

Original Research Article
**ASSESSING THE IMPACT OF ONION JUICE ON THE LEVELS OF
LEUTINIZING
HORMONE AND FOLLICLE STIMULATING HORMONE IN FEMALE
MICE EXPOSED
TO TRANSFLUTHRIN SMOKE.**

ABSTRACT

Aim: Transfluthrin is a fast-acting insecticide used in household mainly against mosquito. Its uses have not been without health risk to humans. The study aims at evaluating the effect of onion juice on follicle stimulating hormone and leutinizing hormone levels in mice exposed to transfluthrin smoke.

Study Design: This is an animal study conducted in the Animal House, College of Medical Science in Rivers State University.

Material and Methods: Thirty (30) female mice were used in this study. They were divided into six groups of five mice each; Group 1 (negative control, exposed to normal environmental air), Group 2 (exposed to 6g of transfluthrin coated insecticide paper smoke and given 0.2ml of onion juice), Group 3 (exposed to 6g of transfluthrin coated insecticide paper smoke only), Group 4 (exposed to 12g of transfluthrin coated insecticide paper smoke and given 0.2ml of onion juice), Group 5 (exposed to 12g of transfluthrin coated insecticide paper smoke only), Group 6 (given 0.2ml of onion juice only) through the oral gavage method daily. The smoke inhalation lasted for 15 minutes daily for 28 days.

Results: There was a significant difference in the mean values of Follicle Stimulating Hormone in groups 2, 3, 4, 5 & 6 when compared to group 1 ($P=0.004$). There was no significant difference observed in the mean values of Leutinizing Hormone in the various groups ($P=0.068$). The ovaries in groups 2, 3, 4, 5 & 6 showed several distortion and fluid congestion.

Conclusion: This study shows that the exposure significantly altered the hormonal levels and the ovarian cyto architecture of the mice. It also reveals that exposure to transfluthrin is detrimental to the reproductive functions of female mice however, the Onion juice administered to the mice in this study showed no ameliorative effect on the ovarian distortion, hence not improving the reproductive status of the mice.

KEYWORDS: Transfluthrin smoke, LH, FSH, Onion Juice, fertility, reproductive health.

1. INTRODUCTION

Lifestyle factors, which include the practices we adopt in our daily life, have a significant role in shaping our overall health. Several factors have been claimed to affect women's fertility; lifestyle-related factors, in particular, have received great attention in the last decade. Transfluthrin is a commonly used synthetic pyrethroid insecticide that is widely utilized for its insecticidal properties in various household products [1]. Transfluthrin is very active in controlling malaria by killing mosquitos via inhalation or contact with the substance (World Health Organization [2, 3]). Its usage has become a daily lifestyle practice for most homes. However, concerns have been raised regarding its potential adverse effects on endocrine function, particularly in female mammals [4]. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) play critical roles in regulating the reproductive system. Any disruption in the normal secretion or balance of these hormones can have profound implications for female fertility and reproductive health [5,

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Comment [AS2]: Check space, secondly Do authors have ethical clearance as this study is based on animals?

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Comment [AS4]: 1. What is the basis of selection of 6g and 12g of dose?
2. Why the dose of 0.2 ml of onion juice is selected and upon higher concentration of transfluthrin still the same dose of onion juice is selected why the authors have not increased or decreased the dose of onion juice?
3. As per my observation groups should be rearranged
Group 1 should be control
Group 2 should be treated one i.e with transfluthrin
Group 3 should be treated one with onion juice
Group 4 should be treated one with higher dose
Group 5 should be treated one (higher dose) with onion juice
Group 6 should be onion juice
First, treatment with transfluthrin should be given after that we can observe the effect of onion juice on it.

