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Assessment of Ovarian Integrity, Reproductive Hormones, and Oxidative Stress in Albino Rats Exposed to Tartrazine Azo Dye

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Author's contributions

This work was carried out in collaboration among all authors. Author IE designed the study, performed the statistical analysis and wrote the protocol. Authors IE, OLH, IGA and SIA wrote the first draft of the manuscript. Authors EON and HAW managed the analyses of the study. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To assess the effect of tartrazine at ADI doses on ovarian integrity (the weight and histology of the ovary), reproductive fertility hormones (luteinizing hormones (LH), follicle stimulating hormone (FSH) and prolactin (PRL)) and oxidative stress markers (glutathione peroxidase (GPX) and superoxide dismutase (SOD)) over a period of 30 and 60 days in albino rats.
Study Design: A total of 63 female rats weighing approximately 0.2kg were divided into two phases. In phase 1 (30 days treatment period), the rats were divided into 2 groups - designated tartrazine treated group (TTG_1) consisting of 20 rats and control untreated group (CUG_1) consisting of 15 rats. In phase 2 (60 days treatment period), the rats were again divided into 2 groups – tartrazine treated group (TTG_2) consisting of 16 rats and control untreated group (CUG_2) consisting of 12 rats. The acceptable daily intake (ADI) of 7.5mg/kg of the dye was administered orally while the control groups were given food and water only.
Methodology: At the end of the study, the animals were anaesthetized and 5 mL of whole blood samples was collected by means of cardiac puncture into plain bottles, later spun at 4000 rpm for 5

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minutes to obtain serum. The laboratory analysis of LH, FSH and PRL as well as GPX and SOD activity were based on Enzyme Linked Immunosorbent Assay (ELISA) Technique using rat-specific kits. Ovarian tissues collected were weighed using electronic balance, washed in normal saline, fixed in 10% formalin saline, embedded in paraffin wax, 5µm thick sections were obtained by rotary microtome, stained using Haematoxylin & Eosin and examined using digital Olympus microscopic.
Results: Non-significant higher values in the absolute weight of the ovary (WOV), FSH, LH, and PRL while non-significant lower values in GPX and SOD were observed in the treated rats against control rats over a period of 30 and 60 days at *P*=.05. The histological examination over a period of 30 days did not indicate any alteration but hydropic dilation and structural alterations were seen after 60 days.
Conclusion: The administration of ADI doses of tartrazine over a period of 60 days affected the integrity of the ovary mildly as observed in the histology but did not markly reflect on the biochemical markers in the plasma as well as the weight of the ovary.

Keywords: Ovarian integrity; hormones; FSH; LH; PRL; anti-oxidants; azo dyes; albino rats.

1. INTRODUCTION

Colour in the form of powder, pastes or semi fluid is added in a wide variety of food items like beverages, baking flavors, candies and other food items to improve appearance, attractiveness and taste [1,2]. These colors are able to influence or stimulate appetite, giving the consumer a quality perception [3]. Several scientific studies have reports adverse effect on health, affecting human population especially children [4]. This has led in the establishment of safety measures in the use of food colorant in food products [5]. Food dyes come in natural and synthetic forms. Natural food dyes are derived within a biological system with sources especially from plants (e.g. carotenoids, cochineal, turmeric, chlorophylin, etc.), and can be synthesized, accumulated or excreted from the living cells and are characterized by chemical instability resulting in loss of colour on exposure to oxidizing agents [3,6]. Synthetic dyes are non-nutritional chemically active components generally of coal-tar based [3,4,6]. Example of synthetic food dye is tartrazine, carmoisine, brilliant green, acid red, etc. [4,6].

Tartrazine is an azo dye of chemical formula 3-carboxy-5-hydroxy-1 (p-sulfophenyl)-4-(sulfophenyl azo) pyrazolone that is derived from coal tar and is readily soluble in water and has a variety of usage in cosmetic, food, and pharmaceutical industries [3,7]. Several studies have reported toxic effects of tartrazine and other synthetic dyes on some organ-systems like that of the renal, hepatic, and blood using experimental animals but data on tartrazine toxicity when administered at ADI dose on gonads, and gonadal hormones have not reached a scientific research benchmark. Tartrazine toxicity is said to result from the in-*vivo* metabolism of its azo bonds which occurs in the liver and the intestine, thereby leading to production of toxic oxidative metabolites such as aryl amines, reactive amines, and free radicals [8,9]. Also, tartrazine dye have been found to covalently react with and destroy the protein active site and configuration of enzymes resulting in loss of normal functioning to the enzyme which may lead to several metabolic consequences [8,9]. Excess in-take of tartrazine dye has been reported to cause attention deficit disorders, allergic and intolerance reactions such as itching, migraines, sleep disturbance, anxiety, blurred vision, and general weakness in children [7,9]. Nephrotoxicity and hepatotoxicity in rats have also been reported [3,7,9]. Some studies reported that tartrazine exerts mutagenic, teratogenic and adverse effects to the brain, immune system, genetic materials, cardiovascular and reproductive organs [10,11,12,13]. Metabolism of tartrazine involves some processes such as hydrolysis, reduction, oxidation via enzymes (Cytochrome P450 reductases, monoamine oxidases, azo-reductases, dehydrogenases, etc.) to yield less toxic water soluble substances [8].

