



Research Paper

EVALUATION OF ESTROGENIC ACTIVITY OF ALCOHOLIC EXTRACT OF FRUITS OF *SOLANUM XANTHOCARPUM* USING UTERINE WET WEIGHT, UTERINE GLYCOGEN CONTENT AND UTERINE HISTOLOGY AS PARAMETER OF ASSESSMENT

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The objective was to conduct a comparative study of estrogenic activity of alcoholic extract of fruits of *Solanum xanthocarpum* with diethylstilbestrol in bilaterally ovariectomized young albino rats using uterine wet weight, uterine glycogen content and uterine histology as parameters of assessment. **Materials and Methods:** Bilaterally ovariectomized albino rats were divided into five groups (n=7) receiving different treatments, consisting of vehicle (distilled water), ethanolic extract of fruits of *Solanum xanthocarpum* 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 drugs were administered orally daily for 7 days. Estrogenic activity was assessed by taking uterine wet weight, uterine glycogen content, and uterine histology as parameters of assessment. **Results:** The results proved the estrogenic activity of extracts for dose 200 & 400 mg/kg body weight by exhibiting the significant ($p < 0.05$ & $p < 0.01$) result for various parameters like uterine wet weight, uterine glycogen content and uterine histology. But the dose 100 mg/kg of *Solanum xanthocarpum* was proved statistically insignificant in above mentioned parameters. **Conclusion:** *Solanum xanthocarpum* showed moderate estrogenic activity in a dose dependent manner compared to diethylstilbestrol.

Keywords: *Solanum xanthocarpum*, estrogenic activity, ovariectomized rats.

INTRODUCTION

Phytoestrogens are nonsteroidal compounds with estrogenic activity occurring naturally in a variety of plants as coumestans, flavonoids and

lignans¹. They have attracted attention because they might be capable of preventing development of estrogen related cancers and also blunting the symptoms of menopause. *Solanum xanthocarpum*

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(Fam: Solanaceae) is a prickly diffusely bright green, perennial, 2-3 m high, it is woody at the base, with zig-zag stem, branches numerous, leaves 5-10 by 2.5-5.7 cm ovate, purple hairy on both sides with yellow sharp prickles. Berry 1-3 cm diameter yellow or white green veins found in different regions of the Indo-Pakistan subcontinent². Since the plant (*Solanum xanthocarpum*) contains phytoesters such as sitosterol, carpesterol and other sterols and phenolic substances and it extensively used in the treatment of sexual debility, facilitating conception, gonorrhoea (Cassia R Overka *et al.*, 2008). It may possess estrogenic activity, but no scientific data is available on the endocrine effects of this plant. Hence the present study was undertaken to evaluate the possible estrogenic activity of alcoholic extract of fruits of *Solanum xanthocarpum*.

The introduction of cheap, plentiful, orally active phytoestrogens at a time when the natural estrogens are scarce will become a milestone in the development of effective endocrine therapy for menstrual disorders, control of fertility and postmenopausal osteoporosis. Bhavamisra specially mentions the plant as useful in facilitating conception (SPARC, 1992).

Formulations containing *Solanum xanthocarpum* are being promoted for use in conditions like irregular menses, menopause, breast cancer and infertility (Kurian, 2004). Thus the evaluation of the estrogenic activity of *Solanum xanthocarpum* was carried out to know whether its beneficial effect in various gynecological problems and breast cancer is due to its estrogenic activity.

MATERIALS AND METHODS

Fruits of *Solanum xanthocarpum* were collected from field's areas of Manjeshwar in the month of

December and its identity was confirmed by Mrs Noelin J. Pinto, HOD Dept of Botany, St Agnes College, and Mangalore.

