CHANGES IN THE BIOCHEMICAL COMPONENTS WITHIN THE OVARY OF FEMALE ALBINO MICE DUE TO THE ADMINISTRATION OF SOME PLANT ROOT EXTRACTS, USED BY THE SANTAL TRIBAL PEOPLE OF THE DISTRICT BANKURA, W.B., INDIA, FOR THE CONTROL OF FERTILITY

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ABSTRACT

The present study was conducted to evaluate the biochemical changes within the ovarian tissue especially (cholesterol, ascorbic acid, alkaline phosphatase and acid phosphatase) due to the administration of three indigenous plant root extracts with the dosages (Vitex negundo Linn – 1000mg/kg B.W.; Rauwolfia serpentina Benth. ex Kurz - 1000mg/kg B.W; Piper betle Linn - 1000mg/kg B.W) on the female albino mice. These medicines were used by the medicine men of the Santal tribal people of the District Bankura, West Bengal, India, for the control of fertility. To know the feasibility, reliability and efficacy, the present set up of experiments were conducted on the adult female albino mice (20-25gm B.W.) for seven consecutive days. The results of the present experiments clearly indicate some remarkable changes in the biochemical components which clearly point out the effectiveness of the ethnomedicines and can be used as the positive antifertility agent for the welfare of the human being.

KEYWORDS Indigenous, antifertility, biochemical components, efficacy, methanolic extracts.

INTRODUCTION

India harbors a remarkable number of tribal populations with their own culture and tradition. Santal tribal people in West Bengal also are quite enormous than others. These people are
quite loyal, hard working and conservative. Most of the tribal people now a days try to live within the forest and besides hilly areas. Due to the forest dwelling habit, they have a good knowledge about the plants, which are usually used for medicinal purposes.

Numerous herbs have been used historically to reduce fertility, and modern scientific research has confirmed the antifertility effects of some of the herbs tested. Herbal contraception may never reach the level of contraceptive protection as the pills, but it offers alternatives for women who have difficulty with modern contraceptive options or who just want to try in a different way.

Some scientists reported an account of the use of 25 plant species (and some mineral and animal products) by the tribals of Rayalaseema region of Andhra Pradesh for family planning and birth control.[1]

An ethnobotanical survey of tribal area of southern Rajasthan was carried out for ethnosexicological herbal medicines. During ethnobotanical survey, 53 plants belonging to 33 families have been reported from the study area, which are used to cure sexual diseases, and for family planning.[2]

The antifertility properties of various medicinal plants have been described by many workers.[3,4,5,6,7,8]

Aims of the Study

The medicinal plants have been used since ancient times for the treatment of human ailments. Traditional healers and Pharmacists in developing countries are an important source of information about plant sources of new drugs.

The tribal people of Bankura district are rich in their traditional knowledge about ethnomedicines. They used several plants and plant products for controlling fertility. But all the ingredients and their efficacy had not been tested thoroughly. But the medicine men have strong belief of their own medication.

For the present study roots of three types of plants e.g. *Vitex negundo* Linn., *Rauwolfia serpentina* Benth.ex.Kurz, and *Piper betle* Linn. were extracted with methanol and their actions were observed on the female albino mice separately and in combination. Changes in
some biochemical compound are mainly observed due to the administration of the three plant root extracts in the female albino mice.

**MATERIALS AND METHODS**

All the experiments were performed on the adult, cyclic virgin female albino mice (weighing 20-25 gm body weight). The mice were housed in the metallic cages and maintained under uniform laboratory conditions with a 12:12 hrs light and dark cycle for each 24 hrs periods and ambient room temperature (23-25°C). Only females with a normal estrus cycle were selected for the experiment.

