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Exposure to uppercott pesticide induces reproductive toxicity in male and female rats

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Abstract
In this study, the effect of Uppercott was investigated in parents and first generation offspring of albino *Wistar* rats. The rats were distributed into ten groups of nine (9) rats each, five groups each for male and female rats respectively. Groups 1 & 2 were taken as normal and oil control respectively for both male and female rats, groups three (3) to five (5) were given 2.5%, 5% and 7.5% of LD_{50} Uppercott value for 28 days. The rats were then mated in the ratio of 2:3 (2 male rats to 3 female rats) until pregnancy was established. Result indicated that Uppercott exposure produced a significant ($p<0.05$) decrease in the testosterone & progesterone levels in male and female rats respectively, and an increase in estradiol levels in female rats exposed to the pesticide, as compared to their controls. Furthermore, the anthropometric indices showed varying results from the treated groups as compared with the control. These results indicated that exposure to Uppercott may be implicated in reproductive toxicity in rats.

Keywords: Uppercott, Pesticide, testosterone, progesterone, estradiol.

Introduction

Background to the study

According to [1], pesticides are substances meant for attracting, seducing, destroying, or mitigating any pest. Generally, pesticides are either chemical or biological agents which slow, weaken, or deter pests. It is undisputable that there are obvious advantages attached to pesticide use, but disadvantages also abound like potential toxicity to man and other desired species. Pesticides are most commonly used as plant protection products, which generally guide against attack from weeds, plant diseases or insects. Pesticides are used in pest control. For instance, pesticides have been very effective against mosquitoes which are known to help in the transmission of diseases like yellow fever and malaria. Also, insects such as bees or wasps which have the potential to induce allergic reactions can also be killed by pesticides.

Uppercott is a type of pesticide composed of two main components namely cypermethrin (30g/l) and dimethoate (250g/l). Cypermethrin is a synthetic pyrethroid used as an insecticide in large-scale commercial agricultural applications and used in consumer products for domestic purposes. It has also been shown to behave like a fast-acting neurotoxin in insects [2]. Excessive exposure to cypermethrin produces the following symptoms in man: headaches, nausea, seizures, shortness of breath, salivation, and muscle weakness [2]. In man, metabolism of cypermethrin occurs by the hydrolysis of enzymes to produce numerous metabolites of carboxylic acids, which are subsequently excreted by the kidney in the form of urine [3]. By measurement of the urinary metabolites, the exposure to the chemicals by workers can be effectively monitored. Furthermore, quantification of cypermethrin in blood or plasma may help confirm severe over dosage [4]. Male rats have been shown to be susceptible to cypermethrin exposure. A study by [5] showed that cypermethrin exposure produced a toxic effect on the reproductive system. It was shown that both androgen receptor levels and serum testosterone levels were greatly reduced after 15 days of continuous dosing. These data gave credence to the suggestions that there is a possibility that the seminiferous tubules could be impaired after exposure to cypermethrin, and therefore affect sperm production in male rats. It is also important to point out that research has indicated if exposed to cypermethrin when pregnant, rats have a very high chance of producing litters with some developmental delays [6]. Also, the proportion of abnormal sperm increases in male rats that have been exposed to cypermethrin [7]. A study by [8] revealed that cypermethrin causes genetic damage: it was revealed that when mice were exposed to cypermethrin, chromosomal abnormalities were shown to increase in bone marrow and spleen cells [8].

Dimethoate is classified as an organophosphate insecticide and is widely applied to destroy aphids and termites to mention but a few [9]. According to [10], the population as a whole is not generally subject to exposure to dimethoate from air, water or food; however occupational exposure may occur during manufacture, formulation and use which mainly occurs through inhalation and dermal absorption. There has not been enough evidence showing it either as a teratogen, carcinogen, or ability to have harmful reproductive effects on humans. Research directed towards mice and rats have indicated certain negative effects on reproduction and also some carcinogenic effects [11].

