Evaluation of the estrogenic effect of Menosan using the rat models of uterotrophic assay

ABSTRACT
The estrogenic activity of Menosan was studied using the rat models of uterotrophic assay. Screening methods for the selection of appropriate models for the uterotrophic assay was based on the need to have a non-functional hypothalamic-pituitary-gonadal axis in order to ensure a consistent and sensitive uterine response to the administered estrogens or antiestrogens. Menosan was administered orally as an aqueous suspension at a dose of 500 mg/kg b.wt. for 21 days to OVX and immature rats. Treatment with Menosan resulted in a significant increase in uterine weight and uterine glycogen levels as compared to the OVX controls without altering the hormone levels. Histopathological evaluation of the uterine section revealed changes characterized by atrophy of the uterus in the OVX controls, while the OVX rats treated with Menosan showed increased endometrial response as indicated by proliferation of the endometrial glands, epithelial hyperplasia, dilatation of the lumen and increased vascularity. Menosan treatment in the immature rats showed a significant increase in uterine weight ratio. Further, the treatment with Menosan also resulted in histological changes like hydrometra, epithelial proliferation and endometrial glandular hyperplasia. The observed estrogenic effect in OVX rats without altering the hormone levels following Menosan treatment suggests that Menosan could possibly act directly on the estrogen receptors without enhancing the endogenous hormone levels. Menosan showed the desired effects on the physical, histological and biochemical parameters of the uterine tissue, thereby indicating its beneficial role in the treatment of postmenopausal symptoms.

INTRODUCTION
Menopause is an experience that is unique to each woman. Some women notice little difference in their physiology while others find the change extremely troublesome and upsetting. Ovarian failure and the
accompanying decline in estrogen production are responsible for the changes of menopause. Globally more than 470 million people suffer from menopausal symptoms and the average age of attaining menopause is 50 years. Menopausal symptoms may manifest themselves 2 or 3 years before the actual menopause starts and continue for 2 to 5 years. During menopause, major physiological, gynecological and social changes occur. Aging is the most obvious risk factor for menopause. Genetic factors may predispose some women to an early menopause (under age 50). The only modifiable risk factor for early menopause is smoking, with heavy smokers starting earlier than light smokers. Menopausal and postmenopausal women have a greater risk of osteoporosis, heart disease, Alzheimer’s disease, colon cancer, and diabetes. The incidence of these conditions is also largely affected by genetic and lifestyle factors.

Conventional treatment for menopausal symptoms usually consists of HRT. Concerns about the safety and effectiveness are causing a retreat from the blanket use of HRT. Side effects such as breast tenderness and breakthrough bleeding, concerns about breast and ovarian cancer, gall bladder disease, and thromboembolic events may all contribute to low adherence rates to the treatment with HRT. Any new agent that offers convenience, safety and protection against the long- and short-term effects of menopause will be of clinical value. Natural therapies using phytoestrogens are receiving increased attention as dietary components that can affect several aspects of human health and are beneficial in countering the manifestations of postmenopausal state.

The traditional system of Indian medicine has cited several plants that are useful in the management of menopausal syndrome. Menosan is one such polyherbal formulation comprising Saraca indica, Asparagus racemosus, Terminalia chebula, Sida cordifolia, Glycyrrhiza glabra, Centella asiatica, Kukkutandatvak bhasma and Zaharmohara bhasma as its main constituents.

Glycyrrhiza glabra has been found to be a rich source of flavanoids and isoflavonoids, which are responsible for its estrogenic activity. Glabridin, an isoflavone isolated from Glycyrrhiza glabra, was found to inhibit LDL oxidation, which could in turn be beneficial in the attenuation of atherosclerosis. Glycyrrhiza glabra is also reported for its memory enhancing and anxiolytic activity. Saraca indica is well known in Ayurvedic medicine for its use as a stimulant of the endometrium and ovarian tissue. In various studies, Asparagus racemosus has been shown to possess immunomodulatory and antibacterial activities. Terminalia chebula possesses antimicrobial activity and is active largely against Staphylococcus aureus and Klebsiella species. Sida cordifolia has potent analgesic and anti-inflammatory properties, which could be beneficial in relieving bone and joint pain. Centella asiatica has anxiolytic and antistress activity, as well as cognitive enhancing effect. Kukkutandatvak bhasma and Zaharmohara bhasma are rich sources of natural calcium. In the present study, Menosan was evaluated for its uterotrophic effect in rat models.

**MATERIALS AND METHODS**

**Standardization of Menosan:** Two or more batches of preparations from raw materials of different origin were standardized by fingerprint analysis for characterization using high-performance thin layer chromatography (HPTLC). Three grams of Menosan were weighed and extracted by refluxing on water bath with 20 ml dichloromethane. The extract was filtered and concentrated to 2 ml. Ten µl of the concentrate was spotted on pre-coated silica gel plate. Plate was developed using dichloromethane: methanol (97:3) and scanned using densitometer at 254 nm. Fingerprint of Menosan is shown in Figure 1.

**Animals:** Laboratory-bred Wistar rats were used in the study. All the experimental procedures were performed according to the guidelines of the Institutional Animal Ethics Committee.

![Figure 1: HPTLC fingerprint pattern of Menosan](image-url)
female rats were used for the study. The animals were housed at a temperature of 22 ± 2 °C, relative humidity of 50-55% and 12 hour light-dark cycle. Drinking water and balanced pelleted diet were supplied ad lib throughout the study period. The study was conducted after obtaining the approval from the Institutional Animal Ethics Committee (IAEC). All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the National Institutes of Health.