**Preparation of the Extract**

Roots of three types of plants used by the medicinemen of the district Bankura, W.B., India were collected from the medicine men. After thorough identification of the plants, the roots were shade dried and powdered separately. The powder was submerged in 80% methanol for 48-72 hrs. Extracts were filtered and dried in thermostatically controlled water both maintained at 50-55°C temperature to yield a reddish brown color that was the crude extract and stored in a refrigerator (at 4-6°C temperature). The crude extract was weighed and diluted with distilled water (40mg/kg) before use. The aqueous suspension was administered orally by specially designed blunt syringe needle.

**Plants used in the Experimental set up**

- **a) Nishinda – Vitex negundo Linn (Verbenaceae)**
  This plant is an erect, branched shrub 2 to 5 meters in height. The roots of *V. negundo* contain flavonoid glycosides, cardiac glycosides, alkaloids, amino acids and neutral resin.

- **b) Sarpagandha – Rauwolfia serpentina Benth.ex Kurz (Apocynaceae)**
  *Rauwolfia serpentina* is an erect, perennial shrub, generally 15-45 cm high. Roots are greenish-yellow externally and pale-yellow inside, extremely bitter in taste. More than 50 alkaloids have been reported from *Rauwolfia serpentina*. Most important alkaloids used in medicine are reserpine, rescinnamine and deserpidine etc.

- **c) Pan – Piper betle Linn (Piperaceae)**
  *Piper betle* is a slender creeper with adventitious roots. Column chromatography of the alcoholic extract of *Piper betle* roots furnished aristololactam A-II and a new phenyl propene,
characterized as 4-allyl resorcinol, while the petroleum-ether extract yielded a diketosteroid, viz. stigmaster-4-en-3,6-dione.

**Experimental Protocol**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drugs</th>
<th>Dose (mg/Kg. Body wt.)</th>
<th>Days of treatment</th>
<th>Autopsy Date</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>No treatment</td>
<td>Food and water ad-libitum</td>
<td>10</td>
<td>11th day</td>
<td>Normal physiology</td>
</tr>
<tr>
<td>A4 (8)</td>
<td>Root extract of <em>Vitex negundo</em></td>
<td>1000</td>
<td>10</td>
<td>11th day</td>
<td>Almost normal</td>
</tr>
<tr>
<td>B4 (8)</td>
<td>Root extract of <em>Rauwolfia serpentina</em></td>
<td>1000</td>
<td>10</td>
<td>11th day</td>
<td>Almost normal</td>
</tr>
<tr>
<td>C4 (8)</td>
<td>Root extract of <em>Piper betle</em></td>
<td>1000</td>
<td>10</td>
<td>11th day</td>
<td>Almost normal</td>
</tr>
<tr>
<td>D3 (8)</td>
<td>Combination of root extracts of <em>Vitex negundo</em>, <em>Rauwolfia serpentina</em> and <em>Piper betle</em></td>
<td>750</td>
<td>10</td>
<td>11th day</td>
<td>Almost normal</td>
</tr>
</tbody>
</table>

**Gravimetric Study**

On the day of autopsy, the body weights of all the mice were recorded and the animals were sacrificed by cervical dislocation. The ovaries and uteri were carefully dissected out after autopsy and weighed quickly on an electronic balance. The weights of ovary and uterus were taken separately, calculated for each animal and compared the results between the control and the treated groups.

**Biochemical Study**

For biochemical estimation of the ovarian tissues the methods which were followed are mentioned below:

1. Total Cholesterol\(^9\)
2. Acid Phosphatase\(^{10}\)
3. Alkaline Phosphatase\(^{10}\)
4. Ascorbic Acid\(^{11}\)

All the measurements were made at various wavelengths with the help of a Spectrophotometer (Spectronic 20 Genesis). After thorough processing all the mentioned biochemical components were measured, recorded properly, calculated and analyzed statistically.
RESULTS

Gravimetric study showed a significant increase both in the wet weight of ovary and uterus were found in the group A4. A significant decrease only in the weight of uterus was found in the group B4. Significant decrease was noticed in both the groups of C4 & D3. Gravimetric changes in the ovary and uterus of the control and other experimental groups have been presented in the table 1 & fig 1.

