PRE AND POST-IMPLANTATION CHANGES IN THE UTEUS OF RATS: RESPONSE TO MORINGA OLEIFERA LAM.EXTRACT

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ABSTRACT: Aqueous extract of Moringa oleifera Lam. (root) has been studies on pre and post-implementation stages of the uterus of rats so as to elucidate its antifertility mode of action. Results on the biochemical estimation in the uterus of control pregnant rats at different stages of pregnancy revealed a successive increase in the total proteins, glycogen content and the activity or acid and alkaline phosphatase from day 2 to 5 post-coitum. When aqueous extract of M. oleifera Lam. Was administered, there was a significant reduction in all these biochemical constituents when compared to their respective control groups. The role of these biochemical transformations has been discussed in relation to anti-implantation action of the extract.

INTRODUCTION

Moringa oleifera Lam. which is commonly known as Sahajana (Drumstick) has been included in the category abortifacient plants on the basis of number of reports from various author’s.1 Its different extracts have been scientifically tested in laboratory animals and have been reported to possess significant antifertility activity2-5, however, some of the authors could not observe any remarkable antifertility effect6,7. Soejarto8 from World Health Organization (WHO) while describing the potency of antifertility plants has also emphasized on the contraceptive effect of this plant. Recently, the aqueous extract of its roots has been reported to inhibit implantations in rats9 when administered at a dose of 200 mg/kg for 7 days (1-7 post-coitum.) Although its antifertility profile has been reported10 but nothing is known about its mechanism of action in pregnant animals. Therefore, present findings deal with the effect of its aqueous extract on the biochemical constituents of the uterus during pre and post-implanting stages in rats so as to pinpoint its antifertility mode of action.

MATERIALS AND METHODS

Roots of M.Oleifera Lam. were collect from the Campus of Jiwaji University, Gwalior and its aqueous extract was prepared as described earlier9. 200 mg/kg dose of aqueous extract was prepared in gum acacia suspension2 and was administered orally with an intragastric catheter.

Mature healthy female albino rats of Sprague Dawley strain (150 ± 10 g) were selected for the present study. These animals were kept under uniform husbandry conditions and were given “Hindustan Lever” Gold Mohar rat pelleted diet and water ad libitum.
The vaginal smear of each adult female rat was examined daily to identify the stage and when the animals showed proestrus stage, these were caged with mature healthy adult male rats of proven fertility (2 females: 1 male). The mating was further confirmed by the presence of vaginal plug and spermatozoa in the vaginal smear next morning and the day was considered as day 1 of pregnancy. The rats were randomly divided into two groups, control and experimental. A dose of 200mg/kg body weight of aqueous extract was fed orally for different days of pregnancy ranging from pre to post-implementation periods (ranging from day 2 to 6 post-coitum) and animals were killed after 24th of last treatment. Control pregnant rats for each set were maintained simultaneously and received vehicle only. At autopsy both the uteri were excised, freed from adhering tissue, weighed and processed for biochemical estimation of local proteins, glycogen content and activity of acid and alkaline phosphatase. The results were analyzed statistically using student’s t test.

**RESULTS**

The changes in the biochemical constituents in the pre and post-implanting uterus of control and treated animals are shown in Table - 1. It reveals that in the control animals the protein and glycogen concentration gradually increases from day 2 to 6 of the pregnancy. However, the activity of acid and alkaline phosphates is increased gradually from 2nd day to 5th day of pregnancy. The maximum activity, however, is achieved on day 5 of pregnancy.

### TABLE -1

**Effect of aqueous extract of Moringa oleifera Lam. on biochemical constituents of pre and post-implanting uterus of rat**