**Effect of Menosan in OVX rats:** Twenty-four female Wistar rats of 3-4 months of age (225-250 g) were randomized into 3 groups of 8 animals each. Rats from all the groups were OVX except the rats from the Group I that served as sham-operated controls. Ovariectomy was performed by ligation and excision of the ovaries along the upper horn under general anesthesia with pentobarbital sodium (35 mg/kg b.wt., i.p.). In the sham-operated controls, the ovaries were exposed and gently manipulated but not excised. Rats from the Group II received vehicle orally (water 15 ml/kg) and served as OVX controls. Group III received 500 mg/kg b.wt., p.o. of Menosan in the form of an aqueous suspension. The respective treatments were given for 21 days. At the end of the treatment period, the uterus was excised and weighed. The weight of uterus was expressed as mg/100 g b.wt. Uterus was fixed in 10% neutral buffered formalin and processed using the paraffin technique. Sections of 5 µ thickness were cut and stained using routine H&E method for histological evaluation.

**Statistical analysis:** Data were expressed as mean ± SEM and analyzed statistically using one way ANOVA followed by Dunnett’s multiple comparison test. The minimum level of significance was fixed at p<0.05.

**RESULTS**

**Effect of Menosan in OVX rats:** Treatment with Menosan resulted in a significant increase in uterine weight, which was not observed in OVX controls. Menosan treatment for 21 days also showed a significant increase in the levels of uterine glycogen as compared to OVX controls (Table 1). No change in the serum estrogen and progesterone levels were observed in the Menosan treated group as compared to OVX controls. Histopathological evaluation of the uterine section revealed changes characterized by atrophy of the uterus in the untreated OVX rats, while in OVX rats treated with Menosan, there was increased endometrial response as indicated by the proliferation of the endometrial glands, epithelial hyperplasia, dilatation of lumen and increased vascularity (Figures 2-4).

**Effect of Menosan on immature rats:** Twenty-four immature female rats weighing between 75 and 80 g were used for the study. The rats were randomized into 2 groups of 10 each. The rats in the Group 1 received 10 ml/kg b.wt. of water as vehicle and served as controls. The Group 2 rats received 500 mg/kg b.wt. of Menosan in the form of an aqueous suspension. The respective treatments were given for 21 days. At the end of the treatment period, the uterus was excised and weighed. The weight of uterus was expressed as mg/100 g b.wt. Uterus was fixed in 10% neutral buffered formalin and processed using paraffin technique. Sections of 5 µ thickness were cut and stained using routine H&E method for histological evaluation.

**Statistical analysis:** Data were expressed as mean ± SEM and analyzed statistically using one way ANOVA followed by Dunnett’s multiple comparison test. The minimum level of significance was fixed at p<0.05.

**RESULTS**

**Effect of Menosan on immature rats:** Menosan treatment in the immature rats resulted in a significant increase in uterine weight ratio (Table 2). Further, treatment with Menosan resulted in a significant increase in uterine weight, which was not observed in OVX controls. Menosan treatment for 21 days also showed a significant increase in the levels of uterine glycogen as compared to OVX controls (Table 1). No change in the serum estrogen and progesterone levels were observed in the Menosan treated group as compared to OVX controls. Histopathological evaluation of the uterine section revealed changes characterized by atrophy of the uterus in the untreated OVX rats, while in OVX rats treated with Menosan, there was increased endometrial response as indicated by the proliferation of the endometrial glands, epithelial hyperplasia, dilatation of lumen and increased vascularity (Figures 2-4).
with Menosan also resulted in histological changes like hydrometra, epithelial proliferation and endometrial glandular hyperplasia (Figures 5 and 6).

**DISCUSSION**

Experimental models for uterotrophic assay are based on the need to have a non-functional hypothalamic-pituitary-gonadal axis in order to ensure a consistent and sensitive uterine response to the administered estrogens or antiestrogens. The organization for economic cooperation and development (OECD) considered 2 possible models to be highly sensitive and equivalent. One model uses the immature female before significant ovarian estrogen synthesis and regulation by the hypothalamic-pituitary-gonadal axis begins; the other model uses the OVX adult female, without the primary source of estrogen synthesis. The present study using the above-mentioned models provides the basis for the uterotrophic assay of Menosan.

Treatment with Menosan showed an evidence of uterotrophic activity as indicated by uterine weight and characteristic histological changes in both OVX and immature rats. These uterotrophic activities of Menosan could be attributed to the flavonoid content of *Glycyrrhiza glabra*, which is proven to possess high estrogenic activity. The mechanisms responsible for the effects of phytoestrogens are not clearly understood but there is suggestive evidence that phytoestrogens could act through two possible mechanisms namely, estrogen receptor-dependent and -independent. Many studies have shown that phytoestrogens bind to estrogen receptors and show significant estrogenic effects in animals, in man and in cell cultures. The insignificant change in the levels of estrogen and progesterone in the present study suggests that Menosan may act directly on the estrogen receptors without enhancing the endogenous estrogen levels.

The energy source for female reproductive system is ovarian glycogen, which is estrogen-dependent. Estrogen is known to increase the glycogen content in the uterus of rats. The decrease in the levels of uterine glycogen in the OVX rats is perhaps due to estrogen deficiency. These findings also correlated with the earlier reports. Increased uterine glycogen concentration in the Menosan-treated group as compared to OVX control indicates the estrogenic effect of Menosan.

In this study, Menosan showed the desired effects on the physical, histological and biochemical parameters of the uterine tissue, thereby indicating its beneficial role in the treatment of postmenopausal symptoms.

**REFERENCES**