Materials and Methods

Chemicals and Reagents

All chemicals and reagents used were of analytical grade. Uppercott pesticide (liquid form) was purchased from Agro chemical company in Calabar, Cross river state, Nigeria.

LD_{50} determination

The LD_{50} was determined to be 14.14 mg/kg b.wt using the method of Lorke, 1983.

Experimental animals

Ninety (90) Wistar albino rats (45 males and 45 females) weighing between 150-180g were used for the experiment. They were obtained from the animal grooming section of the department of Biochemistry, University of Calabar, Calabar, Nigeria. All animals were maintained under standard conditions (ambient temperature, 28.0 + 2.0 ºC and humidity, 46%, with a 12 hr light/dark cycle). All the rats in both test and control groups were allowed free access to food and water *ad libitum*, throughout the experimental period. They were divided into ten (10) groups; five (5) groups of nine (9) rats each for males and five (5) groups also of nine (9) rats each for females.

Table 1 shows the animal distribution into experimental groups. The rats were acclimatized in the experimental animal house for one week before the experiment commenced.

Table 1: Distribution of albino wistar rats into experimental groups

Groups	No. of male animals	Number of female animals	Treatments
Group 1	9	9	NC
Group 2	9	9	OC
Group 3	9	9	2.5% (0.35 mg/kg b.wt) of LD_{50}
Group 4	9	9	5% (0.71 mg/kg b.wt) of LD_{50}
Group 5	9	9	7.5% (1.06 mg/kg b.wt) of LD_{50}

KEY: NC – Normal control, untreated; OC – Oil control, administered only olive oil

Stage 1

After one week of acclimatization, the male and female rats in group 3 to 5 respectively were exposed to oral administration of uppercott through gavage at the different doses for 28 days to allow for ample exposure time. At the end of the exposure, 5 male rats and 5 female rats were selected at random from each group and sacrificed and the testosterone levels were checked for the male rats while the progesterone and estradiol levels were checked for the female rats.

Stage 2

The next stage involved the mating of the male rats with the female rats in the same treatment group in the ratio of 2:3, that is, 2 male rats to 3 female rats until pregnancy was established. This is seen in Table 3.

Once pregnancy was established, the animals were withdrawn from exposure. Pregnancy was determined using the rat progesterone ELISA test kit and they were withdrawn from treatment 7days after pregnancy was confirmed. Blood samples from the female rats were collected, left to clot at room temperature and analysed within 24 hours

Immediately after delivery, several parameters were checked namely; litter number, tail length, death ratio out of the total number of litters, birth ratio, body weight, body length and physical appearance of the rats' litters. Three weeks after delivery body weight was checked once again. All the animal experiments were carried out in accordance with the guidelines of the Institution's Animal Ethical Committee.

Collection and preparation of blood samples for analyses

Blood samples were collected by cardiac puncture using 18G needle w/5ml syringe into plain screw-cap sample bottles for the sex hormonal and lipid peroxidation tests. The blood samples collected for the tests were allowed to clot at room temperature, and the serum extracted with Pasteur pipette after spinning with MSE model (England) table-top centrifuge at 2000 rpm for 5 minutes. All test analyses were carried out within 24 hours of serum separation.

Statistical analysis

Data obtained was expressed as Mean ± Standard Deviation and analyzed using the SPSS package 19.0. One-way Analysis of Variance (ANOVA) was used. Values at $P<0.05$ was regarded as significant in comparison with appropriate controls.

Results

Testosterone as shown in figure 1 was significantly ($p<0.05$) increased in the oil control group (OC) compared to the male control (NC) and significantly lowered ($p<0.05$) in all other treatment groups (2.5%, 5% and 7.5%). The 7.5% treatment group had the lowest testosterone level.