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6, 7]. Onion (*Allium cepa*) is a well-known vegetable with a rich composition of bio active compounds, including flavonoids and sulfur-containing compounds. Previous studies have suggested that onion possesses antioxidant and anti-inflammatory properties, and it may also exert modulatory effects on hormone levels. Specifically, onion has been reported to influence the endocrine system by altering hormone secretion or activity, making it a potential agent or intervention for mitigating the adverse effects of endocrine disruptors. It is ~~also prominent~~ **also prominent** for its reproductive health boosting properties [8]. ~~It~~ **It** is estimated that at least 51 synthetic chemicals are ubiquitous in our environment, all of which are suspected reproductive toxicants [9]. As a result of this, pyrethroids, where Transfluthrin belongs ~~are able to can~~ **can** bind to mammalian steroid receptors and disrupt the steroid signaling pathways [10], ~~thereby adversely~~ **thereby adversely** affecting reproduction and sexual development [11]. ~~The~~ **The** transfluthrin exposure ~~might cause~~ **might cause** poor oocyte maturation and competency, meiotic maturation of oocytes, embryonic ~~defects~~ **defects**, and poor IVF outcomes in animal studies [12]. Therefore, the essence ~~of evaluating~~ **of evaluating** the effect of onion juice on LH and FSH levels in female mice exposed to transfluthrin smoke.

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2. MATERIALS AND METHODS

2.1 Animals

Thirty (30) female mice were obtained from Animal care unit at Rivers State University, NkpoluOroworukwo, Port Harcourt, ~~Nigeria~~ **Nigeria**. They were housed in semi-transparent plastic cages under standard laboratory conditions with a 12 hours daylight cycle and have access to feed and water ad libitum. They were acclimatized to laboratory conditions for 2 weeks before grouping for the ~~experiments~~ **Animal experiments**. ~~Animal~~ **Animal** laboratory conditions room ~~temperature, 40~~ **temperature, 40** to 50% humidity and proper ventilation. Experimental procedures involving the animals and their care were conducted in conformity with International, National and Institutional guidelines for the care of laboratory animals in Biomedical Research [13].

2.2 Animal Feed

~~Pelletised~~ **Pelletized** Vital Feed (ultima) was obtained from a store in Rumuokoro market, River State, Nigeria and used to feed the mice during the experiment.

2.3 Transfluthrin Coated Insecticide Paper (TCIP)

The ~~Transfluthrin insecticide~~ **Transfluthrin insecticide** paper (Rambo® Bayer Crop science, Gongoni Company Limited) was purchased from a retail store in Mile One Market, Port Harcourt, ~~Rivers~~ **River's** state Nigeria. This insecticide paper weighed 6 g and contained 0.45% of transfluthrin, 2.5% of Essential Oil and 97.05% of inert ingredients.

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2.4 Transfluthrin Smoke Exposure

6grams and 12 grams of Transfluthrin coated insecticide papers respectively were burnt and the experimental mice were exposed to the transfluthrin smoke. The papers were placed inside a transparent plexiglass box (0.6x0.5x0.1 m³) with flow ports which acted as an inhalation chamber to perform the smoking process using a modified smoking machine. After placing the mice inside the inhalation chamber, a vacuum pump withdrew smoke from burned repellent papers inside the chamber to produce a smoky environment. The smoking procedure lasted for 15 minutes' duration daily for 28 days. Onion juice was administered once daily for period of 28days after they are exposed to the smoke. After the experimental period, the mice were sacrificed under chloroform euthanasia on the 29th day, blood samples were collected for the biochemical analysis of follicle stimulating hormone and Leutinizing ~~hormone~~ **hormone**. The ovaries were harvested and placed into a sample container containing formaline then followed ~~by histological~~ **by histological** examination.

2.5 Onion Juice Preparation

Onion bulb was obtained from mile one market, River State, Nigeria. ~~The fresh~~ **The fresh** onion bulbs were washed, cut into small ~~pieces~~ **pieces**, and rubbed against a grater. It was squeezed and filtered through a fine cloth to obtain the juice. 0.2mls of ~~the onion~~ **the onion** juice was administered to the mice with the aid of a flexible oral gavage tube for a period of 28 days. Fresh onion juice was prepared daily in the morning for the experimental purpose.

2.6 Experimental Groups/ Design

Group 1 (Negative Control) was given feed and distilled water and exposed to normal environmental air for 28 days.