The ovary as a reproductive organ is primarily responsible for the production of female reproductive hormones and the periodical production of matured oocytes [14]. The ovary produces steroid and protein hormones needed for reproduction. Follicle stimulating hormones (FSH), luteinizing hormones (LH) and prolactin (PRL) are protein hormone strongly associated with reproduction. These hormones function collectively to cause maturation of the ovarian follicle and development of corpus luteum with ovulation, and are also regulated by

Gonadotrophic Releasing Hormone (GnRH) [14]. Xenobiotics like food dyes have been reported to adversely affect the ovaries, leading to declined fertility and reproduction [9,10,11,12]. Though, the Department of Health, Australian Government [15] in 2014 reported no deleterious effect of food dye on reproductive parameters. Similarly, Tanaka [16]; Elhkim & Heraud [17] also reported no adverse effect of food dye on reproductive parameters. However, Sharma [18]; Mehedi et al. [19], reported that the use of tartrazine at high doses caused testicular and ovarian structural abnormalities resulting in significant decrease in testicular and ovary weight.

In addition, anti-oxidant enzymes were also considered in this study, since it has been documented that tartrazine produces reactive oxygen species (ROS), reactive nitrogen species (RNS) and free radicals during its metabolism. Glutathione peroxidase (GPX) and superoxide dismutase (SOD) are anti-oxidative enzymes produced by the body to mitigate or remove ROS/RNS, and free radical and their disastrous impact from the body. The balance between ROS/RNS and anti-oxidant enzymes is very crucial to prevent macromolecule and cellular damages due to oxidative stress especially when there is overproduction of ROS/RNS and free radicals that does not match the amount of anti-oxidants generated by the body. These anti-oxidant enzymes work by enhancing or facilitating the detoxifying of ROS/RNS thereby halting or mitigating the effect oxidation on cellular and macromolecules. Khayyat et al. [8] reported that the ingestion of tartrazine induced reduction in the anti-oxidant capacity in experimental rats.

Therefore, the main focus of this research is to evaluate the effect of tartrazine on the ovarian integrity (weight and histology), reproductive fertility hormones (LH, PRL, and FSH) and oxidative stress markers (GPX and SOD) after chronic exposure (orally) of adult female rats to the azo dye for over a period of 30 and 60 days.

2. MATERIALS AND METHODS

2.1 Reagents and Equipment

The reagents used include commercially available rat-specific ELISA kits; Luteinizing Hormone (LH), Follicle stimulating Hormone (FSH), and Prolactin Hormone (PRL) which were purchased from Bioassay Technology Laboratory (Shangai, China) while glutathione peroxidase (GPX) and superoxide dismutase (SOD) were purchased from Elascience, (Houston, Texas, USA). Equipment used were Ohaus Scout-Pro Electronic weigh balance (Ohaus Corporation, New Jersey, USA), bucket centrifuge (MPW, Poland), incubator at 37ºC (Merment, Germany), Microplate reader Stat-Fax 4500 (Awareness Incorporated, California, USA), Polypropylene gavage tubes (Intech Laboratory Incorporated, Plymouth Meeting, USA), Haier thermocool refrigerator (China), Albino rats, Leica automatic tissue processor (Leica Biosystems, USA), Shandon AS325 rotary microtome (Fisher Scientific, United Kingdom), digital Olympus microscopic with Camera (Olympus, Tokyo, Japan), Tartrazine dyes (E102) purchased in granular form from Fiorio Colori SPA Gessete, Italy. Other materials used were automatic pipettes, hypodermic syringe and chloroform.

2.2 Experimental Female Albino Rats

A total of 63 adult female albino rats (18 weeks old) weighing approximately 200g were used. All the rats used were breed and housed at the animal house, in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria. Prior to the study, the rats were allowed to acclimatize for 14 days in well ventilated cages and were fed with grower's marsh and water *ad libitum*.