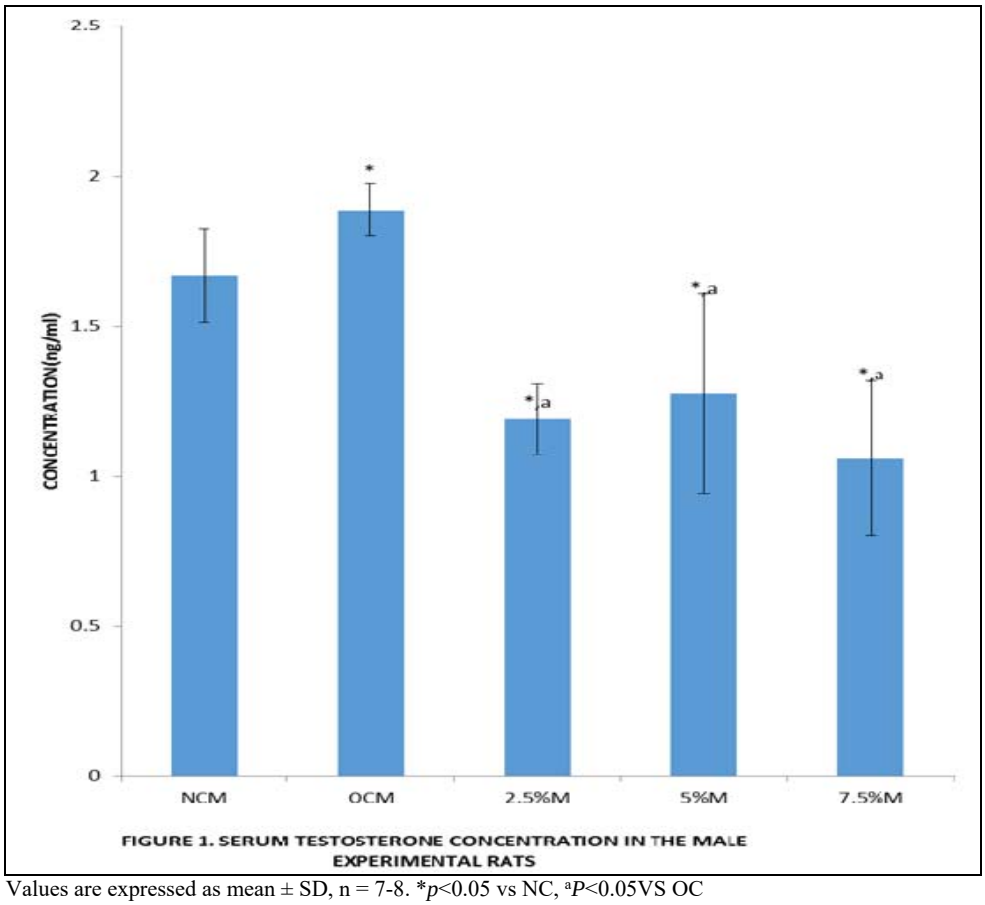


FIGURE 1. SERUM TESTOSTERONE CONCENTRATION IN THE MALE EXPERIMENTAL RATS

Values are expressed as mean ± SD, n = 7-8. *p<0.05 vs NC, [a]P<0.05VS OC

Progesterone levels as shown in figure 2 significantly decreased relative to dose concentration. The higher the uppercott dose, the lower the progesterone level.

