

CONTRACEPTIVE PROPERTIES AND ANTIFERTILITY EFFECTS OF ANDREWS LIVER SALT ON THE HISTOMORPHOLOGY OF THE UTERUS OF ADULT FEMALE WISTAR RATS**Anyanwu G. E.¹, Atuadu V. O.*², Nwannadi V. I.¹, Ogbe P. C.¹, Omire Oluedo O. L.¹, Ugwu P. I.¹, Nweke M. L.¹, Ezeofor, Nwaelom³**¹Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria, Enugu campus (UNEC), Enugu State, Nigeria.²Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, Enugu State University of Science and Technology (ESUT), Enugu State, Nigeria.³Department of Radiation Medicine, Faculty of Medical Sciences, College of Medicine, University of Nigeria, Ituku Ozalla, Enugu.***Corresponding Author: Atuadu V. O.**

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ABSTRACT

Background: Globally, many unplanned pregnancies end up being aborted under safe or unsafe conditions. Andrews liver salt have been perceived by young females as an emergency contraceptive, even though it has not been proved by any scientific research or being specified by manufacturers. **Objective:** This work is designed to investigate the contraceptive potentials of Andrew liver salt and its antifertility effect on the histo morphology of the uterus in adult female wistar rats. **Methods:** Adult female Wistar rats were randomly assigned into 3 groups (A-C). Normal Saline (10 mLkg), Postinor 2 (30 mgkg) and Andrew liver salt (100 mgkg) were administered to rats in group A, B and C respectively after mating. Animals were observed and sacrificed using cervical dislocation on the 15th day. The uteri were harvested for histological examination. **Results:** Microscopic examination of the uterus revealed endometrial mucosa lined by low columnar cells with basal nuclei, well vascularized sub-epithelial stroma. Irregular cytoplasmic vacuolation of the mucosal lining cells and the glandular cells in normal saline and postinor 2 treated rats. Andrew liver salt treated rat uteri revealed thick endometrium and myometrium, apoptosis and vacuolation of both luminal and glandular epithelial cells with marked endometrial infiltration by eosinophils and neutrophils. Multifocal hyperplasia of the epithelial lining was also observed. **Conclusion:** This study shows that excess use of Andrews liver salt causes endometrial hyperplasia and might prevent pregnancy.

KEYWORDS: Andrews liver salt, Oral contraceptives, Uterus.**INTRODUCTION**

Globally, many unplanned pregnancies end up being aborted under safe or unsafe conditions.^[1] The use of plants and other substances for prevention of unplanned pregnancies has been practiced in African societies for centuries. In 2012, approximately 213 million pregnancies occurred worldwide; over 40% of these pregnancies were unplanned^[2], while 50% seek unsafe methods get rid of the unplanned pregnancies.^[3] Research have revealed many reasons some women give for getting rid of unplanned pregnancies, some of which include but not limited to postponement or end of childbearing, socioeconomic concerns—including disruption of education or employment; lack of support from the father; desire to provide schooling for existing children; poverty and unemployment.^[4] Globally, an estimated 22 million unsafe abortions occur each year.^[5] According to the research, Andrew liver salt is one of the

substances most used contraceptive in prevention of unplanned pregnancies amongst female undergraduates.^[6] Andrew liver salt is an antacid and laxative for mild stomach complaints. It is sold as powder which is added to water and mixed creating effervescence before being swallowed. The powder contains two antacids sodium bicarbonate, citric acid, and a laxative magnesium sulphate.

Research on Knowledge and perception of female undergraduates showed that Andrew liver salt was one of the often used agent of contraception.^[7] Despite the lack of scientific evidence backing the indiscriminate use of some oral contraceptives it is important to note that it could be harmful to the body and may cause secondary infertility problems later in life. Hence the need to investigate the effect and contraceptive potency of this substance.

MATERIALS AND METHODS

Experimental animals

Twelve (18) adult female wistar rats of 12 weeks old (110 ± 10 g) and 9 male wistar rats of 14 weeks old (180 ± 10 g), were purchased from Enugu State University of science and technology Animal house for the experiment. The rats were acclimatized for 2 weeks prior to the experiment. The animals were housed in appropriate cages and are allowed free access to standard rat feeds and clear drinking water *ad libitum*. The animals were handled in strict compliance with the guidelines for animal research in National Institute of Health guidelines for care and use of laboratory animals.^[8]

Determination of estrus cycle and Mating

Vaginal secretion was collected from the female rats with the aid of a plastic micropipette. The micropipettes were filled with 10 μ L of normal saline (NaCl 0.9%) and inserted into the vagina of the rats. The vaginal fluid obtained was placed on a glass slide and the unstained fluid was examined via a microscope at X40 magnification. Three types of cells were recognized: the epithelial cells (round and nucleated); the cornified cells (irregular cells without nucleus); the leukocytes (little round cells). The proportion of each type of cell was used for the determination of the estrous cycle phases.^[9] The females in pre-estrus cycle will be caged with the

male rats overnight for mating. The following morning detection of vaginal plug was considered an evidence of mating and considered day 1 of pregnancy.