2.3 Study Design

The experiment was divided into two phases depending on the duration of exposure of the rats to tartrazine dye. In phase 1, the rats were divided into 2 groups - designated tartrazine treated group (TTG_1) consisting of 20 rats and control untreated group (CUG_1) consisting of 15 rats. The rats were further separated randomly into 7 groups of 5 rats per cage. The tartrazine treated groups were given 7.5mg/kg (ADI dose) of tartrazine orally using gavage tube for 30 days while the control groups were given food and water only. Similarly, in phase 2, the rats were again divided into 2 groups – tartrazine treated group (TTG_2) consisting of 16 rats and control untreated group (CUG_2) consisting of 12 rats. The rats were further distributed into cages with 4 rats per cage. In this phase, the tartrazine treated groups were given 7.5mg/kg (ADI dose) of tartrazine orally using gavage tube for 60 days while the control groups were given food and water only. At the end of the study, the animals were anaesthetized with chloroform, blood

samples were collected and ovarian tissues harvested by for investigations.

2.4 Tartrazine Azo Dye, Preparation and Administration

Tartrazine food dye of industrial grade of 86.7% purity was used. 1.50g of tartrazine was dissolved in 1.0litre of water. That is, 1.0millitre of the solution will have an equivalent tartrazine concentration of 0.0015g which is equivalent to ADI of 7.5mg/kg body weight of tartrazine when given to 200g (0.2kg) weight of rat. The method of administration was strictly oral using the oro-gastric technique to ensure complete delivery of the azo dye. The administration was performed with gavage tube carefully inserted into the esophagus of the rats through the mouth. The rats were restrained by holding them by the loose skin of the neck and back to immobilize the head avoiding the introduction of the dye into the lungs or rupturing the esophagus or stomach.

2.5 Study Area

The study was carried out in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria. However, serum samples collected were analyzed at the Chemical Pathology Unit, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria.

2.6 Specimen Collection, Preparation and Analysis

At the end of the study, the animals were anaesthetized with chloroform and 5mL of whole blood samples was collected by means of cardiac puncture into plain bottles. The collected whole blood samples were allowed to clot and later retracted and spun at 4000rpm for 5 minutes to obtain serum. The laboratory analysis of the hormonal parameters was based on ELISA Technique. The ELISA procedure for the determination of rat-specific LH, FSH and PRL concentrations were based on method described by Engvall & Perlmann [20]. In addition, GPX was determined by the method described by Moron et al. [21] while SOD activity was determined by the method described by Marklund et al. [22]. The weights of the ovaries were measured in grams.

2.6.1 Histological preparation and examination of ovarian tissue

Ovarian tissues were collected from sacrificed animal. Ovaries were washed in normal saline and fixed in 10% formalin saline, processed in ascending grades of alcohol, cleared and embedded in paraffin wax using Leica automatic tissue processor. 5µm thick sections were obtained by rotary microtome and stained using Haematoxylin & Eosin as described by Stevens [23]. The ovarian sections were examined using digital Olympus microscopic and photomicrographs were taken using digital Olympus microscopic camera with a magnification of 400X.

2.7 Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 8.02 (San Diego, California, USA). Results were presented as Mean ± Standard deviation (SD). Inferential statistics using Students' statistical t-test was employed to compare values of the treated rats and control rats as well as the treated rats over a period of 30days against 60 days. Statistical significance was set at P=.05.

3. RESULTS

3.1 Results of Reproductive Hormonal Parameters and Oxidative Markers in Female Rats Chronically Treated with Tartrazine Over a Period of 30 and 60 Days

Table 1 and Table 2 shows non-significant higher in the values of FSH, LH, and PRL in the treated rats over a period of 30 and 60 days (except PRL that indicated a non-significant decrease over a period of 30 days). Also, non-significant higher values were seen in the absolute weight of the ovary (WOV) in the 30 and 60 days treated rats respectively compared to their respective controls. However, GPX and SOD showed non-significant lower values in the treated rats over a period of 30 and 60 days when compared with their respective control rats at P=.05.

3.2 Comparison of 30 and 60 Days Results of Reproductive Hormonal Parameters, Oxidative Markers, and Weight of Ovary in Female Rats Treated with Tartrazine

Table 3 shows non-significant lower values of FSH, LH, PRL , GPX and SOD as well as the in the absolute weight of the ovary (WOV) in the 60 days treated rats compared against the 30 days treated rats at P=.05.

Table 1. Results of reproductive hormonal parameters, oxidative markers, and weight of ovary in treated with tartrazine over a period of 30 days

Parameters	Control rats (n=15)	Treated rats (n=20)	*P*value	Tvalue	Remark
LH (miU/ml)	0.41±0.25	0.51±0.26	0.778	1.198	NS
FSH (miU/ml)	0.91±0.30	1.06±0.91	0.671	1.401	NS
PRL (ng/ml)	1.25±0.41	1.20±0.45	0.873	0.367	NS
SOD (U/ml)	276.3±179.9	186.4±89.32	0.067	1.819	NS
GPX (U/ml)	12.16±6.54	10.19±8.45	0.658	0.535	NS
WOV (g)	1.59±0.65	1.67±0.54	0.686	0.407	NS