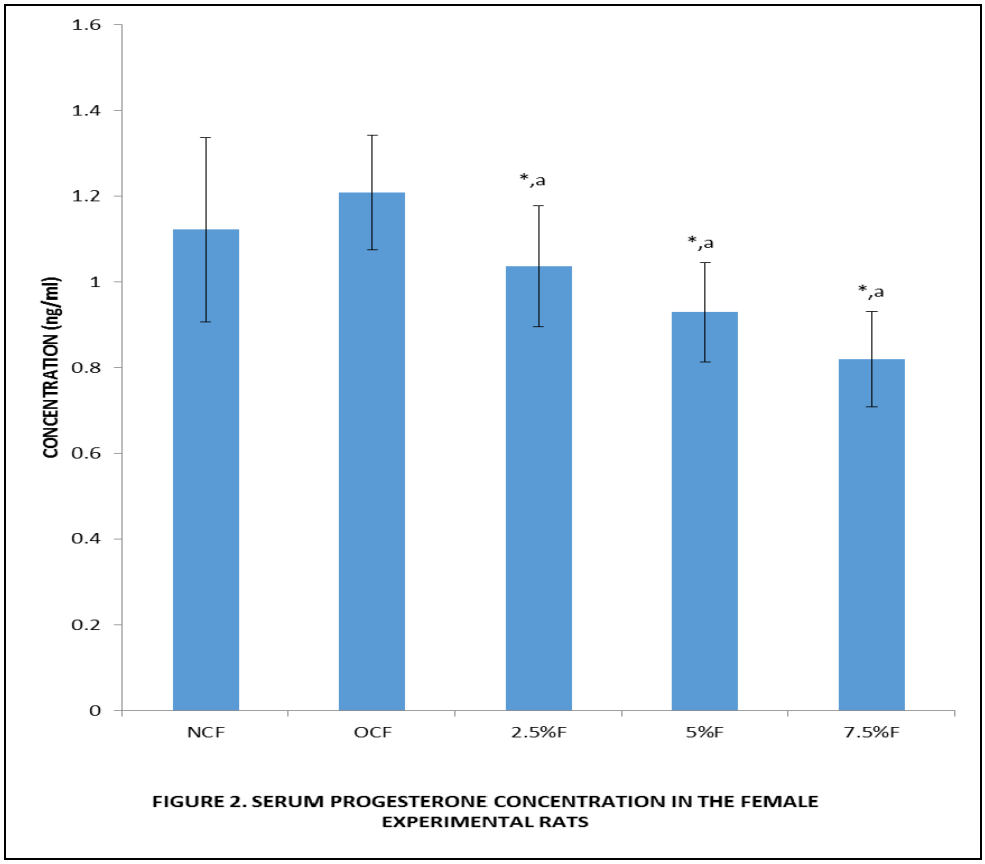


FIGURE 2. SERUM PROGESTERONE CONCENTRATION IN THE FEMALE EXPERIMENTAL RATS

Values are expressed as mean ± SD, n = 7-8. *p<0.05 vs NC, [a]P<0.05VS OC.

The result in figure 3 shows estradiol was significantly increased (p<0.05) in the female animals receiving higher doses of uppercott 5% and 7.5% respectively compared to normal control (NC) and compared to all the test group (OC, and 2.5%).