Table 1: Effect of root extract of Vitex negundo, Rauwolfia serpentina, and Piper betle on body weight and reproductive organs weight of female albino mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg body weight)</th>
<th>Body weight (gm)</th>
<th>Ovaries (mg/100g body weight)</th>
<th>Uteri (mg/100g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(8)*</td>
<td></td>
<td>22.63±0.595**</td>
<td>25.13±0.295</td>
<td>387.0±1.08</td>
</tr>
<tr>
<td>A4(8)</td>
<td>1000</td>
<td>23.13±0.294#</td>
<td>28.5±0.422***</td>
<td>390.0±2.90***</td>
</tr>
<tr>
<td>B4(8)</td>
<td>1000</td>
<td>22.75±0.366#</td>
<td>25.25±0.25#</td>
<td>321.9±1.64****</td>
</tr>
<tr>
<td>C4(8)</td>
<td>1000</td>
<td>21.88±0.693#</td>
<td>20.63±0.324****</td>
<td>312.6±2.57****</td>
</tr>
<tr>
<td>D3(8)</td>
<td>750</td>
<td>21.75±0.558#</td>
<td>24.0±0.587****</td>
<td>338.5±3.10****</td>
</tr>
</tbody>
</table>

* Number of animals used
** Mean±SEM
*** p<0.05
**** p<0.01
# Not significant (p>0.05)

Fig: 1 Effect of different treatments on the relative weight of ovaries and uterus

The results of all the biochemical parameters of various groups (control and treated) have been presented in table no.2 and figures no.2, 3, 4 & 5. A significant increase in the cholesterol (Fig.2) and ascorbic acid level (Fig.3) showed in the groups B4, C4 and D3 as compared with the control animals. Except group A4, acid phosphatase and alkaline phosphatase level decreased in all other treated groups (B4, C4, and D3) compared to the control animals. In A4
group acid phosphatase did not change but alkaline phosphatase increased significantly (Fig 4&5).

Table 2: Changes in some biochemical components of ovary after the administration of the root extracts of *Vitex negundo*, *Rauwolfia serpentina* and *Piper betle* on the female albino mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (µg/mg of tissue)</th>
<th>Ascorbic acid (µg/mg of tissue)</th>
<th>Acid phosphatase (mM/100mg of tissue)</th>
<th>Alkaline phosphatase (mM/100mg of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(8)*</td>
<td>5.88±0.131**</td>
<td>0.56±0.01</td>
<td>0.18±0.002</td>
<td>1.04±0.01</td>
</tr>
<tr>
<td>A4(8)</td>
<td>4.29±0.247***</td>
<td>0.55±0.01#</td>
<td>0.18±0.002#</td>
<td>1.56±0.05****</td>
</tr>
<tr>
<td>B4(8)</td>
<td>7.47±0.23****</td>
<td>0.67±0.01****</td>
<td>0.06±0.001****</td>
<td>0.04±0.01****</td>
</tr>
<tr>
<td>C4(8)</td>
<td>6.37±0.187****</td>
<td>0.58±0.007****</td>
<td>0.01±0.001****</td>
<td>0.70±0.01****</td>
</tr>
<tr>
<td>D3(8)</td>
<td>8.72±0.217****</td>
<td>0.76±0.01****</td>
<td>0.05±0.002****</td>
<td>0.22±0.05****</td>
</tr>
</tbody>
</table>

* Number of animals used
** Mean±SEM
*** p<0.05
**** p<0.01
# Not significant (p>0.05)

Fig: 2 Changes in the Cholesterol level (µg/ mg of tissue) of ovary after various types of treatments.
Fig: 3 Changes in the Ascorbic acid level (µg/ mg of tissue) of ovary after various types of treatments.

Fig: 4 Changes in the Acid phosphatase level (mM/100mg of tissue) of ovary after various types of treatments.