*Values are mean ± S. E. Five rats were taken in each group.*

| Days of pregnancy | Animals* | Proteins (Mg/100gm) | Glycogen (mg/100gm) | Acid phosphatase (mg/100g/hr) | Alkaline phosphatase (mg/100g/hr) |
|-------------------|----------|---------------------|---------------------|-------------------------------|----------------------------------|
| 2                 | C        | 13.81 ± 0.82        | 65.55 ± 3.65        | 210 ± 12.35                   | 284.85 ± 13.25                   |
|                   | E        | 10.95 ± 0.56        | 60.65 ± 3.85        | 244.65 ± 13.85                | 268.95 ± 19.65                   |
| 3                 | C        | 15.25 ± 0.82        | 72.65 ± 3.96        | 240.60 ± 12.25                | 320.60 ± 16.80                   |
|                   | E        | 13.76 ± 0.78        | 68.77 ± 3.95        | 221.80 ± 13.25                | 300.50 ± 16.35                   |
| 4                 | C        | 18.60 ± 0.95        | 85.56 ± 4.50        | 302.65 ± 16.25                | 425.60 ± 22.65                   |
|                   | E        | 15.62 ± 0.85        | 68.95 ± 4.20        | 215.76 ± 12.25                | 265.85 ± 12.35                   |
| 5                 | C        | 20.24 ± 1.20        | 98.65 ± 5.25        | 380.80 ± 20.65                | 585.65 ± 30.85                   |
|                   | E        | 16.52 ± 0.96        | 70.54 ± 4.25        | 201.57 ± 10.55                | 245.55 ± 14.65                   |
| 6                 | C        | 21.55 ± 1.32        | 110.53 ± 6.75       | 360.78 ± 18.92                | 410.44 ± 23.25                   |
|                   | E        | 16.55 ± 0.98        | 72.62 ± 4.35        | 240.70 ± 12.35                | 240.32 ± 14.35                   |

*C = Control; E = Experimental*
When the animals are administered with aqueous extract of *Moringa oleifera* Lam., there is an increase in the pattern of concentration gradually, however, when compared to the control animals, the values are significantly lower. The glycogen content in the treated animal is significantly reduced when compared to their respective control animals. Similarly, the activity of acid phosphatase in the treated animals is significantly reduced, although, the maximum inhibition has been recorded at day 5 of pregnancy. Similarly, alkaline phosphatase activity is increased on 2nd day of pregnancy but thereafter it decreased throughout the experiment when compared maximum inhibition has been observed on day 5 of pregnancy.

**DISCUSSION**

An mammals, the genital tract undergoes cyclic alterations in their morphological and physiological aspects with respect to various reproductive phases. After fertilization, the uterus encounters many biochemical changes in order to prepare itself for the reception of fertilized eggs14-17, and all these transformations depend upon the action of estrogen and progesterone18-21. Protein is considered to be the building materials and is involved in the alteration of almost every physiological function. Cellular functions are changed when new proteins are formed. Total proteins and the pattern of nucleic acid synthesis is generally changed during the period of pre and post implantation stages as these are urgently required for the survival of developing foetus17,22. Present studies also reveal that total proteins are increased successively in the uterus of normal rats at different stages of implantation. When pregnant rats are administered with aqueous extract of *Moringa oleifera* Lam., although the total proteins are increased gradually from day 2 to 6 post-coitum but the values remained relatively low when compared to their respective control groups. Therefore, on the basis of these observations it is safely said that the aqueous extract of *Moringa oleifera* Lam., marginally reduces the protein concentration in the uterus of rat during the early stages of pregnancy, which can be attributed due to an activity of the extract which induces the formation of certain enzymes, catalyzing few key reactions during the process of protein synthesis. Furthermore, the decreasing pattern of the total proteins in the uterus of treated rats respect to their control groups also revealed that aqueous extract of *Moringa oleifera* Lam., does not promote the favourable conditions to the blastocysts for their proper development and attachment.

Presence of glycogen and its synthesis during early pregnancy has been reported by a number of workers 23, 24. The formation of deciduoma on 5th day of pregnancy is a characteristic feature during the normal implantation25. At this stage, the uterus becomes very rich in the presence of glycogen material and its mobilization is controlled by various hormonal metabolic pathways26. On the basis of these and other evidences, it is considered that the glycogen content is increased successively during the stages of implantation. An increase in the glycogen mobilization consequently provides a nutritive support to the developing blastocysts for their survival present studies also corroborate these findings in normal pregnant rats where a successive increase in the glycogen content has been observed from day 2 to 6 of pregnancy. When aqueous extract of *Moringa oleifera* Lam., is administered to pregnant rats, there is a marginal increase in the glycogen content from day to 2 to 6 of pregnancy; however, the values are
significantly low when compared to their respective control group especially after 4th day post-coitum.