The collected fruits were cleaned from adhering soil and other materials, and then it was dried under shade for two weeks. The dried fruits were chopped and pulverized in an electric grinder. The powdered plant material was subjected to Soxhlet extraction with about 80%w/v ethyl alcohol. The extract obtained was concentrated over a hot water bath. Percentage yield of thus obtained crude extract was calculated. Accordingly alcoholic extract of *Solanum xanthocarpum* was prepared in sufficient quantity and stored in the refrigerator for further use.

Animals and experimental set-up: Estrogenic activity of the alcoholic extract was assessed in bilaterally ovariectomized young albino rats weighing 150-200 g using a standardized method with few modifications, uterine wet weight, uterine glycogen content and uterine histology as parameters of assessment (Jonathan *et al.*, 1995). The ovariectomized rats were divided into 5 groups each consisting of 7 animals. Estrogenic activity of phytoestrogens ranges from 1/500 to 1/1000 to the activity of diethylstilbestrol (DES) (Cassidy, 1999). Based on this assumption a dose range between 100 to 400 mg/kg of *Solanum xanthocarpum* extract was taken.

Group 1 (Control): Received distilled water at a dose of 10 ml/kg.

Group 2 (Standard): Received aqueous suspension of diethylstilbestrol (NEMESTROL) at a dose of 2 mg/kg.

Group 3 (Test): Received alcoholic extract of *Solanum xanthocarpum* in distilled water at a dose of 100 mg/kg.

Group 4 (Test): Received alcoholic extract of *Solanum xanthocarpum* in distilled water at a dose of 200 mg/kg.

Group 5 (Test): Received alcoholic extract of *Solanum xanthocarpum* in distilled water at a dose of 400 mg/kg.

All drugs were administered orally daily for 7 days.

After 24 hours of last treatment, hysterectomy was performed in all rats under pentobarbitone anesthesia. Harvested uteri were cleaned carefully from adhering connective tissue and weighed. The three excised uteri from each group were fixed in Bouins fluid and processed for histological preparations. Haematoxylin and eosin stained slides were examined under microscope for changes in cellular organization. The remaining uteri were used for glycogen estimation by anthrone method (Dayton *et al.*, 1980). This

study was conducted in accordance with the latest CPCSEA guidelines and the experimental protocol was approved by Institutional Animals Ethics Committee.

Statistical Analysis: One way analysis of variance (ANOVA) followed by Dunnet's 't' test was used to analyze the difference in uterine wet weight, uterine glycogen content between different groups of treatment.

RESULTS

Assessment of estrogenic activity of alcoholic extract of *Solanum xanthocarpum* was done by taking uterine wet weight, uterine glycogen content and uterine histology as parameters.

A) Uterine wet weight (Table 1)

The alcoholic extract of *Solanum xanthocarpum* showed a dose dependent, statistically significant increase in uterine wet weight compared to control

Table 1: Effect of Alcoholic Extract of *Solanum xanthocarpum* on Uterine Wet Weight, Uterine Glycogen Content and Uterine Histology in Bilaterally Ovariectomized Albino Rats

Group	Treatment (route)	Dose (mg/kg)	Uterine Wet Weight (mg)	Uterine Glycogen Content ($\mu\text{g}/\text{mg}$ of uterine tissue)	Uterine Histology
1	Control distilled water (p.o)	10	97.50 \pm 0.4601	0.3056 \pm 0.004	The uterine endometrium was disintegrated.
2	Standard DES (p.o)	2	221.8 \pm 0.6700 ^x	0.9201 \pm 0.005 ^a	Height of luminal epithelium was increased and number of glands increased.
3	<i>Solanum xanthocarpum</i> extract (p.o)	100	97.93 \pm 0.3881 ^{ns}	0.3950 \pm 0.0117 ^{ns}	
4		200	104.53 \pm 0.1315 ^{xx}	0.4163 \pm 0.018 ^b	
5		400	110.23 \pm 0.111 ^{xx}	0.4926 \pm 0.020 ^a	
	ANOVA		F=180.80 D.F:(4, 15) p<0.001 b/w grps.	F=72.02 D.F:(4, 15) p<0.001b/w grps.	