Group 2 (6g of TCIP smoke plus onion juice) mice were exposed to (6g) transfluthrin coated insecticide ~~paper smoke~~~~paper smoke~~ for 15 minutes daily for 28 days, then administered 0.2mls of fresh onion juice daily using the oral gavage method.

Group 3 (6g of TCIP smoke without onion juice) mice were exposed to low concentration (6g) of transfluthrin coated insecticide paper smoke for 15 minutes ~~daily without~~~~daily without~~ administration of onion juice (0.2ml).

Group 4 (12g of TCIP smoke with onion juice) mice were exposed to high concentration of transfluthrin coated insecticide paper smoke for 15 minutes daily (12g) and also administered 0.2ml of fresh onion juice daily using the oral gavage method.

Group 5 (12g of TCIP smoke without onion juice) mice were exposed ~~to high~~~~to high~~ concentration of transfluthrin coated insecticide paper ~~smoke~~~~(smoke (12g) without the~~~~without the~~ administration of ~~fresh~~~~of fresh~~ onion juice

Group 6 (only Onion juice) mice were orally administered fresh onion juice (0.2ml) ~~using~~~~using~~ the oral gavage method daily without exposure to the transfluthrin smoke for 28 days.

2.7 Blood collection

3mls of blood sample was collected through cardiac puncture into plain sterilized centrifuge bottles and allowed to clot. The serum was separated by centrifugal force at 3000rpm for 15 minutes and collected in sample bottles for determination of FSH ~~and~~~~AND~~ LH levels.

2.8 Hormone Analysis

LH and FSH levels were quantified using enzyme-linked immunosorbent assay (~~ELISA) kits~~~~ELISA) kits~~ from Fortress diagnostics Ltd (Antrim, UK)

2.9 Histological Examination

The ovary was immersed in 10% formal saline solution in tissue sample bottle. The paraffin-embedded samples were sectioned at 5 ~~mm~~~~thickness. The~~~~thickness. The~~ samples passed through each stage of tissue processing. They were grossed, and placed in labeled tissue cassettes and introduced into ascending grades of alcohol, (70%, 90%, Absolute 1 and 2) for dehydration and further introduced into two changes of xylene for clearing. The tissues were infiltrated and embedded with molten paraffin wax and solidified with cold ice packs. The prepared tissues were sectioned using a rotary microtome and the selected ribbons was picked in clean grease free slides. They were heat fixed on a hot plate and dewaxed in xylene, hydrated in descending grades of alcohol and taken to water bath before proceeding with the staining procedure. ~~Haematoxylin~~~~Hematoxylin~~ and Eosin staining technique was used to stain the tissues. finally, the slides containing the tissue sample was covered with a cover slip after the application of DPX for mounting and then observed under the light ~~microscope.~~~~Photomicrographs~~~~microscope. Photomicrographs~~ of the sections where obtained.

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2.10 Statistical Analysis

GraphPad Prism version 9.0.4 of Apple Macintosh HD Big Sur (version 11.0) statistical package was used for data analysis. ANOVA was used to compare means between the various groups for inferential evaluation. Tukey's multiple comparison test was used to compare mean values in groups that were significantly different. The probability (p) value less than 0.05 was considered statistically significant.

3.RESULTS

Table 1: Mean ± SD of Follicle Stimulating Hormone and Leutinizing Hormone levels in Mice with different treatment requirement for two weeks.

Group/Treatment Design	Follicle Stimulating Hormone ng/ml	Leutinizing Hormone ng/ml
Group 1(Negative control)	0.967±0.355 ^a	1.40±0.515
Group 2(low dose from TCIP smoke + onion)	0.590±0.370 ^b	0.85±0.530
Group 3(low dose from TCIP smoke smoke)	0.580±0.130 ^c	0.81±0.175
Group 4(high dose from TCIP smoke + onion)	0.643 ±0.1007 ^d	0.92±0.140
Group 5(high dose from TCIP smoke)	1.083±0.205 ^e	1.547±0.295
Group 6(positive control) onion only	1.453±0.095 ^f	1.673±0.467
F value	6.412	3.944
P value	0.004	0.068
Remark	Significant	Not significant

N=5, values ~~with different~~with different super scripts are significantly different from each other ($P=0.05$)

Comment [AS8]: Why upon high dose of insecticides the value of FSH has been increased while in other cases the FSH value has been decreased?