NS=Not significant at P=.05. LH=Luteinizing Hormone, FSH=Follicle Stimulating Hormone, PRL= prolactin, GPX= Glutathione peroxidase, SOD= Superoxide dismutase, WOV=Weight of ovary, n= No of rats. Results were expressed as Mean±SD

Table 2. Results of reproductive hormonal parameters, oxidative markers, and weight of ovary in treated with tartrazine over a period of 30 days

Parameters	Control rats (n=12)	Treated rats (n=16)	*Pvalue*	Tvalue	Remark
LH (miU/ml)	0.43±0.29	0.45±0.18	0.106	0.137	NS
FSH (miU/ml)	0.69±0.26	0.84±0.23	0.624	1.539	NS
PRL (ng/ml)	1.08±0.54	1.14±0.38	0.257	0.286	NS
SOD (U/ml)	194.6±121.9	173.0±74.09	0.554	0.687	NS
GPX (U/ml)	11.89±8.12	9.42±5.02	0.460	0.843	NS
WOV (g)	1.38±0.63	1.44±0.86	0.397	0.858	NS

NS=Not significant at P=.05. LH= Luteinizing Hormone, FSH= Follicle Stimulating Hormone, PRL= Prolactin, GPX= Glutathione peroxidase, SOD= Superoxide dismutase, WOV=Weight of ovary, n= No of rats. Results were expressed as Mean±SD

3.3 Histological Examination of Ovaries

Histological examinations of the ovaries are shown in the following photomicrographs (Fig. 1).

4. DISCUSSION

The use of synthetic azo food dyes and their effect on organs and well-being is still scientifically controversial especially at the acceptable daily intake (ADI) dose [5,9]. In addition, data regarding their effects on fertility, gonads, and reproductive hormones are still very sparse and obscure. This study was designed to ascertain the effect of tartrazine at ADI doses on the weight and histology of the ovary (integrity), reproductive fertility hormones such as LH, FSH, and PRL as well as oxidative stress markers like GPX and SOD over a period of 30 and 60 days in albino rats. When the parameters over a period of 30 and 60 days were considered, non-significantly higher values were seen in LH, FSH and PRL (except PRL in 30 days treated rats that indicate non-significant lower value) as well as the weight of the ovary (WOV) in the treated rats compared to the control rats. However, non-significantly lower values were seen in GPX and SOD oxidative stress markers while the histology result indicated mild hydropic dilation over a period of 60 days.

The non-significant differences seen in LH, FSH, and PRL are in line with the reports of Tanaka [16]; Tanaka [16] stated that at a high dose of 773mg/kg, tartrazine did not induce any change in reproductive parameters. In addition, EFSA [24], also reported in their separate work that ADI doses of tartrazine did not cause harmful effect on reproductive hormonal parameters. However, the non-significant difference seen in LH over the period of 30 and 60 days of treatment is contrary to the report of Khiralla et al. [25]. They observed a significant reduction in LH hormones with a corresponding fall in testosterone when rats were treated with tartrazine at ADI dose and at a dose 5 times that of the ADI dose. More so, It was observed that tartrazine at ADI doses did not affect noraldrenaline hormone and dopamine viz-a-viz PRL. This finding on PRL is in agreement with our work. In addition, our result on LH and FSH also contradicts the findings of Abbas & Al-Hamadavi [26]. They reported significant higher values of LH and FSH in rats treated compared with control rats with chocolate brown azo food dye at a high dose of 200 mg/kg and 400 mg/kg for 8 weeks. It was further documented that

Table 3. Comparison of 30 and 60 days results of hormonal parameters, oxidative markers, and weight of ovary in female rats treated with tartrazine

Parameters	Treated rats 30 days (n=20)	Treated rats 60 days (n=16)	*Pvalue*	Tvalue	Remark
LH (miU/ml)	0.51±0.26	0.45±0.18	0.182	0.901	NS
FSH (miU/ml)	1.06±0.91	0.84±0.23	0.143	2.414	NS
PRL (ng/ml)	1.20±0.45	1.14±0.38	0.300	0.390	NS
SOD (U/ml)	186.4±89.32	173.0±74.09	0.785	0.334	NS
GPX (U/ml)	10.19±8.45	9.42±5.02	0.751	0.342	NS
WOV (g)	1.67±0.54	1.44±0.86	0.125	2.433	NS

NS=Not significant at P=.05. LH=Luteinizing Hormone, FSH=Follicle Stimulating Hormone, PRL= Prolactin, GPX= Glutathione peroxidase, SOD= Superoxide dismutase, WOV=Weight of ovary, n= No of rats, Results were expressed as Mean±SD

