Hormonal and Morphological Effects of Averrhoea Carambola Fruit Extract on Female Reproduction

ABSTRACT

Medical herbs are dazzling resources that influences female fertility. The effect of *Averrhoa carambola* ethanolic fruit extract on gonadal function and structure in female Wistar rats was investigated in the study. 15 Wistar rats weighing 100 to 143g were divided into three groups of five rats each. Food and distilled water were given to Group A, 500mg/kg of ethanolic fruit extract of *A. carambola* was given to Group B, and 1000mg/kg of ethanolic fruit extract of *A. carambola* was given to Group C every day for 21 days through oral garvage. The animals were anesthetized with chloroform at the end of the experiment, and serum was taken for hormone testing. When compared to the control, there was a significant ($p<0.05$) weight gain in the test groups. In addition, there was a substantial rise in ovarian and uterine weight in groups B and C after receiving the *Averrhoa carambola* fruit extract compared to the control group. However, when compared to the control group, LH increased ($p>0.0$) while progesterone declined significantly. According to the findings, the extract's structural effects on the gonads of female rats were shown to be adverse. In conclusion, *Averrhoa carambola* fruit extract may be more beneficial as a contraceptive than a pro-fertility agent, as traditional medicine practitioners claim.

Key words: *Averrhoa carambola*, Hormonal, Morphological, Reproduction.

Introduction

In recent years, medicinal plants have acquired substantial prominence in the management of a variety of human illnesses, outperforming synthetic medications with few side effects. Secondary metabolites found in medicinal plants, such as flavonoids, glycosides, tannin, saponin, terpenoids, and others, have been demonstrated to play a part in the healing process of diseases (1). Plant products with higher medicinal properties have been connected to a substantial impact on the development of novel medications.

The fruit of Averrhoa carambola, which belongs to the Oxalidaceae family, is noted for its strong therapeutic potential (2). Star fruit and Chinese gooseberry are the English appellations for it. It's called Kamrakh and Karmal in Hindi, Balimbing and saranate in Phillipino, Belimbing in Indonesian, and Belimbing in Malay (2).

It is widely consumed as a fruit in various tropical places around the world, and it is widely grown in Southeast Asia (3).

Infertility, fever, cough, diarrhea, persistent headache, inflammatory skin problems (eczema), and fungal skin infections are all treated using the fruits and other aerial parts of *A. carambola*, according to an ethnomedical review (4). Different parts of the plant, particularly the ripening fruits, are employed as antipyretics, laxatives, appetite stimulants, sialogogues, astringents, and other medicinal properties. Inflammation of the throat, mouth ulcers, toothaches, cough, asthma, hiccups, food poisoning, colic, diarrhea, jaundice, malarial splenomegaly, hemorrhoids, skin rashes, pruritis, sunstroke, and some eye disorders are all treated with the fruits.

When crushed and applied topically, the leaves are said to treat chicken pox, ringworm, and headache locally. Oliguria, boils and pyodermas, postpartum edema, gastroenteritis, and traumatic injuries are also treated with the leaves. Fever and malaria are treated using the blossoms. Arthralgia, chronic headache, epitaxis, and spermatorrhea are all treated with the roots. When mixed or blended with sugar, it acts as a toxic antidote (2).

A. carambola has antioxidant, hypoglycemic, hypotensive, hypocholesterolemic, antiinflammatory, antiinfective, and anticancer properties, according to a pharmacological review (5). Following the injection of the root extract of *A. carambola* in a diabetic animal, Qin et al. (6) discovered a considerable drop in blood glucose levels. Aladaileh et al (7) found that rats fed high-fat diets had lower serum lipid levels, as well as lower body mass index, atherogenic index, hepatic cholesterol, and triglycerides, and higher fecal cholesterol and bile acids. Leelarungrayub et al. (8) found a substantial increase in overall antioxidant capacity, lower levels of malondialdehyde and protein hydroperoxide, and higher levels of vitamin A and C. Cabrini et al. (9) found that an ethanolic extract of star fruit leaves reduced edema in the croton oil-induced ear edema model of inflammation in a dose-dependent manner. Myeloperoxidase activity was decreased by the ethanolic extract or its fractions, suggesting that these chemicals may influence cell motility during the inflammatory process. TNF alpha, interleukin (IL) 23, and nitric oxide levels were reduced after 4 weeks of star fruit juice drinking, according to Leelarungrayub et al. (8). (NO).