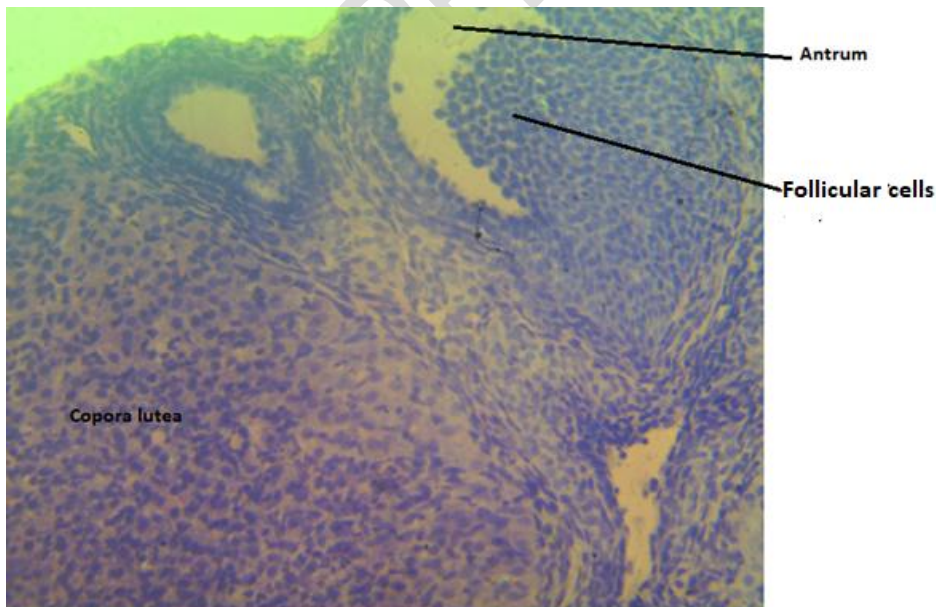


Plate 1: (Group 1) Photomicrograph of the ovary tissue showing primary follicles with developing antrum, Well delineated corpora lutea observed. Normal tissue observed at higher magnification. H & E, X400.