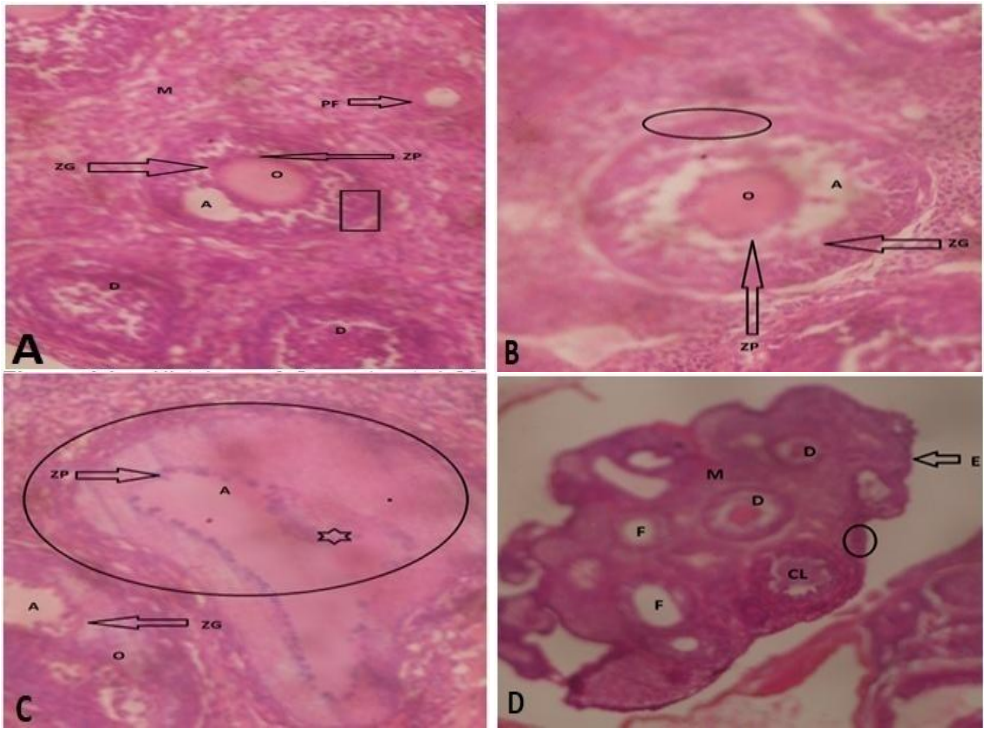

Fig. 1. Histological examination of ovaries

A. Control, 30 days: Section shows Oocyte (O) with zona pellucida (ZP) surrounded with zona granulosa (ZG) with well-defined follicular antrum (A). The medulla (M) of the ovary appears normal with primordial folicles (PF) and degenerating corpus luteum (D) of ovum. The squared area showed theca interna and externa of oocyte. Inference: Normal Ovarian section. B. Treated Rats, 30 days: Normal Oocyte (O) with zona pellucida (ZP) surrounded with zona granulosa cells (ZG) with a developing follicular antrum (A). The circled area showed theca interna and externa of oocyte. C. Treated Rats, 60 days: Oocyte (O) with zona granulosa cells (ZG) with a developing follicular antrum (A). The circled area indicates hydropic dilated oocytic region with zona pellucida (ZP), and atrium. D. 60 days treated Rats: Developing ovarian follicles (F) with corpus luteum (CL), and early-onset degenerating corpus luteum (D) in a well-defined medulla (M). The circled area is the stroma of the ovary with well arranged epithelial (E). H&E stain. X400

comparative analysis of the treated groups did not indicate any significant difference which is also in line with our observation when 30 days and 60 days treated rats were compared. In another related study, Amin [27], also reported significant higher and lower values in FSH and LH respectively in rats treated with carmoisine azo food dye at a dose of 10.0 and 20.0mg/kg for 30 days. However, when the dose of the azo dye was reduced to 5.0mg/kg bodyweight, LH

showed no significant difference. This finding of LH at low dose of 5.0mg/kg is in agreement with our work. We believe the discrepancies between our results and the results of other authors could be due to the differences in the dose of treatment. Most of the other authors used higher doses above the recommended ADI as treatment dose while we used ADI doses in our work. The non-significant differences seen FSH, LH and PRL over a period of 60 days of treatment with tartrazine could be due the body's capacity to tolerate the toxicological effect of tartrazine at the acceptable daily intake dose of 7.5mg/kg. The non-significant increases seen in the glycoprotein hormones especially in LH and FSH could be due to poor feedback negative estrogen response by the theca cells of the ovary when stimulated by these gonadotrophic hormones. The poor feedback negative estrogen could be as a result of interferences induced by mild oxidative stress or the mimicking of physiological estrogen due to the xenoestrogenic properties of tartrazine dye. The xenoestrogenic activities could have also stimulates the kisspeptin (Kp) from kiss1 neuron in the anteroventral periventricular nucleus (AVPV) of the hypothalamus in a very mild proportion (based on the dose and duration of treatment) triggering a non-significant increase in LH. Probably in a situation where the theca membrane and cells were severely distorted, reduced values of LH and FSH would have been seen due to complete loss of estrogen response. Therefore, it could also be possible that FSH, LH and PRL values may indicate significant derangements (either significantly lower or higher levels) if the duration of treatment was extended judging from the results obtained.