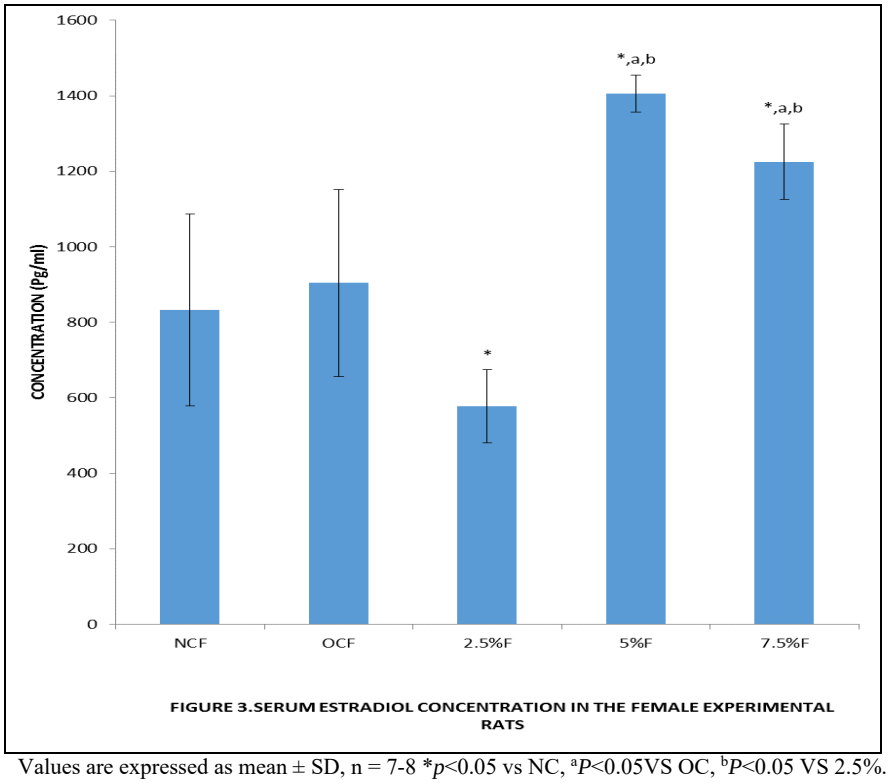


FIGURE 3.SERUM ESTRADIOL CONCENTRATION IN THE FEMALE EXPERIMENTAL RATS

Values are expressed as mean ± SD, n = 7-8 *p<0.05 vs NC, [a]P<0.05VS OC, [b]P<0.05 VS 2.5%.

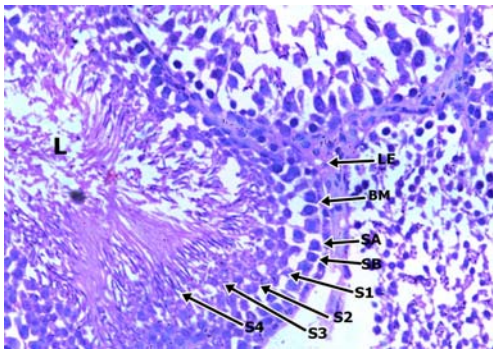
In table 2, different anthropometric parameters were checked. It was observed that the group treated with the least amount of pesticide was the only treated group with litters (5) as compared with Group 1 (20 litters) and group 2 (21 litters). The average body weight of the litters in Group 3 were reduced as compared with Group 1 and 2 after observation for three (3) weeks following birth. There was no much difference in their tail length for any of the groups with litters. All the female rats in group 1, 2 and 3 gave birth as indicated in their birth ratio. The average body length of the litters born in Group 3 were reduced as compared with Group 1 and 2 after one (1) week. There was no deaths recorded among the litters in groups 1 and 2, but one death was recorded in group 3.

Table 2: Anthropometric features of the rats' litters three weeks after birth

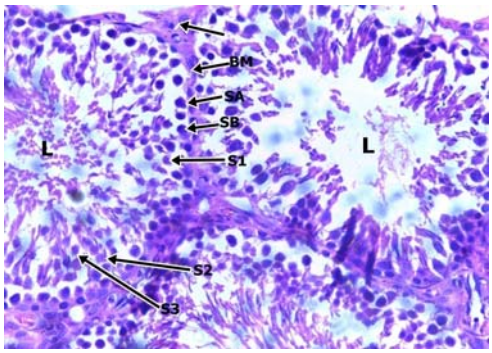
GROUP	RATS (2M:3F)	NL	AV. B.WT (WK1)g	AV. B.WT (WK3)g	TL	BR	AV. BL(cm) (WK1)	DR
NC	5	20	7.99 ±0.61	30.79 ± 0.71*	1.65 ± 0.69	3/3	6.72 ± 0.81	0/20
OC	5	21	5.52 ± 0.41	27.51 ± 0.43*	1.41 ± 0.42	3/3	6.12 ± 0.53	3/21
2.5%	5	5	4.62 ± 1.27	21.75 ± 1.43*	1.6 ± 0.91	3/3	4.00 ± 0.95	1/5
5%	5	0	0	0	0	0	0	0
7.5%	5	0	0	0	0	0	0	0