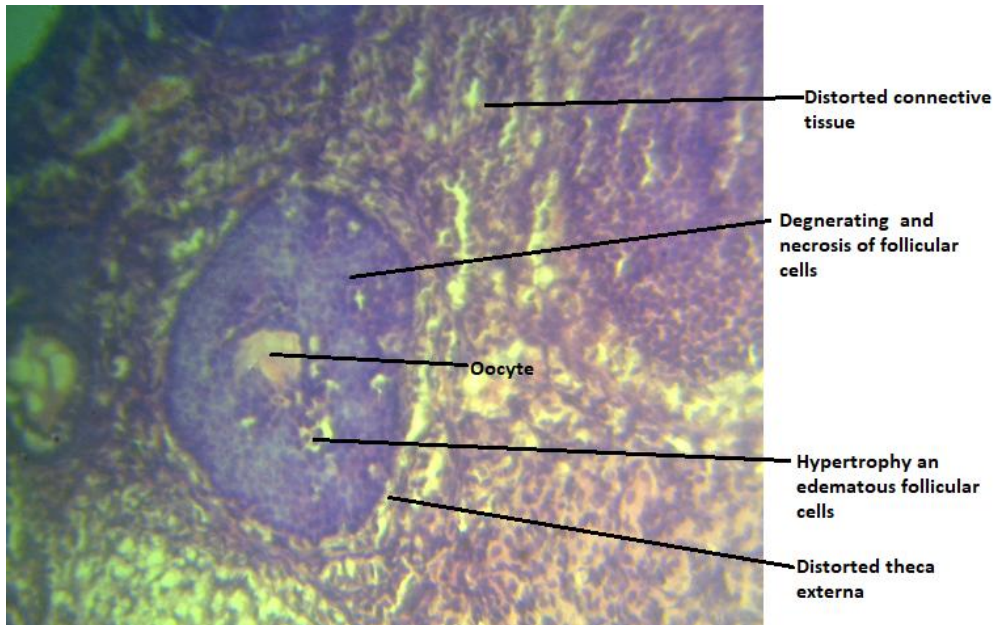


Plate 2: (Group 2) Photomicrograph of the ovary tissue showing degenerating, necrosis and hypertrophy of follicular cells, large oocyte. The theca externa shows distortion. The connective tissue also shows distortion. Tissue shows distortion of cytoarchitecture. H & E, X400.

Comment [AS9]: Specify the dose of transferrin and onion juice

UNDER REVIEW

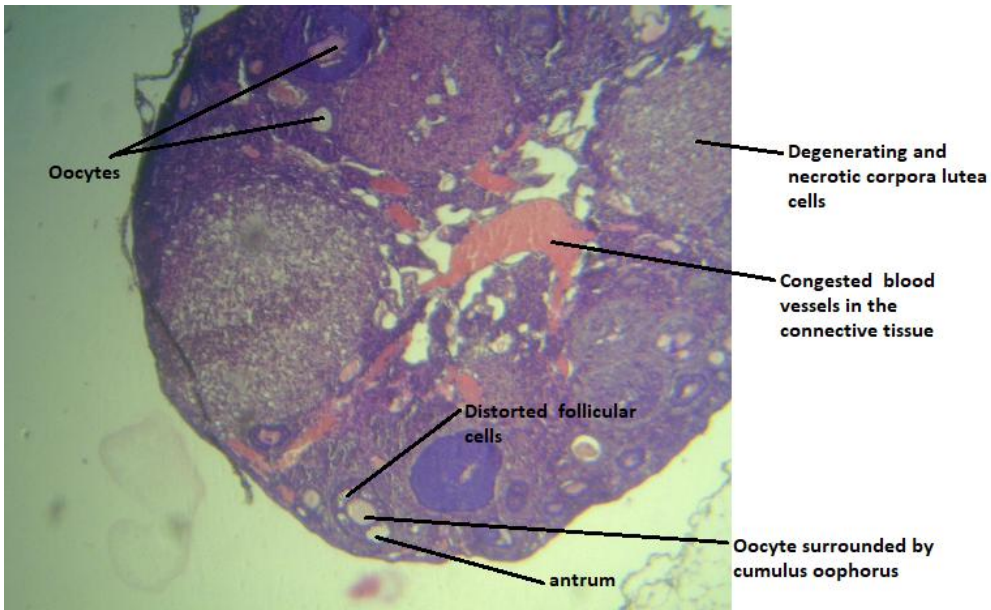


Plate 3: (Group 3) Photomicrograph of tissue showing severe congestion of blood in the connective tissue space, distorted follicular cells of a secondary follicle and degeneration/necrosis of corpora lutea cells. Several primordial and primary follicles are observed. Severe tissue distortion. H & E, X100.