Note: ^xp<0.01 compared to control group; ^ap<0.01 compared to control group; ^{xx}p<0.05; ^bp<0.05 compared to control group; D.F: 15; D.F: 15; Values are mean \pm S.E.M of 7 animals in each group. Data were analysed by one-way ANOVA followed by Dunnet's 't' test.

[F=180.80; df=4, 15 at p<0.01].

A 0.004, 0.06 and 0.128 fold increase in uterine wet weight at doses 100, 200, 400 mg/kg respectively, were seen compared to control. But 0.004 fold increase in uterine wet weight was not statistically significant [F=180.80; df = 4, 15 at p<0.05]. On the other hand DES at a dose of 2 mg/kg showed a statistically significant, 1.27 fold increase in uterine wet weight compared to control [F=180.80; df = 4, 15 at p<0.01].

B) Uterine glycogen content (Table 1)

A dose dependent statistically significant increase in the uterine glycogen following the administration of *Solanum xanthocarpum* extract was seen. A dose of 100 mg/kg showed a statistically insignificant [F=72.02; df=4, 15, at p> 0.05] 0.292 fold increase. Whereas 200 & 400 mg/kg dose showed statistically significant [F=72.02; df=4, 15, at p< 0.01] 0.3622 & 0.6119 fold increase

respectively, compared to control.

Diethylstilbestrol extract also showed a statistically significant [F=72.02; df=4, 15, at p< 0.01], 2.01 fold increase compared to control.

C) Uterine histology (Table 1)

The alcoholic extract of *Solanum xanthocarpum* induced proliferative changes in the uterine endometrium as evidenced by increased height of luminal epithelium, with loose stroma and increased number of glands, compared to control (Figure 1) for 200 mg/kg and 400 mg/kg dose (Figures 4 and 5). But for 100 mg/kg no proliferative changes occurred (Figure 3). On the other hand DES showed proliferative changes as evidenced by increased height of epithelium, with loose stroma and increased number of glands (Figure 2 and Table 1). In the control rats, uterine

Figure 1: Photomicrograph (X100) of Haematoxylin and Eosin Stained Transverse Section of Uterus of Control Rat, Showing Disintegrated Endometrium