In addition, when the weight of the ovary was evaluated, significant differences were also not seen in treated rats compared to control rats over the period of 30 and 60 days. Our finding is consistent with the report of Mehedi et al. [19]. They reported no significant change in the absolute and relative weight of the ovary when rats were treated with tartrazine at a dose of 0.1%, 0.45%, 1% and 2.5% for a period of 13 weeks. More so, our finding agrees with the report of Elekima et al. [1]. They also observed no significant change in the absolute weight of the ovary when rats were given tartrazine at a dose of ADI doses over a period of 30 and 60 days. However, the reports of Sharma [18] contradict our observation. The author observed meaningful reduction in the weight of the ovary when kerasi powder was fed to rats at higher dose above the ADI for over 35 days. The non-significant difference in the ovarian weight seen in our work against the reports of other authors could be as a result of the amount of azo dye (dose) administered. Change in the weight of organs, whether increase or decrease are good predictors of toxicological effect of a substance. The non-significant higher weight of the ovary seen could be related to the retention of fluid within the ovary which probably resulted in the distention of the atrium seen as hydropic dilation in the histology.

Again, the non-significantly lower values seen in GPX and SOD of the treated rats over a period of 30 and 60 days when compared to their respective control rats at P=.05 is contrary to the finding of several authors such as Saxena [28], Amin et al. [29], and Ali et al. [30], also reported significant reduction in GPX and SOD when high doses of tartrazine above ADI doses were given to rats for over a period of 30 and 60 days respectively. In addition, at ADI doses, Khayyat et al. [8] documented significant reduction in total antioxidant capacity (TAC) levels in rats treated with tartrazine at a dose of 7.0mg/kg bodyweight for 30 days. Albasher et al. [31], also recorded significant fall in GPX and SOD oxidative stress markers in treated rats at ADI dose compared to control rats but recorded no significant differences at lower doses of 2.5mg/kg and 5.0mg/kg. However, the results of Adele et al. [32] are in line with findings. They observed a non-significant decrease in GPX and SOD activities in rats fed with tartrazine at ADI doses for 60 days. They further reported significant increase in Malondialdehyde (MDA) activities over the aforementioned specified period. The non-significant lower values observed our work with respect to GPX and SOD in the tartrazine treated rats against the control rats may be due to progressive gradual depletion of anti-oxidant enzymes (state) in the system of the treated rats while trying to counteract the toxic effect of reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced in course of tartrazine metabolism in the hepatic and gastrointestinal organ-system. In other words, the delicate balance between free radicals and antioxidants over the 30 and 60 days treatment period was not completely disrupted due to either mild production of free radicals from the dye during metabolism as a result of the ADI dosages administered or the steady decline of antioxidant defense capacity or both.

Furthermore, the examination of the ovarian section of the control group indicated normal ovarian histology with well-defined the oocyte, zona pellucida, surrounded by zona granulosa cells, follicular antrum and intact theca membrane of the oocyte. In addition, the medulla appeared normal with primordial follicles and degenerating corpus luteum. The 30 days histological results in the treated rats indicating normal oocyte, zona pellucida surrounded with zona granulosa cells, a developing follicular antrum and intact theca interna and externa of oocyte is consistent with reports of Elekima & Nwachuku [9]. They reported normal ovarian histology when chronic toxicity of tartrazine (E102) in albino rats was evaluated but reported ovarian distortions after a period of 90 days treatment at ADI doses. Similarly, Amin [27] observed no ovarian distortions at low dose of 5mg/kg of carmoisine azo dye fed to female rats for 30 days. However, our finding contradicts the reports of Sharma [18]. He reported ovarian distortions when *kerasi* powder was fed to rats for over 35 days. In addition, the histological results over 60 days treatment showing normal oocyte, zona granulosa cells and a developing follicular antrum but with mild hydropic dilation of the oocytic region that was seen affecting the anatomical and architectural arrangement of the zona pellucida, atrium and zona granulosa area in one of the sections as well as the observation of developing ovarian follicles with corpus luteum, and early-onset degenerating corpus luteum in a well-defined medulla in another section is also in line with the reports of Elekima & Nwachuku [9]. They also observed unorganised follicular cells, normal theca membrane and corpus luteum over a period of 60 days treatment with tartrazine at ADI doses. More so, Mehedi et al. [19], recorded ovarian distortion when tartrazine at dose of 0.1%, 0.45%, 1% and 2.5% were fed to rats for 13 weeks. The mild structural changes observed in the ovarian sections of the treated rats are suggestive of oxidative damages maybe due effect of reactive oxygen species (ROS) or reactive nitrogen species (RNS) with a degree of tolerance maybe as a result of the treatment dose and duration. This might further explain why the reproductive hormones were not severely deranged as seen in our results. In other words, the hormones were not significantly affected probably because sometimes, marked significant changes in biochemical markers or parameters also depend on the degree of anatomical or structural derangements. As documented by Dermirkol et al. [33], free radicals and ROS are toxic by-products of aerobic activities generated endogenously and exogenously by chemicals, xenobiotics and pollutants. These radicals are capable of inducing biochemical and physiological lesions that may lead to cell distortion, damage and eventually cell death as a result of oxidative derangements involving macromolecules such as lipids, proteins, and nucleic acids.