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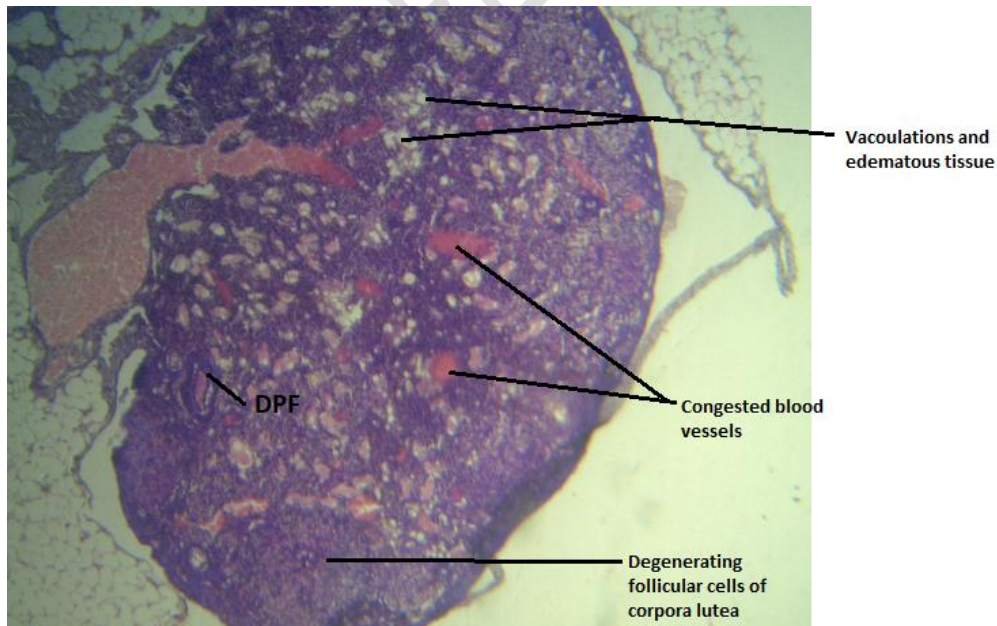
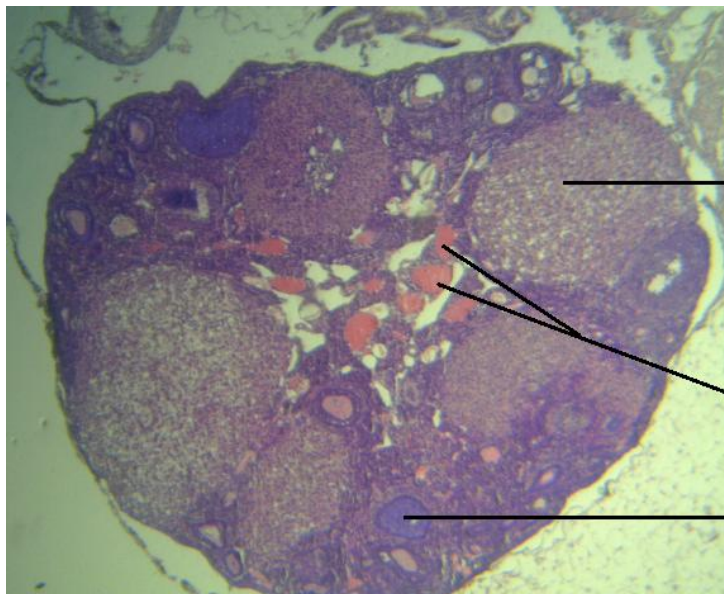


Plate 4: (Group 4) Photomicrograph of ovary tissue showing severe distortion of connective tissue

cytoarchitecture, diffused vacuolation and distortion of primary follicle (DPF) and congested blood vessels and fluid filled tissue. Severe distortion of ovary tissue. H & E, X100.

Comment [AS11]: Please specify the dose concentration



Degenerating and necrotic cell in corpora lutea with several vacuoles

Congested blood vessels in connective tissue

Degenerating primordial follicle

Plate 5: (Group 5) Photomicrograph of ovary showing degenerating and necrotic cells in corpora lutea, and congested blood vessels in the connective tissue. The follicular cells in primary follicle indicate degeneration. Distortion of tissue cytoarchitecture is indicated. H & E, X100.