Experimental design

Adult female Wistar rats were randomly assigned into 3 groups of 6 rats each (A-C). Female rats would be caged with males of proven fertility in the ratio of 2:1. Rats in group A received distilled water (10 mL/kg, p.o.) and served as normal control. The rats in groups B and C received Postinor 2 (30 mg/kg, p.o.) (10) and Andrew liver salt (100mg/kg) respectively from day 1 post mating to day 14.

Sacrifice

Two rats from each group was sacrificed using cervical dislocation. The abdominal cavity was incised to expose the reproductive organs. The uteri were harvested for histological examination following Haematoxylin and eosin (H&E) staining.

Data Analysis

Data were analyzed using SPSS version 22 and presented as Mean \pm Standard deviation (SD). Statistical difference in the mean was done using one-way ANOVA and Students T-test for multiple and paired variables respectively. The statistical analysis was considered significant at $P < 0.05$.

RESULTS

RESULT OF ANTIFERTILITY EFFECT OF ANDREW LIVER SALT ON BODY WEIGHT OF ADULT WISTAR RATS

Initial and final mean body weights of rats

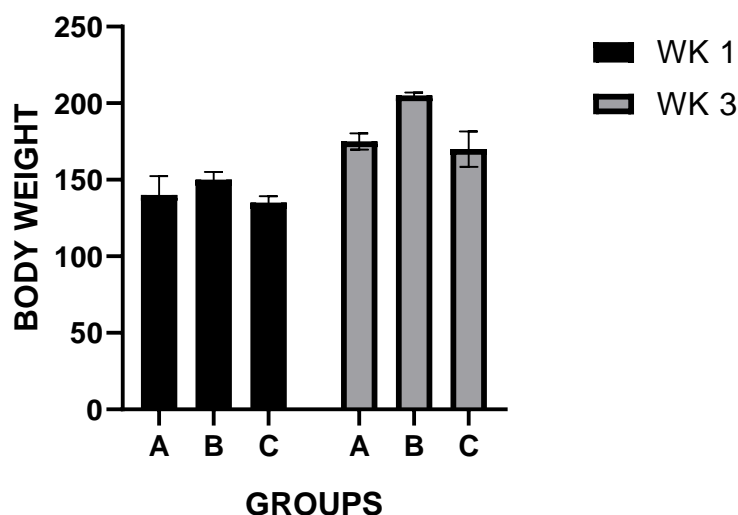


Fig 1: Graph shows initial and final mean body weight of the rats in different groups. There was an increase in weight in all the groups at the end of the experiment. The highest increase in weight was seen in group B treated with postinor 2 (30 mg/kg).

Change in body weight of rats in various groups

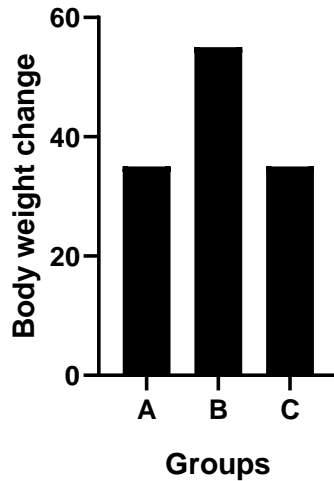


Fig 2: Graph shows the increase in body weight among the groups. It shows that all groups increased in weight during the experiment. Group B which is the positive control for contraception had the highest increase in weight, followed by group A, the negative control and then Group C, the test (Andrew liver salt) group.