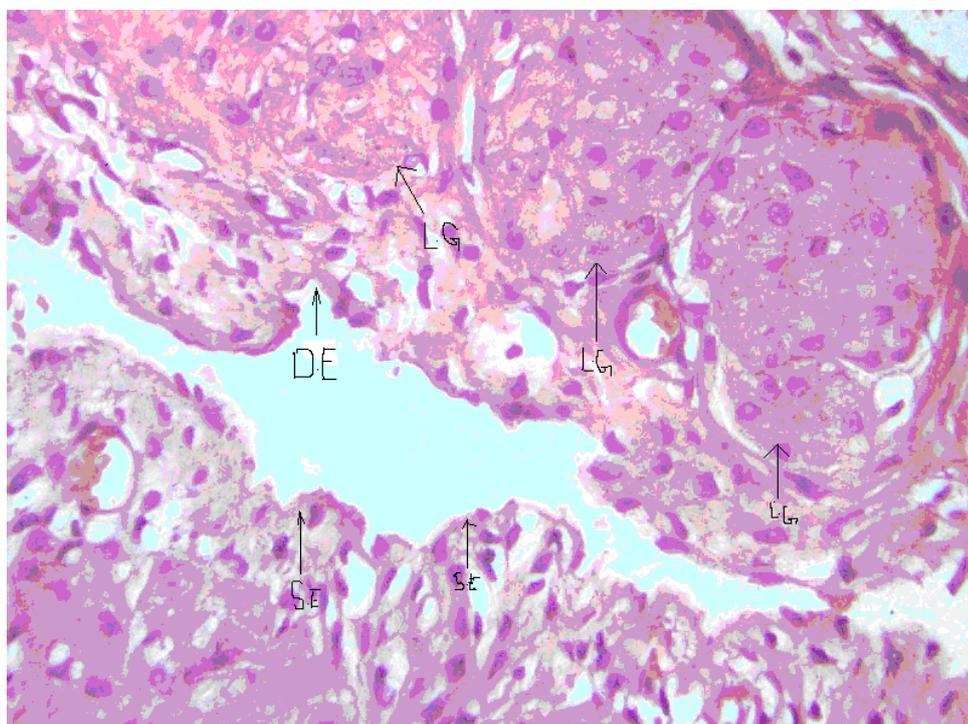


Figure 2: Photomicrograph (x100) Of Haematoxylin and Eosin Stained Transverse Section of Uterus of Diethylstilbestrol (2 mg/kg, P.O) Treated Rat, Showing Proliferative Stage (i.e., Stimulated Endometrium With Loose Stroma and Glands

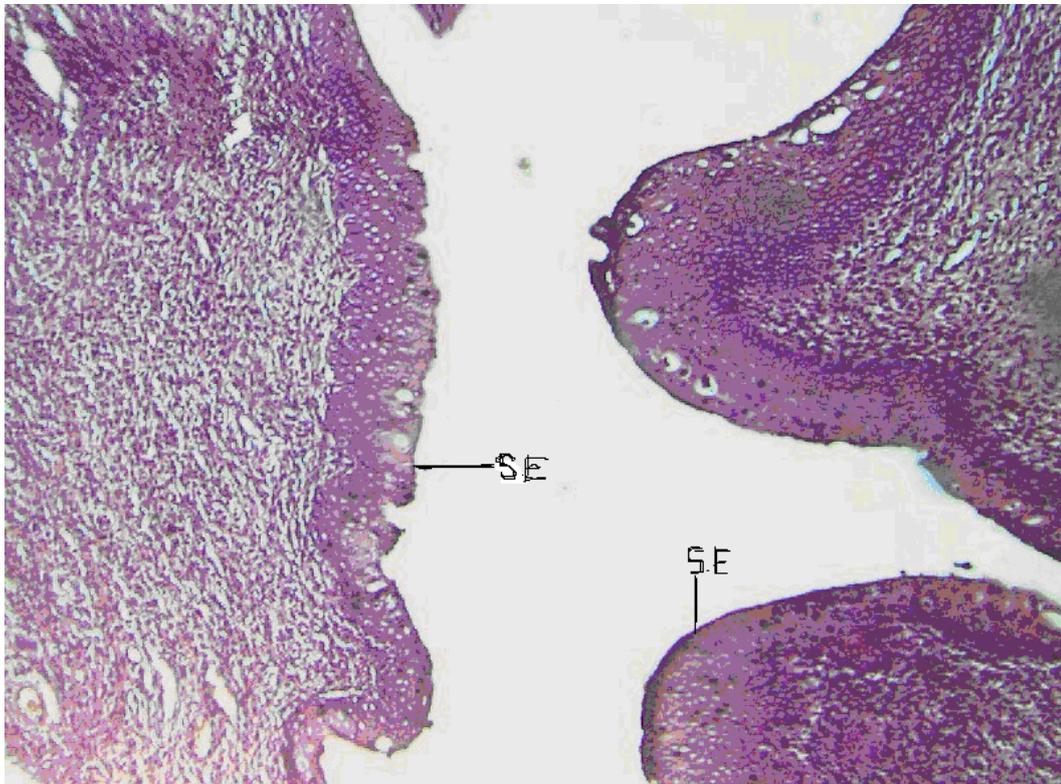


Figure 3: Photomicrograph (x100) of Haematoxylin and Eosin Stained Transverse Section of Uterus of *Solanum xanthocarpum* Extract 100 mg/kg, P.O) Treated Rat, Showing No Proliferative Stage (i.e., Unstimulated Endometrium With No Loose Stroma)