Comment [AS12]: Same write the dose concentration

UNDER PEP

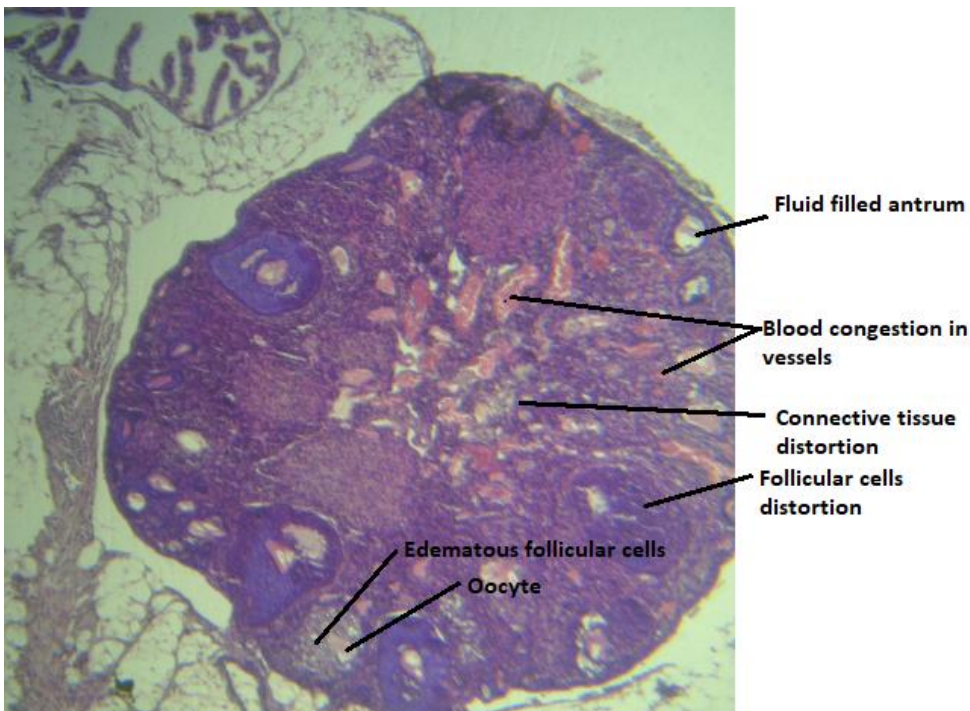


Plate 6: (Group 6) Photomicrograph showing pockets of edematous follicular cells in primary follicle. There is also mild distortion of the connective tissue. The corpora lutea show distinct appearance. Mild distortion of tissue indicated. H & E, X100.

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4. DISCUSSION

The function of the female reproductive system depends upon hormone concentrations and their balance. Endocrine disruption may result in disturbances in the reproductive system, such as modulation of hormone concentrations, ovarian cycle irregularities, and impaired fertility [14]-[1].

The result showed a significant difference in the mean values of follicle stimulating hormone levels in the various groups as showed in Table 1. From the result, there was a significant increase in Group 6 the positive control group (FSH-1.453±0.095) which was not exposed to the ~~TCIP-smoke~~TCIP smoke but was administered with onion juice when compared with Group 1, the negative control group (FSH-0.967±0.355). The result showed that the onion juice ~~had juice an effect on~~influenced follicle stimulating hormone with no effect in LH. This is at variance with the study by Khaki *et al* [15] where the levels of LH significantly increased only in the group that received the high dose of fresh onion juice ($P < 0.004$), however the level of FSH did not differ between the experimental and control ~~groups~~. This groups. This observation might have been attributed to the specific compounds present in the onion juice that could have had a direct impact on the regulation of FSH levels. Onion contains phytochemicals and antioxidants that have been shown to affect hormone levels and improve reproductive health [16]. These compounds might have interacted with the hypothalamic-pituitary-gonadal axis, which is responsible for regulating the production and release of FSH and LH. It is also possible that the compounds in onion juice stimulated the production of FSH, leading to an increase in its levels, while not affecting the production of LH as in a study by Narges *et al* [17]. ~~However~~. However, a study by Neda & Mehrdad [18] showed no significant changes in FSH and LH levels when onion juice were used to check effects on reproductive hormones.