NC= Normal control untreated rats not exposed to uppercott, M= Male rats, F= Female rats, OC=Normal rats given oil, NL= Number of litters, TL= Tail length, DR= Death ratio, BR= Birth Ratio, AV. B.WT (WK1) = Average body weight in week one (1), AV. B.WT (WK3) = Average body weight in week three (3), AV. BL = Average body length

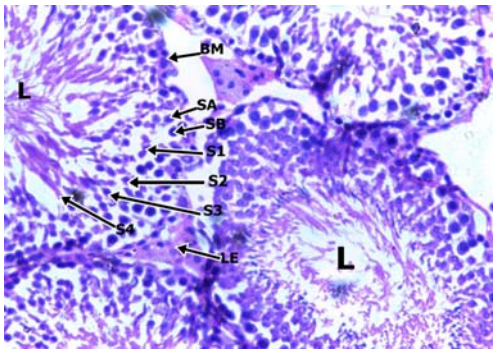
*= p<0.05 against average B.WT (week1) across all test groups with litters



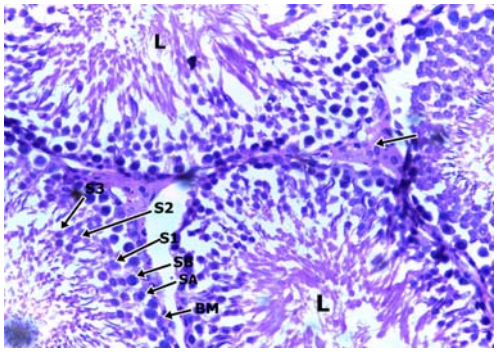
(a1) Photomicrograph of normal control (NC) rat testes. (Mag. x 400)



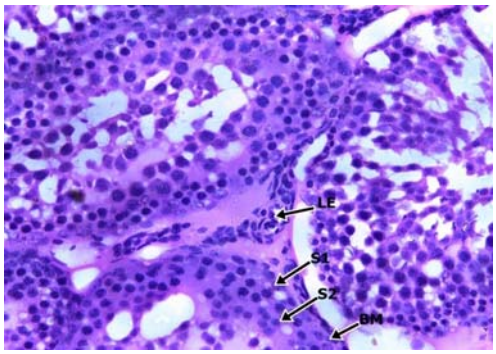
(a2) Photomicrograph of oil control rat testes. (Mag x 400)



(a3) Photomicrograph of rat testes exposed to 2.5% Uppercott pesticide (Mag. x 400)



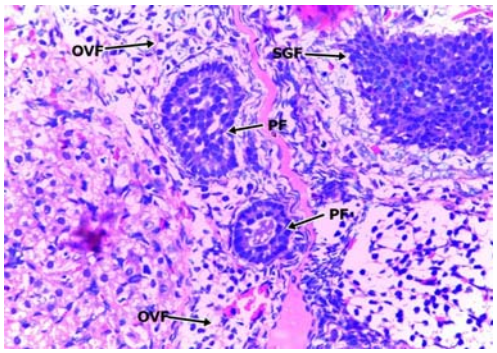
(a4) Photomicrograph of rats testes exposed to 5% Uppercott pesticide (Mag.x 400)



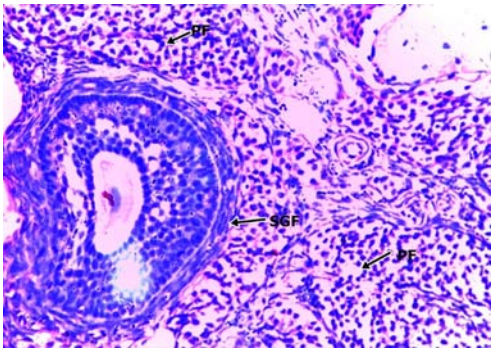
(a5) Photomicrograph of rat testes exposed to 7.5% Uppercott pesticide (Mag. x 400)