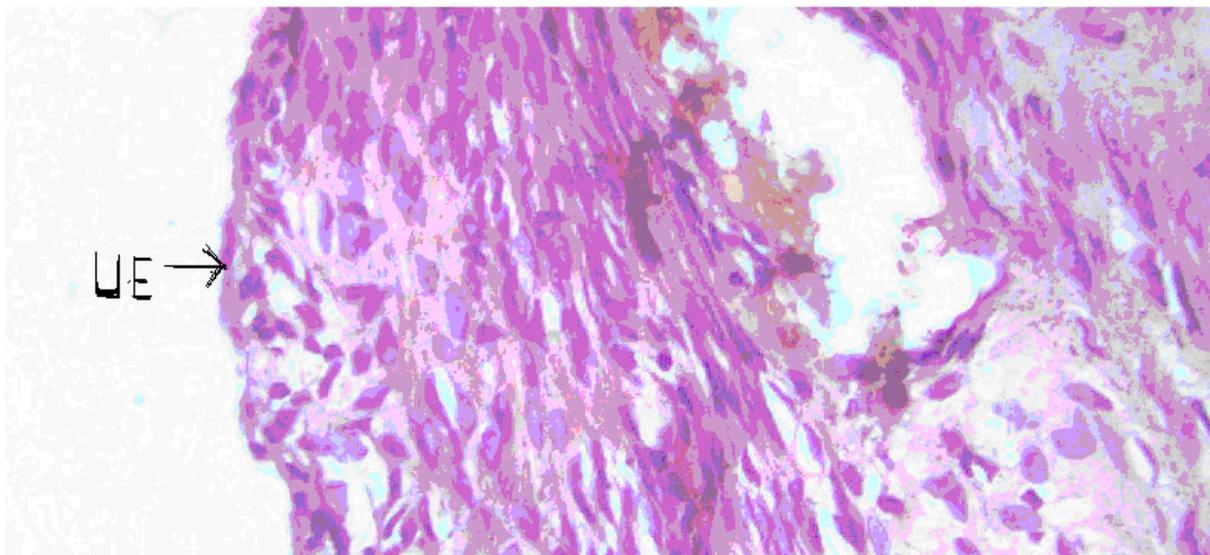


Figure 4: Photomicrograph (x100) of Haematoxylin and Eosin Stained Transverse Section of Uterus of *Solanum xanthocarpum* Extract (200 mg/kg, P.O) Treated Rat, Showing Proliferative Stage (i.e., Stimulated Endometrium With Loose Stroma)

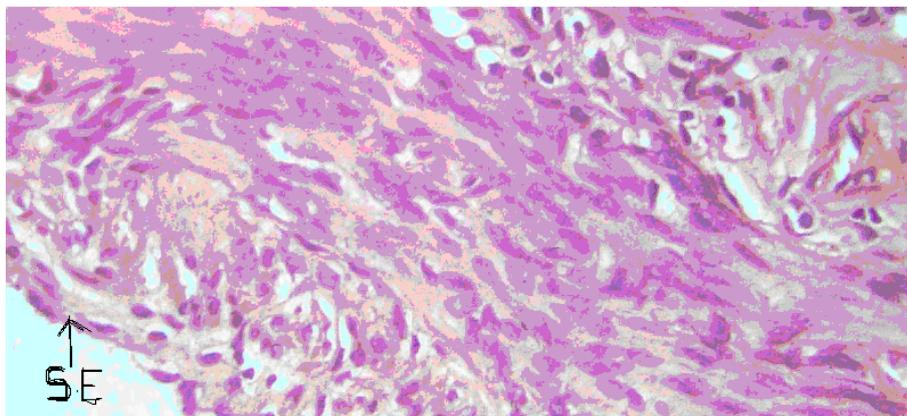


Figure 5: Photomicrograph (x100) of Haematoxylin And Eosin Stained Transverse Section Of Uterus Of Solanum Xanthocarpum Extract (400 mg/kg, P.O) Treated Rat, Showing Proliferative Stage (i.e., Stimulated Endometrium With Loose Stroma)