The photomicrograph of Group 3 (Plate 3) exposed to low dose of TCIP smoke only, showed severe congestion of blood in the connective tissue space, distorted follicular cells of a secondary follicle and degeneration/necrosis of corpora lutea cells. Several primordial and primary follicles were observed with severe tissue distortion. The photomicrograph of ~~Group of Group~~ Group 5 (Plate 5) which was exposed to high dose of TCIP smoke ~~only~~, only, showed degenerating and necrotic cells in corpora lutea and congested blood vessels in the connective tissue. The follicular cells in primary follicles indicate degeneration while there was distortion of tissues in ~~the cytoarchitecture~~ the cytoarchitecture. From ~~these observations~~ these ~~observations~~, it is indicative that ~~the result~~ the result was consistent with ~~the report~~ the report by Marettova *et al* [19] which showed the effect of pyrethroids on female genital system as, resulting in atresia of follicles, decrease in the number of follicular cells, oocytes, corpora lutea and also induced vesicular atrophy of the endometrial glands. Whereas, the photomicrograph of the ovaries of Group 2 (Plate 2) mice exposed to low dose (6g) of TCIP smoke and administered onion juice showed degenerating necrosis and hypertrophy of follicular cells, large oocyte with the theca externa showing distortion hence no ~~ameliorating~~ ameliorating effect seen by the onion juice administered. ~~Likewise~~ Likewise, the same observation was noticed in the Plate 4 (Group 4) mice exposed to high dose (12g) of the ~~TCIP smoke~~ TCIP smoke with the administration of the onion juice. There was a severe distortion of connective tissue cytoarchitecture, diffused vacuolation, distortion of primary follicle (DPF), congested blood vessels and fluid filled tissue ~~with severe~~ with severe distortion of ~~ovarian tissues~~ ovarian tissues. This result is not in agreement with a similar study by Jeje *et al* [20] who evaluated the protective role of *Allium cepa* Linn (onion) juice on maternal dexamethasone induced alterations in reproductive functions of female offspring of Wistar rats with the results suggesting that *Allium cepa* ameliorated deleterious reproductive alterations and ovarian oxidative stress in offspring of lactating Wistar rats treated with dexamethasone.

Onion juice has been shown to have antioxidant properties and potential protective effects in various studies [20]. However, the ~~ameliorating effect~~ ameliorating effect of the onion juice on toxicity induced ovarian ~~damage~~ damage was not clearly ~~observed~~ in observed in this ~~study~~ study. The reason could have been the bio availability of the phytochemicals or bioactive compounds, dosage ~~or~~ the or duration of exposure ~~of the~~ of the experiment which lasted for 4 weeks as compared to the study of ~~Khakiof~~ Khakiof *et al* [21] which lasted for a ~~period of~~ period of 2 months with resultant significant effect of ~~Onion of Onion~~ onion juice on the increases in the number of Leydig cells.

The results observed in this study also suggests that the transfluthrin smoke are ~~detrimental~~ detrimental to the ovaries hence inflicting a negative impact which might lead to infertility. The photomicrograph of group 6 mice (Plate 6) treated with onion ~~juice without~~ juice without exposure to transfluthrin insecticide smoke showed enlarged oocyte surrounded by corona ~~ary~~ radiata cells, pockets of distortion of follicular cells in primary follicle, well distinct corpora luteal and mild distortion of follicles. This shows certain developmental or structural changes in the ovaries which is suggestive that the transfluthrin affects the ovaries despite the administration of onion juice [22]. However, from the result obtained there were discrepancies between the biochemical and histology report which could be as a result of autoregulatory mechanisms leading to biochemical derangement, these mechanisms can lead to variations in biochemical markers, making it difficult to accurately reflect the underlying histological changes [23]. Another reason could be random selection of histology slides which can lead to inconsistency with biochemical reports [24]. This is because variations in slide staining and scanning procedures, such as dye batch, slice thickness, and whole slide scanner used, can compromise feature stability [25]. Therefore, random selection of histology slides without considering feature stability can result in inconsistencies between histomorphometric features and biochemical reports [26].

5. CONCLUSION

The onion juice was able to cause an increase in the Follicle stimulating hormone with no effect on the Leutinizing hormone and there were damages in the ovaries due to the toxicity of the Rambo paper smoke ~~and co~~ and co-administration with the onion juice did not ameliorate the effect caused by the ~~exposure~~ exposure. Use of transfluthrin coated insecticide paper damages the ovary and can lead to infertility.

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Comment [AS14]: Check references and spaces

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