The strong flavonoids activity was linked by Vastra et al. (10) to its substantial anti-ulcer action in gastric damage produced by free radicals. On indomethacin and pylorus ligation-induced ulcer models, Kulal et al. (11) demonstrated a gastro protecting and anti-ulcerogenic effect. Also, indomethacin and pylorus ligation have potent ulcer-protective efficacy of up to 71.36 percent and 76.48 percent, respectively, as well as a reduction in stomach volume, total acidity, free acidity, and

ulcer index, as well as a rise in gastric pH. According to a phytochemical analysis, A. carambola contains saponins, flavonoids, alkaloids, tannins, and pyrogallic steroids (12). Phenols, anthocyanin and anthocyanidin, chalcones and aurones, leucoanthocyanidins, catechins, and triterpenoids were all isolated from different portions of the star fruit (13).

Despite its vast medicinal efficacy, research on the effects of Start fruits on gonadal function and structure in female wistar rats is sparse.

Materials and methods

Preparation of extract

Averrhoa Carambola (Star Fruit) fruits were cleaned under running tap water and air-dried at room temperature. Using a local grinder, the dried fruits of Averrhoa carambola were ground into a coarse powder. 250 grams of dried Averrhoa Carambola fruits were macerated for 48 hours in 1000 milliliters of 95 percent absolute ethanol (JHD, China). It was first filtered with a clean porcelain cloth, then with Whatman No 1 filter paper (Sigma Aldrich WHA1001042). A rotatory evaporator (TT-52, Techmel & Techmel, USA) was used to concentrate the filtrate, which was then dried into a gel-like form in a laboratory oven (DHG-9023A, PEC MEDICAL USA) at 45oC. The extract was kept in the refrigerator to be used later. This extraction process was based on Al-(14) Attar's approach with certain modifications.

Acute Toxicity of ethanolic fruit extract of *Averrhoa carambola*

Lorke's (1983) method was used to determine the median lethal dose (LD50) of the Ethanoic fruit extract of *Averrhoa carambola*, which was shown to be greater than 2000mg/kg for more than 28 days.

Experimental design

The animals were separated into three groups of five animals each for the study.

Group A received only food and distilled water.

Group B received 500mg/kg of *A. carambola* ethanolic fruit extract

Group C received 1000mg/kg of *A. carambola* ethanolic fruit extract

The extract was given to the subjects once a day for 21 days via oral gavage.

Preparation of Serum

Blood was collected from rats in each group at random through ocular puncture into sterile containers, spun, and stored in the refrigerator. The rats were then sacrificed through cervical dislocation.

Hormonal Assay

The luteinizing hormone and FSH levels were determined using the enzyme immunoassay technique, as stated in the manufacturer's instructions.

Histological Examination

The control and treatment rats' ovary and uterus were fixed in Bouin's fluid for 6 hours before being transferred to 10% formalin for histological assessment. The tissues were treated and examined under a light microscope on a regular basis. The slide was then photomicrographed (15).