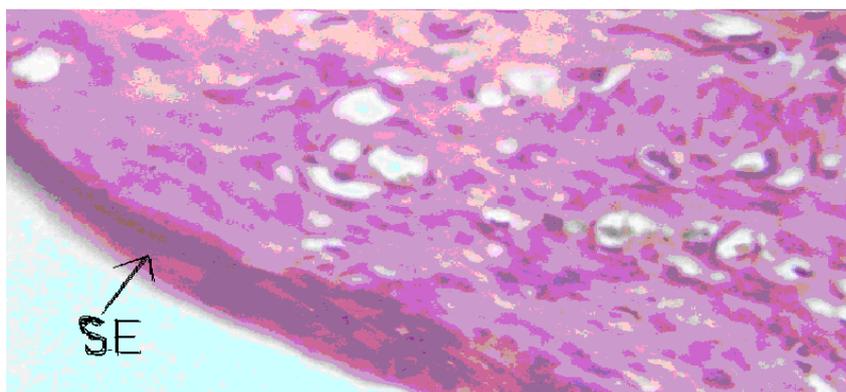


Figure 6: Uterine Epithelial Cell Height

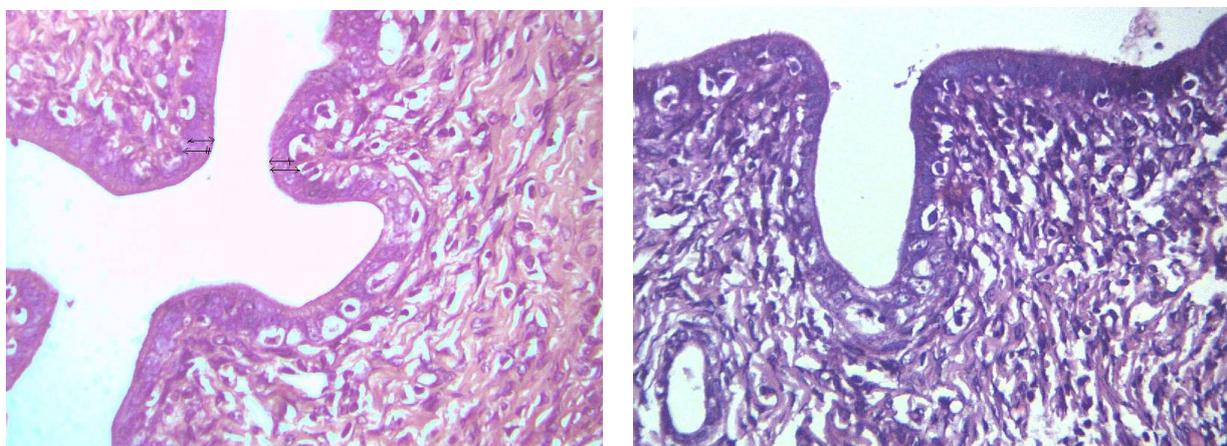


Figure 6 (Cont.)