This marginal increase in the glycogen level of treated rats from 2 to 6 ost-coitum may be explained on the basis of mild estrogenic activity of the aqueous extract which keeps the endogenous level of estrogen high in the uterus and thus causes change in the glycogen metabolism. However, the reduced value with respect to their control set may be achieved by the endogenous estrogen-progesterone interactions with the intrinsic hormonal activity of the aqueous extract as the extract has also been reported to possess multiple hormonal attributes like estrogenic, antiestrogenic and antiprogestational. Therefore, due to this hormonal interactions, the glycogen content is reduced in the pregnant rats treated with aqueous extract of Moringa oleifera Lam. and thus in the deficiency of uterine glycogen reserve, the developing blastocysts may not get full energy from maternal tissue for their proper development and thus this factor may become the cause of infertility.

During early stages of implantation the activity of acid phosphatase is considerably changes. It has been shown histo-chemically that during first four days after fertilization, the activity of acid phosphatase is remarkably increased. Additionally on 5th day of implantation when deciduoma makes its appearance beneath the sites where the blastocyst is lined, he associated cells show strong acid phosphatase reaction. Present findings have also revealed that in control pregnant rat the activity of acid phosphatase is successively increased from day 2 to 5 of pregnancy. When the pregnant rats are treated with the aqueous extract of Moringa oleifera Lam. there is no significant change in the activity of this enzymes from day 1 to 5 but at every stage the values the significantly lower than their significant groups especially on day 5th of pregnancy. Acid phosphatase has been reported to pay an important role in the disintegration of complex organells and the liberation of hydrolytic enzymes, removal of the debris or lysed cells by phagocytosis and the alteration of cell permeability. Therefore decrease in the uterine activity of acid phosphatase in the rats treated with aqueous extract of M. oleifera Lam. reveals that the extract does not permit the uterus to undergo phagocytosis for the preparative changes to welcome the fertilized eggs. Present studies also reveal that in control pregnant rat the activity of acid phosphatase is reduced on day 6th of pregnancy, which clearly indicates that at this stage acid phosphatases may not be libeated for the phagocytosis of the epithelial cells as this enzymes is not much required just after the implantation.

An increase in activity of alkaline phosphatase in the uterus at the time of implantation and deciduoma formation has been reported by a number of workers. There are other evidences which clearly indicate that during early days of pregnancy there is disintegration of uterine epithelial cells which is brought about by the observed changes in the lysosomes including the acid phosphatase. Present findings also clearly reveal that the activity of alkaline phosphatase is increased gradually and significantly in the uterus of normal pregnant rat from day 2 to 5 post-coitum and then declines. When the pregnant rats are administered with aqueous extract of Moringa oleifera Lam., the activity of alkaline phosphatase remained unaltered during he early days of implantation (from day 2 to 6 p.c.). However, the value are significantly educed when compared to their respective control group especially after 4th day post-coitum. The exact function of
alkaline phosphatase during pre and post-implantation stages of the uterus has been reported to be associated with the turnover of DNA within the nucleus\textsuperscript{37}, the differentiation of the tissues and in the formation of deciduoma\textsuperscript{38}. In the present study, as the activity of acid and alkaline phosphatase remained significantly reduced in the uterus of rats treated with aqueous extract of \textit{Moringa oleifera} Lam. when compared to normal pregnant rat probably due to the interactions of its multiple normal properties. It is thus concluded that due to the low enzymic activity, the aqueous extract fails to trigger the action of these physiological transformations to induce the formation of deciduoma and the endometrial bed.

The anti-implantation activity in the aqueous extract of \textit{Moringa oleifera} Lam. as observed earlier\textsuperscript{9} can therefore, be explained on the facts that the administration of aqueous extract to pregnant rat does not induce any biochemical modifications in the uterus so as to prepare itself for providing suitable milieu to welcome the fertilized eggs. Consequently, the unprepared or non-receptive uterus results in the insult of the blastocysts.

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