5. CONCLUSION

The administration of ADI doses of tartrazine over a period of 60 days affected the integrity of the ovary mildly as observed in the histology but did not markly reflect on the biochemical markers in the plasma as well as the weight of the ovary.

6. RECOMMENDATION

It is advised that high doses of tartrazine in foods or food products should be avoided completely. More so, because of the hydropic dilation seen in the 60 days treated rats, it is also advised that duration far above 60 days should be considered in further studies.

7. LIMITATION OF THE STUDY

The study duration was not more than 60 days. In addition, our present findings were in rats and therefore cannot be directly interpreted that these effects observed in rats will be exactly and/or physiologically the same in humans. Therefore, these findings are subject to further research and verification especially in humans.

CONSENT

It is not applicable.

ETHICAL APPROVAL

We hereby declare that the Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Rivers State University research/ethics committee with file No: RSU/CV/APU/74/VOL.VIII/104.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Elekima I, Nwachuku EO, Okwukwu D, Nwanjo UH, Nduka N. Biochemical and histological changes associated with azo food dye (tartrazine) in male albino rats. Asian Journal of Research in Biochemistry. 2019; 5(1):1-14.
2. Meyer KS, Robert MEP, Lakey FA, Axon RA, Leitch CA, William MF, Jowsey AP, Blain GP, Kass ENG, Wright CM. Hepatic effects of tartrazine (E102) after systemic exposure are independent of oestrogen receptor interactions in the mouse. Toxicological Letters. 2017;273:55-68.
3. Amin KA, Al-Sheshri FS. Toxicological and safety assessment of tartrazine as a synthetic food additive on health biomarkers: A review. African Journal of Biotechnology. 2018;17(6):139-149.
4. Zahra N, Kalim I, Saeed MK, Mumtaz Z, Amjad N, Alim N, Hina S, Masood S, Ahmed I, Ashraf M. Effect of natural and synthetic dyes on human health. International Research Journal of Biological Sciences. 2017; 6(10):23-29.
5. Okafor SN, Obonga W, Ezeokonkwo MA, Nurudeen J, Orovwigho U, Ahiabuike J. Assessment of health implications of synthetic and natural food colourants – A critical review. Pharmaceutical and Biosciences Journal. 2016; 2347-2352.
6. Ai-Mashhedy LAM, Fijer AN. Acute toxicity of food additives tartrazine and carmoisine on white male mice. International Journal of PharmTech Research. 2016;9(4):364-367.
7. Ghonimi AMW, Elbaz A. Histological changes of selected wister rat tissues following the ingestion of tartrazine with special emphasis on the protective effect of royal jelly and cod liver oil. Journal of Cytology and Histology. 2015;6(4):346-347.
8. Khayyat IL, Essawy EA, Sorour MJ, Soffar A. Sunset yellow and allura red modulate BCL2 and COX2 expression levels and confer stress-mediated renal and hepatic toxicity in male rats. PeerJ. 2018; 6:e5689.
   Available:https://doi.org/10.7717/peerj.5689
9. Elekima I, Nwachukwu EO. Evaluation of acute and chronic toxicity of tartrazine (E102) on steroid reproductive hormones of albino rats. Asian Journal of Research and Reports in Endocrinology. 2019;2(1):1-15.
10. Yang S, Jia ZC, Chen JY, Hu JX, Zhang LS. Toxic effects of tartrazine on reproductive system of male rats. Biomedical Environmental Sciences. 2014; 27(4): 281-288.
11. Mohamed EG, Abo-laila IM, Masoud MA. Fortification role of curcumin against renal and testicular toxicity of synthetic food dye brilliant blue in rats. Journal of Drug Delivery & Therapeutics. 2019;9(2):1-8.
12. Boussada M, Lamine J, Bini I, Abidi N, Lasrem M, El-Fazaa S, El-Golli N. Assessment of a sub-chronic consumption of tartrazine (E102) on sperm and oxidative stress features in wistar rat. International Food Research Journal. 2017; 24:1473-1481.
13. Iroh G, Weli OB, Adele AU, Briggs NO, Waribo AH, Elekima I. Assessment of atherogenic indices and markers of cardiac injury in albino rats orally administered with tartrazine azo dye. Journal of Advances in Medical and Pharmaceutical Sciences. 2020; 22(6):51-61.
14. Filho CJ, Swanson RJ, Varghese A. Overview of the female reproductive system. Journal of Molecular Endocrinology. 2016; 10(7):3402-4936.

