterine glycogen in a dose-dependent pattern. A triple increase in uterine glycogen content was observed at a dose of 400 mg / kg which is consistent with earlier observations made by Prakash and Mathur, (1979) that ovariectomized rat treatment with estrogen induces a 3-4 fold increase in uterine glycogen content. Uterine glycogen function is not fully understood in rats. Estrogens have been reported to increase the transport of hexose into the rat uterus and thus increase glycogen synthesis in the uterus. An rise in the uterine glycogen content of ovariectomized rats under the influence of *Saraca indica*'s alcoholic extract can be attributed to their estrogenic activity.

The effect of the 200 mg / kg *Saraca indica* extract

was found to be nearly equivalent to the 2 mg / kg DES dose. Therefore it can be inferred that the *Saraca indica* extract has 1/100th the potency of regular DES product. It can also be speculated that the flavonoids and phenolic compounds present in the alcoholic extract may be responsible for the *Saraca indica* bark's estrogenic activity, since it is understood that flavonoids and phenolic compounds have estrogenic activity.

Earlier pharmacological studies of animal models on this plant showed hepato-protective, anti-oxidant and protective effects against DMBA-induced mammary cancer in mice, which can be attributed to the drug extract's estrogenic activity.

Separating the active constituents from the *Saraca indica* bark responsible for its estrogenic effects and evaluating its efficacy on menopausal conditions such as hot flushes and osteoporosis can provide an appropriate non hormonal therapy for many postmenopausal conditions.

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