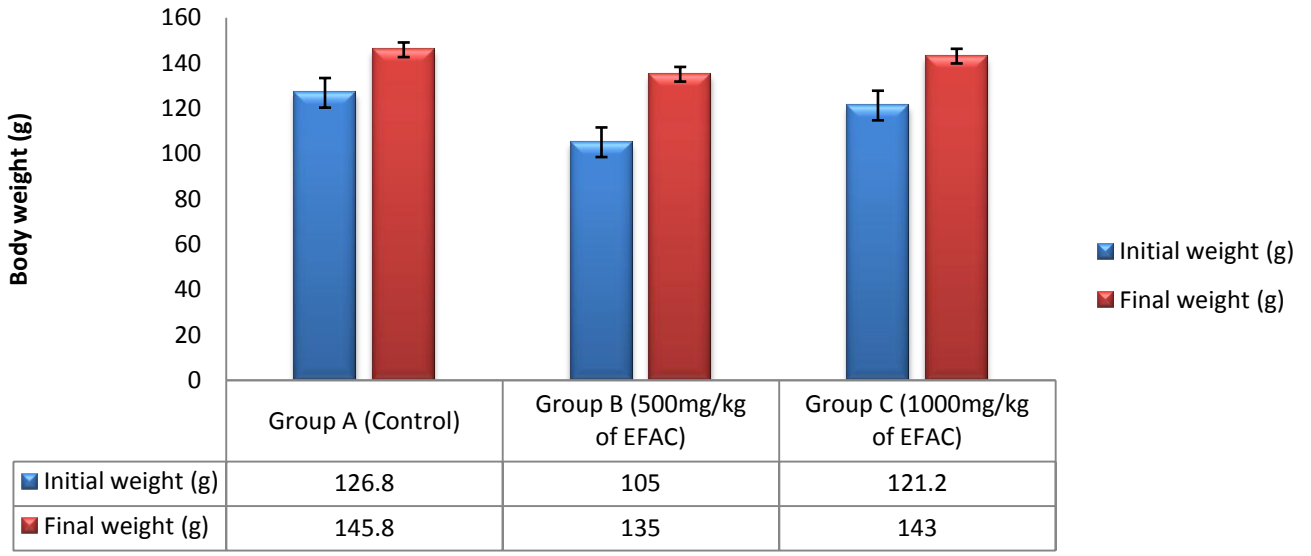
Statistical Analysis

Statistical Science for Social Sciences (SPSS) version 25 was used to analyze the data from this study. The results of the hormonal profile (FSH, progesterone, and LH), as well as the Gonado-somatic index weight (ovary and uterine), were analyzed using ANOVA and post hoc Turkey. The T-dependent test was used to examine body weight. At $p<0.05$, the data was declared significant.

Results

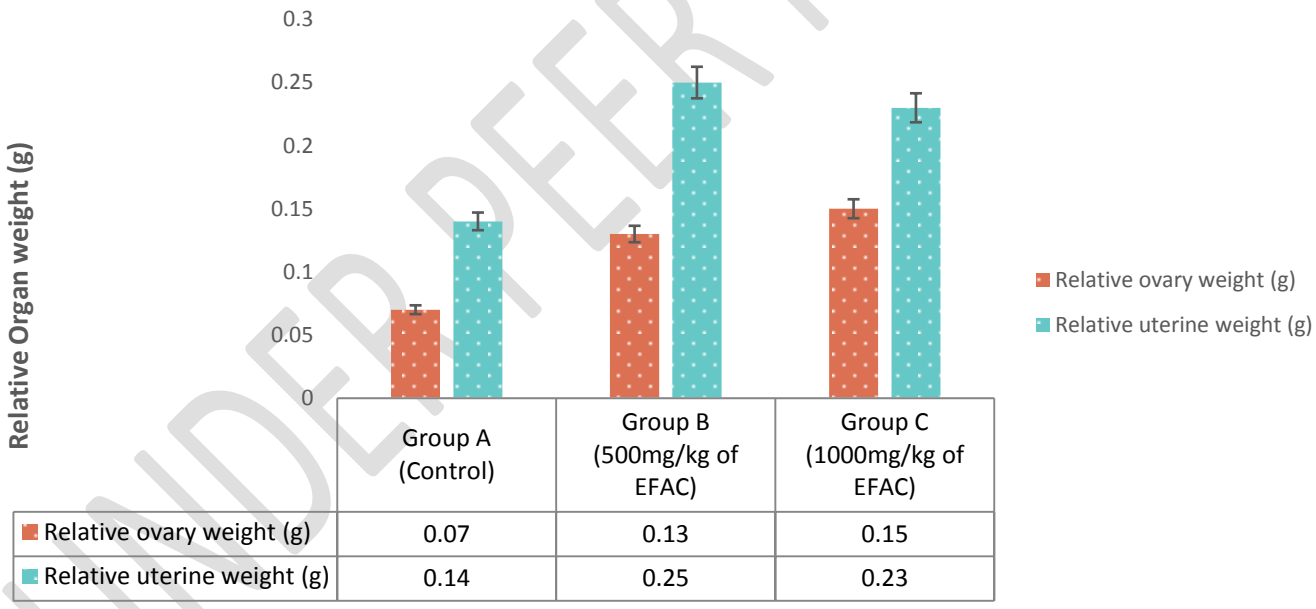
When the initial weight was compared to the final weight, Fig. 1 revealed a substantial ($p<0.05$) rise in groups B and C. Fig. 2 shows that in groups B and C, ovarian and uterine weights increased significantly ($p<0.05$) when compared to group A. In comparison to group A, LH levels in groups B and C increased significantly ($p<0.05$). In comparison to group A, FSH increased insignificantly ($p<0.05$) in groups B and C. Progesterone levels in groups B and C were significantly lower ($p<0.05$) than in group A.