RESULT OF ANTIFERTILITY EFFECT OF ANDREW LIVER SALT ON UTERINE HISTOLOGY OF ADULT WISTAR RATS

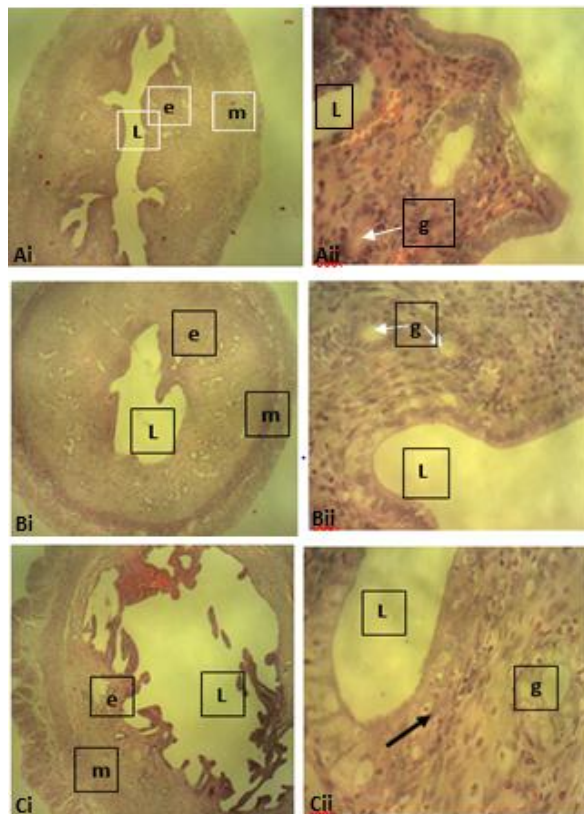


Fig. 3: Photomicrograph sections of the uterine horn of (A) normal saline (10 ml/kg), (B) postinor 2 (30 mg/kg) and (C) Andrew liver salt (100 mg/kg) treated rats; H&E X40 and X400 (i and ii) respectively. There is an increased proliferation of endometrial glands (g), folds of lumen (L) in the uterus of rats treated with normal saline (10 ml/kg) and postinor 2 (30 mg/kg). The endometrial mucosa is markedly thickened, throwing out varying sizes and shapes of mucosal folds into the endometrial lumen (L). The uterus of rats treated with Andrews liver salt (100 mg/kg) showed irregular cytoplasmic vacuolation of the mucosal lining cells and the endometrial glands (g) were sparse. There was endometrial and myometrial hyperplasia and the myometrium

(m) appears to be thinner than the endometrium (e). There was apoptosis and vacuolation of both luminal and glandular epithelial cells with marked endometrial infiltration by neutrophils and eosinophils. Multifocal hyperplasia of the epithelial lining (black arrow) was also observed. H&E x40; x400.

DISCUSSION

Andrew liver salt is used as an emergency oral contraceptive by most female undergraduates.^[7] This research studied the histomorphological effects and histological alterations in the uterus of adult wistar rats, as a result of oral administration of Andrew liver salt. It revealed important alteration in uterus, such as endometrial hyperplasia. Endometrial hyperplasia is one of the effects of oral contraceptives according to a research 'Endometrial changes in women receiving oral contraceptives.'^[9] The findings in the work also corresponds to that of Friedrich in his research on effects of contraceptive hormone preparations on the fine structure of the endometrium.^[10]

Sodium bicarbonate which is one of the constituents of Andrew's liver salt has been discovered to affect the motility of spermatozooids.^[11] A physical application of sodium bicarbonate into the vaginal cavity 10 minutes before coitus had no negative influence on the vaginal mucus membrane or the normal vaginal flora. Furthermore, 2-3 applications of this solution were found to change a previously vaginal cleanliness index of 4 to an index of 3.^[12] As such chronic use of Andrew liver salt as emergency oral contraceptive could lead to vacuolation of luminal and glandular epithelial cells, myometial hyperplasia as were observed in this work.

Research revealed that of all women who used Andrew liver salt as oral contraceptive over the course of 2-3 years, only 22 became pregnant. Hence, bicarbonate of soda was considered to be a contraceptive agent.^[13] This likely effect of sodium bicarbonate could also be associated to its presence in Andrews liver salt and an explanation to the inability of group C to conceive during the period of the research. The lowest increase seen in group C rats treated with Andrew liver salt could be as a result of Andrews liver salt as it is already known to be associated with problems of the Gastrointestinal tract (GIT) according to the manufacturers.

Although in Keburiya's research sodium bicarbonate was applied ectopically, the result from this research is in line with that of Keburiya's work.^[13] Therefore, women who take Andrews liver salt and other sources of sodium bicarbonate who are unable to conceive or still planning on conception should stop taking them or reduce consumption to minimal level as sodium bicarbonate has various and numerous sources especially baked foods which women take on regular basis.

Furthermore, the results of body weight showed that Andrew liver salt decreased the body weight of the rats although the highest decrease in body weight was recorded in rats in group B treated with Postinor 2. This

showed that Andrew liver salt has only a mild effect on body weight.

CONCLUSION

In conclusion, from this research work, it was observed that Andrews liver salt causes endometrial hyperplasia and prevents pregnancy.

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