As shown in figure 4a above, the histological Section of the normal rat testis showed prominent seminiferous tubules consisting of proliferating spermatogonia cells at various stages of maturition. These include spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids. Their lumen were filled with spermatozoa and the intervening interstitium contains round to oval Leydig cells serving as support. The oil control rats showed or maintained a similar testicular cyto-architecture. The rats exposed to uppercott at various percentages (2.5%, 5% and 7.5%) showed seminiferous tubules with indistinguishable sections contaning proliferating spermatogonia cells. These cells are enlarged with prominent nuclei and distinct cell membrane, with coarse chromatin pattern. There was pseudogland formation and their basement membranes are compromised. Showing arrest of spermatogenesis across the various degree of exposures.
SA and SB- Stem cells ; S1, S2,S3, S4 – Spermatogonia cells; BM – Basement membrane; LE- Leydig cells; L- Lumen

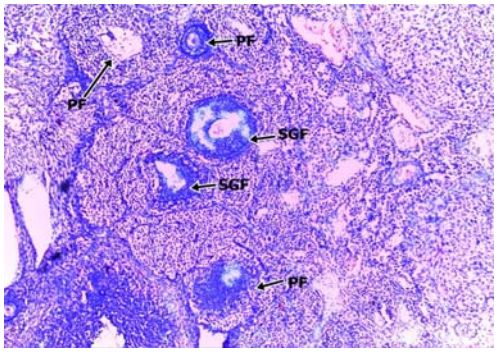
Also, in figure 4b below, the ovarian histology shows a distinct cortex and medullary regions. The cortex contains ovarian follicles at various stages of maturation and the medulla comprised of a fibrovascular cellular stroma. The follicles are mainly primary follicle with both multilayered and single layered typed consisting of a central viable oocytes. Some secondary follicles with antral space are also present in their normal shape. Section of ovarian tissue in uppercott exposure shows inseparable cortical and medullary regions with viable oocytes, and the ovarian stroma is fibrovascular in both oil control and various percentages of exposure, viz 2.5%, 5% and 7.5% respectively.
PF- proliferating follicle, OVF- Ovarian follicle, SGF- Secondary graffin follicle, MGF- matured griffin follicle.



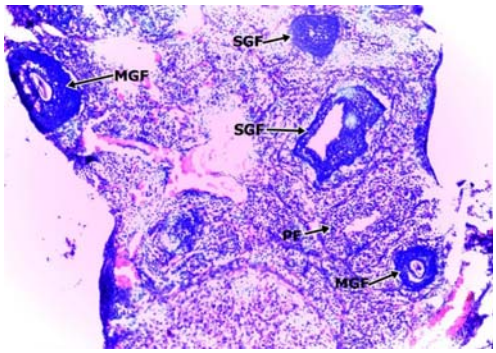
(b1) Photomicrograph of normal control (NC) rat ovary. (Mag. x 400)



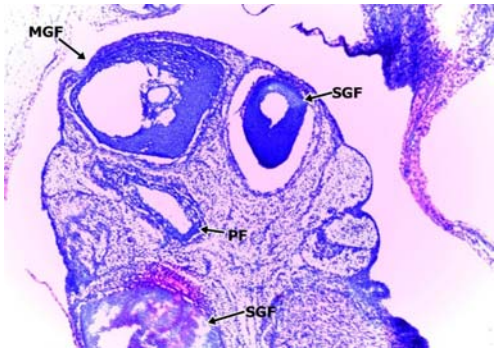
(b2) Photomicrograph of oil control (OC) rat ovary. (Mag. x 400)



(b3) Photomicrograph of ovary of rat treated with 2.5% Uppercott pesticide. (Mag. x 400)



(b4) Photomicrograph of ovary of rat treated with 5% Uppercott pesticide. (Mag. x 400)



(b5) Photomicrograph of ovary of rat treated with 7.5% Uppercott pesticide. (Mag. x 400)

Fig 4: Histology of the testes (a); histology of the ovary (b)

Discussion

Uppercott pesticide was thoroughly investigated in this research. Pesticide exposure has become part of our everyday encounters for varying reasons. Either it is used to spray against pests preying on our crops, or against insects like mosquitoes, or even against rodents. Most times, these pesticides leave residues which we take in either orally, dermally or through inhalation.