Figure 1: Values of extract on body weight of study animals.



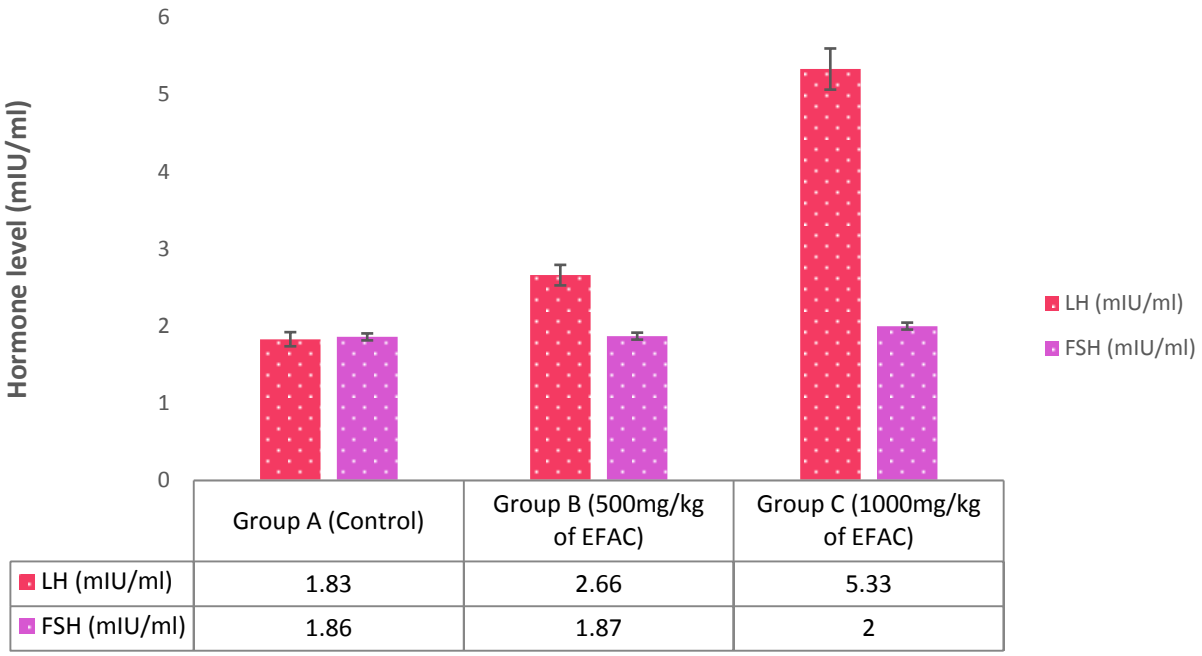
	Group A (Control)	Group B (500mg/kg of EFAC)	Group C (1000mg/kg of EFAC)
Initial weight (g)	126.8	105	121.2
Final weight (g)	145.8	135	143

Figure 2: Values of extract on ovary and uterine weight of study animals.