15. Department of Health, Australian Government. Toxicity of tartrazine: Scientific review report; 2014.
Available: http://www.tge-gov.au
(Accessed 28 July, 2019)
16. Tanaka T. Reproductive and neurobehavioural toxicity study of tartrazine administrated to mice in the diet. Food Chemical Toxicology. 2006; 44:179–187.
17. Elhkim M, Heraud F. New consideration regarding the risk assessment in tartrazine: An updated toxicological assessment, intolerance reactions and maximum theoretical daily intake in France. Regulatory Toxicology Pharmacology, 2007; 47:308–316.
18. Sharma G. Reproductive toxic effect of the synthetic food dye kesari powder in female swiss albino mice (*Mus musculus*). International Journal of Science, Technology and Management. 2015;1: 153-168.
19. Mehedi N, Mokrane N, Alami O, Ainad-Tabet S, Zaoui C, Kheroua O, Saidi D. A thirteen week of libitum administration toxicity study of tartrazine dye in swiss mice. African Journal of Biotechnology. 2013;12(28): 4519-4529.
20. Engvall E, Perlmann P. Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. Immuno-chemistry. 1971; 8(9):871–874.
21. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochemica et biophysica Acta, 1979; 582:67-78.
22. Marklund S, Marklund G. Involvement of the superoxide anion radical in the auto-oxidation of pyrogallol and convenient assay for superoxide dismutase. Journal of Biochemistry, 1974;47:469-474.
23. Stevens A. The haematoxylins. In: Bancroft JD, Stevens A, editors. Theory and Practice of Histological Techniques. 3rd Ed. London, Churchill Livingstone, 1990.
24. European Food Safety Authority (EFSA) Panel on food additives and nutrient sources added to food (ANS: 2009): Scientific opinion on the re-evaluation of tartrazine (E 102) on request from the European Commission. European Food Safety Authority Journal. 2009; 7:1331–1383. 15
25. Khiralla MG, Salem AS, El-malky AW. Effect of natural and synthetic food colouring agents on the balance of some hormones in rats. International Journal of Food Science and Nutrition Engineering, 2015;5(2):88–97.
26. Abbas RJ, Al-Hamadavi AH. Effect of chocolate brown TH E155 on some hormones in male albino rats. Eurasian Journal of Bioscience. 2019;13: 485–489.
27. Amin MAF. Pathophysiological effect of Azorubine on female reproductive organs and hormones in Sprague Dawley rats. International Journal of Health Science. 2018;7(6):57–62.
28. Saxena B. Ovarian toxicity and oxidative stress induced by food colours in albino rats. International Journal of Science and Nature. 2016;7(4):838–842.
29. Amin AK, Hameid II AH, Abd Elsstar HA. Effects of food azo dyes tartrazine and carmoisine on biochemical parameter related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food and Chemical Toxicology. 2010;48:2994–3999.
30. Ali AF, Abdelgayed SAS, El-Tawil OS, Bakeer AM. Toxicological and histopathological studies on the effect of tartrazine in male albino rats. International Journal of Biological, Biomolecular, Agricultural, Food & Biotechnological Engineering. 2016;10(8): 513–518.
31. Albasher G, Maashi N, Alfarraj S, Almeer R, Albrahim T, Alotibi F, Bin-Jumah M, Mahmoud MA. Perinatal exposure to tartrazine triggers oxidative stress and neurobehavioral alterations in mice offspring. Antioxidants. 2020;9(1): 53.
Available:https://doi.org/10.3390/antiox9010053
32. Adele U AU, Iroh G, Briggs NO, Waribo AH, Elekima I. Evaluation of anti-oxidant enzymes, lipid peroxidation, lipid profile and liver function in albino rats orally administered tartrazine. International

Journal of Biochemistry Research & Review. 2020;29(5):19-29.

33. Dermirkol O, Zhang X, Ercal N. Oxidative effect of tartrazine (Cas No. 1934-21-0) and new coccin (Cas No. 2611-82-7) azo dyes on CHO cells. Journal of Consumer Protection and Food Safety. 2012;7:229–236.

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