Testosterone levels are very important in the reproductive health of the male species. A major implication associated with lower testosterone levels is low sperm count. Pesticides are responsible for decreasing testosterone concentration in a number of ways, e.g. inhibiting release of follicle stimulating hormone (FSH) or luteinizing hormone (LH) [12], apoptosis of leydig cells [13]. Additionally, pesticides cause increased secretion of hypothalamic corticotrophin-releasing hormone which stimulates the release of adrenocorticotropic hormone (ACTH) and cortisol [14]. High cortisol level inhibit gonadotropin releasing hormone (GnRH) leading to a reduction in LH and testosterone levels. Leydig cells have a very important role to play in testosterone synthesis. Leydig cells are involved in the synthesis of testosterone and also its secretion into the bloodstream in males. The steroid potential of the testes is reduced by abnormal Leydig cells. The histological analysis showed structural impairments and decreased number of Leydig cells which could lead to reduction in the concentrations of serum testosterone.

Due to the enormous importance of testosterone in male reproductive system, any disruption in its production could lead to impaired reproductive health [15]. It is suggested that Uppercott might suppress male spermatogenesis and induce low daily sperm production by disturbing testosterone biosynthesis. The reduced testosterone might also be responsible for morphological abnormality of testis in Uppercott treated rats in this study. This agrees with the work of [16] who reported impairments of cypermethrin on male reproductive system. It was also observed that the control group receiving only olive oil had an increase in testosterone level as compared to the normal control. Different works on pesticides have made olive oil their choice vehicle [17, 18]. As shown, the olive oil did not interfere with the results as testosterone levels clearly decreased relative to pesticide dosage. Similar conclusions have been reached by [19].

Progesterone levels decreased in a dose dependent manner as observed in the results. This was in tandem with the works of [20-22] where they reported a decrease in progesterone levels of female rats exposed to organophosphorus pesticides or mixture of pesticides. More research is to be carried out on the mechanism of action behind the reduced progesterone levels on exposure to xenobiotics but one possible reason may be by the disruption of the hormonal balance necessary for normal functioning. Reproductive effects that have been associated with pesticide exposure in women are decreased fertility, spontaneous abortions, stillbirth, premature birth, low birth weight, developmental abnormalities, ovarian disorders, and disruption of the hormonal function [23-26].

Also from table 2, we can observe that the group of rats administered higher doses of Uppercott did not give birth at all. This actually agrees with our earlier assertions about the effect of endocrine disruptors where the researchers noted reduced testosterone and progesterone level on increased exposure to uppercott. According to the work of [27], they observed that organophosphorus containing pesticides have the ability to cross the placenta and create genotoxic effects on the fetus. They achieve this by causing damage to the placental tertiary villi thereby disturbing the nutrition supply to the fetus. Damaged placental carrier leads to excess organophosphorous pesticides in the fetus and result in stillbirth and perinatal death [28]. This further confirms the differences in the anthropometric features seen in the rats administered least dose of uppercott as compared with the controls.

Conclusion

Pesticides are an inevitable part of our modern existence. It has shown to be useful in fighting household pests and also has shown agricultural importance. This study has revealed a negative impact on fecundity with Uppercott use and exposure, therefore, caution and moderation are advised in its usage.

Recommendations/suggestions for further study

Given the role of pesticides in national revolution, it is not possible to eradicate their uses. Hence, possible ways of incorporating mammalian protective substances into these chemicals to reduce their effect on human species is needed for further study. Also, a study of the molecular mechanism of the toxicity of this substance on various organs and systems is highly recommended and possible ways of blocking the effects with pharmacological agents of plant origin need to be investigated.

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