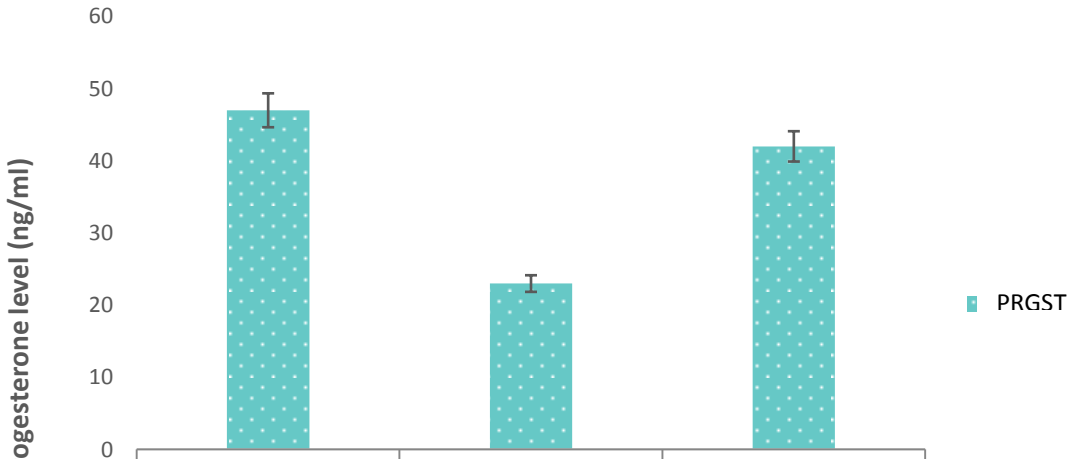
	Group A (Control)	Group B (500mg/kg of EFAC)	Group C (1000mg/kg of EFAC)
Relative ovary weight (g)	0.07	0.13	0.15
Relative uterine weight (g)	0.14	0.25	0.23

Figure 3: Values of extract on LH and FSH of study animals.



	Group A (Control)	Group B (500mg/kg of EFAC)	Group C (1000mg/kg of EFAC)
LH (mIU/ml)	1.83	2.66	5.33
FSH (mIU/ml)	1.86	1.87	2

Figure 4: Values of extract on Progesterone of study animals.



	Group A (Control)	Group B (500mg/kg of EFAC)	Group C (1000mg/kg of EFAC)
PRGST	47	23	42