Fig: 5 Changes in the Alkaline phosphatase level (mM/ 100mg of tissue) of ovary after various types of treatments.
DISCUSSION

Many indigenous plants were used by Ayurvedic physicians in India from the ancient times for the prevention of conception. The medicine men of the district Bankura, used many plants (separately or in combination) for controlling fertility. Amongst the plants, used by the medicine men three plants were selected and tested. These three plants showed positive responses when they were used either alone or in combination. The use of the root extracts of *V. negundo, R. serpentina* and *P. betle* were reported by both the tribal medicine men and many scientists but very little information is available regarding the nature of the active components and their mechanism of action. The present study is mainly dealt with the activity of the methanolic root extract of these three plants separately and in combination on the female albino mice. Results of the study provide evidence of the post-coital contraceptive property of methanolic root extracts of these three plants species.

The A4 group showed a highly significant (*P* < 0.01) increase in ovarian and uterine weight. A significant decrease occurred in the weight of the uterus in B4 group. A significant decrease occurred in the weight of uterus in C4 and D3 groups also. The decrease in the weight of ovary and uterus showed antiestrogenic nature of the extract since antiestrogenic substance decreased the wet weight of the uterus.[12]

Some workers reported that ethanolic extract of *P. betle* exerted antifertility and antiestrogenic effects on female rats. [13] Some scientists investigated the anti-implantation potential of the methanolic extract of the leaves of *Vitex negundo* Linn. [14]

Cholesterol derived from the different sources is the precursor for the steroidogenesis of ovarian endocrine tissue. [15] The significant increase in the cholesterol level of the group receiving extract indicates that cholesterol was not used for steroidogenesis hence accumulated within the ovarian tissue. [16]

Important role of ascorbic acid in the process of gonadal steroidogenesis is well established. [17] Amongst the steroid hormone-secreting gland, the adrenal gland contains a high concentration of ascorbic acid. On stimulation with ACTH, the content of ascorbic acid is reduced before an increase in steroidogenesis can commence. [18] In mammals, ascorbic acid has been found to exert an inhibitory role on steroidogenesis. [17] So accumulation of cholesterol and ascorbic acid in the ovary especially in the D3, treated female mice provided the support to the suppression of ovarian steroidogenesis.
Changes in the phosphatase levels (alkaline and acid phosphatase) within the ovary have been reduced due to the action of three types of root extracts on the experimental albino mice. Experiments conducted on mice,\textsuperscript{[19]} rats \textsuperscript{[20,21]} and sheep\textsuperscript{[22]} showed that the exposure to polychlorinated biphenyls markedly affected sperm motility, follicular growth and maturation, embryonal development with inhibitory effect on the enzymatic system at circulating hormone conversion, and oocyte competence.\textsuperscript{[23,24]} The results of these investigations also pointed out an inhibitory nature of alkaline and acid phosphatases from the ovarian tissue especially due to the treatment of \textit{Rauwolfia serpentina} and \textit{Piper betle}. No remarkable changes were evident with the administration of \textit{Vitex negundo}. The differential action of these three extracts cannot be explained accurately at present. However, for thorough confirmation, it requires further accurate studies, on other experimental animals separately.

**CONCLUSION**

In the present study, methanolic root extract of the three plants (\textit{Vitex negundo} Linn, \textit{Rauwolfia serpentina} Benth. ex Kurz and \textit{Piper betle} Linn.) were used for the study of antifertility activity on female albino mice. Changes in some biochemical parameters (e.g. cholesterol, ascorbic acid, alkaline phosphatase & acid phosphatase) were studied carefully. In this context it can be concluded that 80\% methanolic crude extract of all the three plants roots have antifertility activity. Although the antifertility activities have been noticed in the methanolic root extract treated mice, but due to some differential action of these extracts it requires more thorough and accurate studies using separate animal species.

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**REFERENCES**