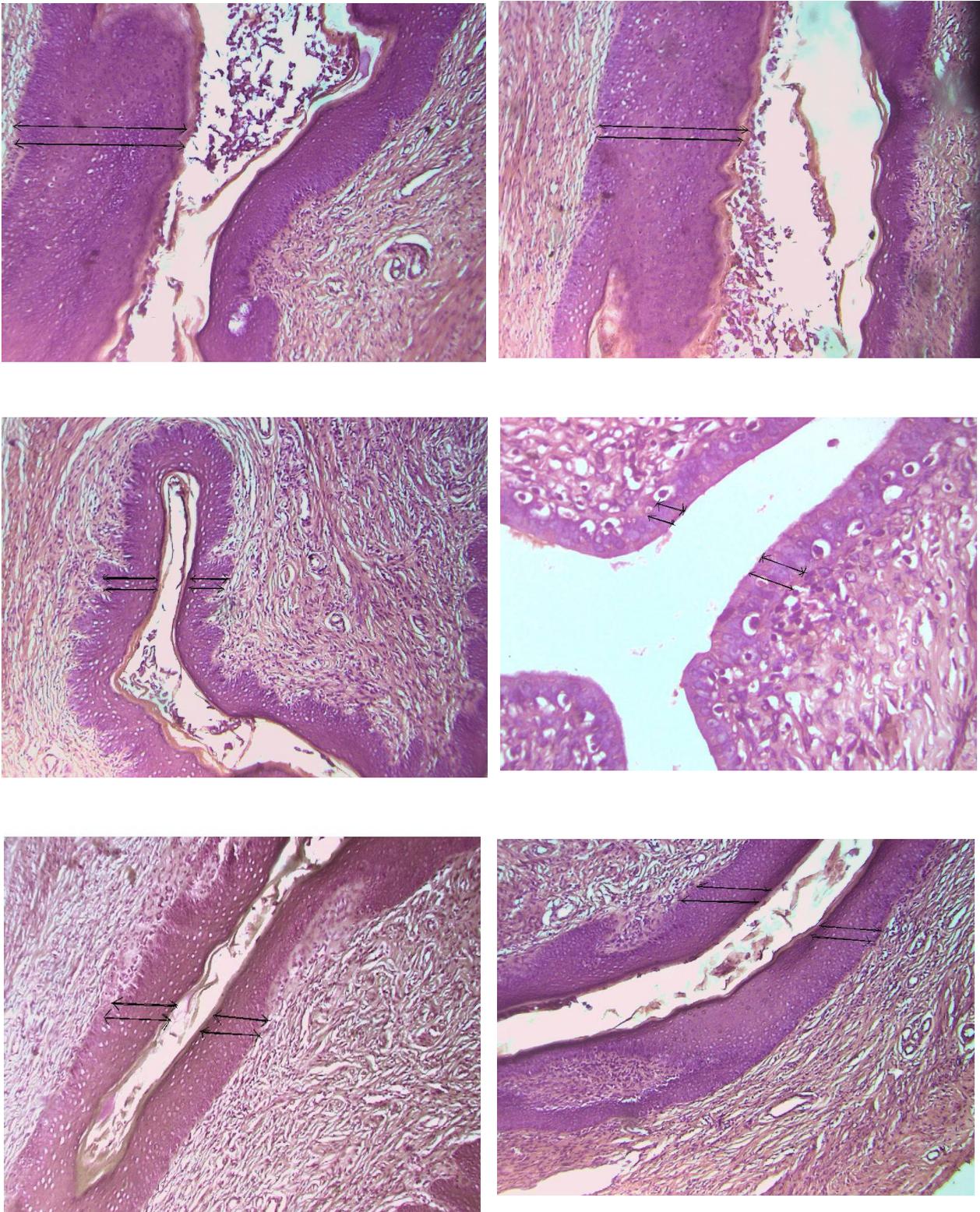
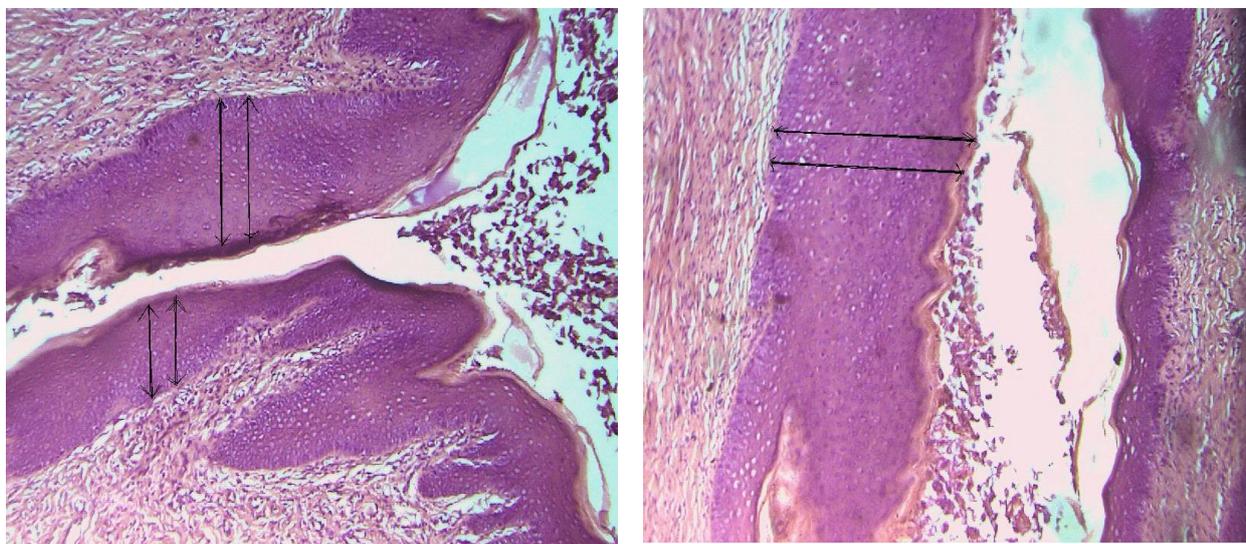