Ovary and Uterus Histology of Extract

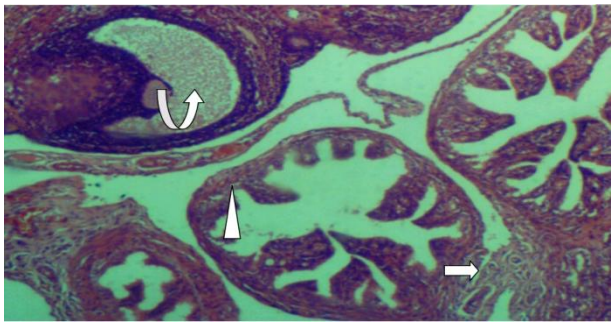


Plate A (H & E) x 100

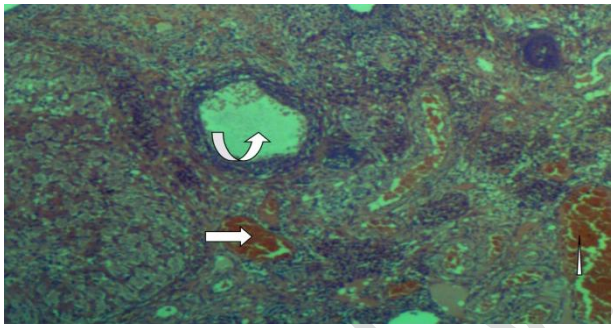


Plate B (H & E) x 100

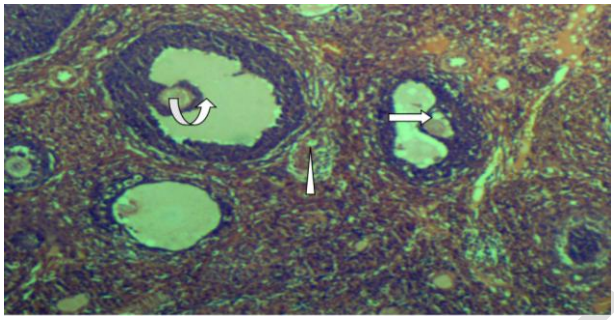


Plate C (H & E) x 100

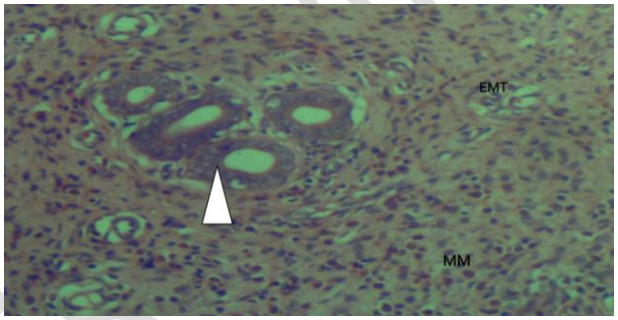


Plate D (H & E) x 400

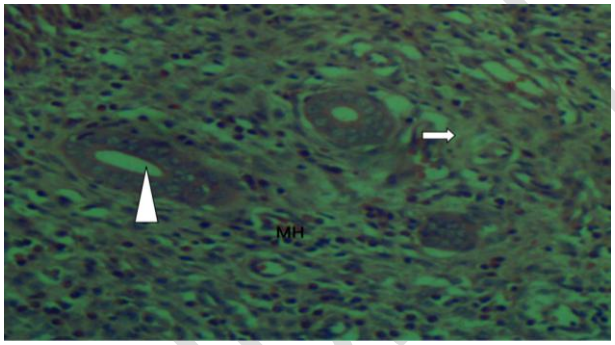


Plate E (H & E) x 400

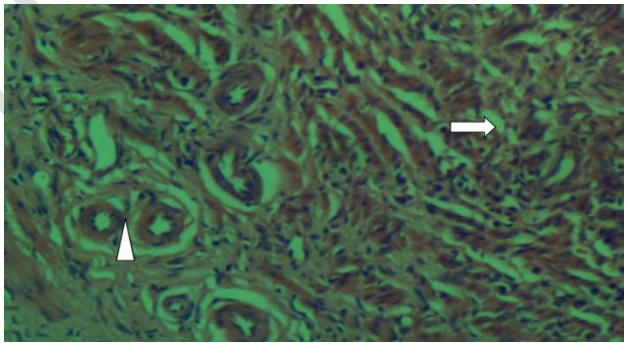


Plate F (H & E) x 400

Plates A-C (Ovary) and Plates D-F (Uterus) show the results of the histological investigation of the rat's ovaries and uterus. Plate A depicts a photomicrograph of ovarian tissue with normal morphology. Normal histology can be seen in the follicular cells (arrowhead), primordial follicles (arrow), and oocytes (curved arrow). Plate B shows minor bleeding in follicular cells (arrowhead), but primordial follicles (arrow) and oocytes (curved arrow) have normal histology. Plate C has normal histology with follicular cells (arrowhead), primordial follicles (arrow), and oocytes (curved arrow). The photomicrograph of the uterus in Plate D demonstrates normal myometrium (MM) and endometrial histology (EMT). The stroma and endometrial glands (arrowhead) are both normal, with no signs of damage. In Plate E, the myometrium and endometrium of the uterus have normal histology. With minor hemorrhage, the endometrial glands (arrowhead) and stromal (arrow) are normal. Plate F shows normal endometrial glands (arrow head) and stroma (arrow), with minimal stroma cell vacoulation.

Discussion

In both animals and humans, female hormones (FSH, LH, and progesterone) are crucial regulators of species continuity (24, 16). Female infertility has also been linked to shortages in these hormones, which has been a source of concern. Averrhoa carambola is a well-known medicinal plant with significant antioxidant properties that is frequently used as a reproductive plant by herbalists without scientific support (17).

The effect of ethanolic fruit extract of Averrhoa carambola on FSH, LH, and progesterone in female rats is investigated in this study. The treatment groups gained weight significantly, which could be due to the elevated cellulose activity that regulates energy homeostasis. Furthermore, the control of metabolism is influenced by the energy regulation of the hypothalamic satiety regions, which regulate food and fluid intake (18). Zhang et al. (19) observed a considerable weight gain in diabetic mice after receiving DMDD from Averrhoa carambola roots, which is consistent with our findings. Li et al., (20) found that DMDD from Averrhoa carambola roots resulted in considerable weight growth, which is similar to our findings. In addition, Singh et al. (21) found no significant weight gain in a hepatocellular carcinoma model after administration of Averrhoa carambola, which contradicts our result. Furthermore, Ramadan et al. (22) observed a considerable rise in weight after consuming Averrhoa carambola, indicating high fiber content, which is consistent with this study. Aba and Amadi (23) reported considerable weight reduction after taking Averrhoa carambola, which contradicts the findings of this study.

In comparison to the control group, the fruit extract of Averrhoa carambola caused a considerable rise in ovarian and uterine weight in groups B and C. The presence of saponin, which has a favorable effect on the organs, could explain the physiology of the increase; nevertheless, the particular mechanism of action is unknown. Aba and Amadi's (23) research contradicted this study's findings, indicating non-significant weight increase organs in rats. Singh et al. (21) reported a substantial increase in organ weight after administration of Averrhoa carambola, which is consistent with this investigation.

Luteinizing hormone is a crucial hormone in the female reproductive system that regulates ovarian function. In comparison to group A, the study found a significant ($p>0.05$) increase in LH levels in

groups B and C. The phytoconstituents in the extract may be the mechanism of action for the rise. In addition, FSH increased insignificantly in groups B and C compared to group A, with the cause unknown. In comparison to group A, the Progesterone result showed a significant drop in groups B and C.

The results of the histological examination of the rat's ovaries and uterus are shown in Plates A-C (Ovary) and Plates D-F (Uterus). The extract produced mild hemorrhage of follicular cells at a low dose (500 mg/kg), while primordial follicles and oocytes were consistent with the normal histology of the ovary of the animals in Group B. The high dose (1000 mg/kg) is inconsistent with the normal histology. Hence, *A. Carambola* can affect a woman's ovary and uterus, which are important reproductive organs.

Oral treatment of the extract at a low dose (500 mg/kg) resulted in modest bleeding of the endometrial glands and stroma of the uterus in the animals in Group E, whereas a high dose (1000 mg/kg) resulted in mild vacuolation of the uterus stroma cells in Group E. When compared to the control (Group D), both doses of the extract have a negative effect on the stroma cells of the uterus of female Albino wistar rats

In conclusion, *Averrhoa carambola* fruit extract may have a beneficial effect on body weight, relative ovary and uterine weight, and FSH and LH activities.

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