Figure 6 (Cont.)



endometrium was disintegrated (Figure 1). Height of luminal epithelium can be seen from Figure 6.

DISCUSSION

Uterus and the female reproductive tract undergo innumerable physiologic and biochemical changes under the influence of ovarian hormones such as estrogen (Prakash and Mathur, 1979). If female rats are ovariectomized, the resultant lack of estrogen causes atrophy of the uterus and the reproductive tract; administration of estrogenic substances to ovariectomized rats leads to uterotrophic effects, increase in uterine glycogen content and proliferative changes in uterine endometrium (Williamson *et al.*, 1996).

Estrogenic potency and efficacy have traditionally been expressed in terms of uterotrophic effects in immature or ovariectomized female rats (Ruentiz, 2003). The increase in uterine wet weight was successive and gradual with increase in the dose of the extract of *Solanum xanthocarpum*.

The histological examination of uterus of extract treated rats showed estrogenic influence, as evidenced by increased height of luminal epithelium with loose stroma and increased number of glands.

Increase in uterine glycogen content in ovariectomized rats under the influence of alcoholic extract of *Solanum xanthocarpum* may be due to their estrogenic activity since estrogens have been reported to increase the hexose transport into the rat uterus and thereby increase the synthesis of glycogen in uterus (Tripathi, 1983). The effect of *Solanum xanthocarpum* extract at 400 mg/kg on uterine glycogen content was found to be almost equivalent to the effect seen with 2 mg/kg DES dose. So it can be agreed that the extract of *Solanum xanthocarpum* has about 1/300th the potency of standard drug DES.

Literature review conducted on *Solanum xanthocarpum* indicated the presence of flavonoids, phytosterols and phenolic compounds (en.wikipedia.org/wiki/Estrogen). Flavonoids and phenolic compounds are known to possess

estrogenic activity (Murad and Jeffrey, 1991; and Kuiper *et al.*, 1998). Thus the estrogenic activity shown by the extract of *Solanum xanthocarpum* can be attributed to the presence of flavonoids and phenolic compounds.

With a further study on the efficacy and safety aspect, the drug in future might be recommended for preventing the development of estrogen related cancers, blunting the symptoms of menopause, lowering the incidence of osteoporosis and providing a cardioprotective effect.

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