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RESEARCH ARTICLE

Protective Potential of Aqueous Extract of *Allium cepa* against Tartrazine Induced Reproductive Toxicity

Chaman Ara[1], Aqsa Arshad[1], Mehwish Faheem[2*], Muhammad Khan[1] and Hafiz Abdullah Shakir[1]

[1]Department of Zoology, University of the Punjab, Lahore Pakistan
[2]Department of Zoology, Government College University Lahore, Pakistan
*Corresponding author: mehwishfaheem@gcu.edu.pk

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A B S T R A C T

Tartrazine is routinely used in food and pharmaceutical industries as an azo dye, that imparts yellow color to the products of different food items. Various medicinal plants and herbs are used as protective agents against natural or man-made toxicants. In this study, the protective effect of aqueous onion extract against tartrazine instigated reproductive toxicity in both male and female mice was evaluated. A total of 80 healthy male and female mice (weighing 14 ± 1g) were divided into four groups (n=20). Group I was marked as control, group II was exposed to tartrazine (10µg/g B.W), group III was exposed to aforementioned concentration of tartrazine and 0.1ml onion extract, while group IV received only 0.1ml onion extract. Mice were treated orally for 30 consecutive days and then euthanized for sample collection. Our findings showed that the total antioxidant capacity of testes and ovaries homogenates reduced significantly after exposure to tartrazine, while co-administration of onion extract and tartrazine improved the anti-oxidant power to a great extent. Hormonal analysis showed that male mice exposed to tartrazine had significantly reduced serum testosterone level while level of estradiol, luteinizing and follicle stimulating hormone was also significantly lower in female mice after exposure of tartrazine. Histopathological lesions were also prevalent in tartrazine exposed testes and ovaries sections as compared to controls. Moreover, exposure to tartrazine resulted in increased number of abnormal sperms and decreased total sperm count in male mice. Overall, it is concluded that onion extract was proved to be a remedial agent against tartrazine induced organ pathologies and biochemical alterations.

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INTRODUCTION

Food coloring has been used to increase food quality as tempting colored food attract more customers (Singh, 2006). Natural food color, like saffron and turmeric, had been used as feed additive, but their tint is dependent on storage conditions, temperature and pH of food (Amchova *et al.,* 2015). Therefore, now a days, artificial food dyes are extensively used in domestic cooking, commercial food production, pharmaceuticals and cosmetics (Delwiche 2004; Wopara *et al.,* 2021). These artificial food dyes are available in powder, gel, paste or liquid form.

Tartrazine, also known as food yellow 4, Acid Yellow 23, Yellow 5, is most commonly used synthetic food dye. It imparts yellow color and therefore it is not only extensively used in food but also in pharmaceuticals like yellow color tablets, syrups and lotions etc. High intake of these dyes leads to subsequent increase of their residues in tissue and serum, resulting in irregular levels of glucose, total cholesterol and lipoproteins in serum (Merinas-Amo *et al.,* 2019). High oxidative stress and onset of various metabolic syndromes like diabetes are also associated with increased intake of tartrazine. Tartrazine is an azo dye, gut microorganisms cleave the azo bond using azoreductases and convert the parent dye in more potent genotoxic compounds (Feng *et al.,* 2014). The metabolites of tartrazine results in allergic reaction such as asthma and urticaria (Titova, 2011). Therefore, tartrazine is considered as one of the most contentious coloring compounds, exhibiting noxious potential for memory, learning and human lymphocytes and adolescent behavioral disturbances (Hannuksela and Haahtela, 2009; Gao *et al.,* 2011).

According to WHO, acceptable daily intake (ADI) of tartrazine ranges between 0-0.7mg/kg/day (Authority

EFSA, 2009). But concern has been aroused about it's unregulated use in various products without labels. In developing countries, like Pakistan, children are more susceptible to tartrazine because they consume comparatively large amount of colored food like bright colored candies and flavored juices etc., which usually exceed their daily intake limit (Husain *et al.,* 2006).

Phytochemicals especially phenols and polyphenols are considered as major bioactive components of plants/herbs that act as valuable source of antioxidants and are responsible for major health benefits (Peschel *et al.,* 2006; Latif *et al.,* 2021). Onion (*Allium cepa* L.) is highly cultivated species that belongs to genus *Allium*, and is known for its antioxidant properties (Bindu and Podikunju, 2015). In a study governed by Nuutila *et al.* (2003), it was reported that onion is effective in prevention of many human related disorders such as arteriosclerosis, inhibiting systematic infections, effective in detoxifying phenomenon. Moreover, onion is a rich source of exogenous and endogenous antioxidants like glutathione and isorhamnetin. It also contains different vitamins like vitamin A, B and C and trace minerals like selenium that can help in preventing damage caused by reactive oxygen species and can also protects DNA from oxidative damage which leads to enhanced fertility in humans (Meeker *et al.,* 2007).

Keeping in view of beneficial aspects of onion, the current study was designed to investigate the toxic effects of tartrazine on reproductive organs and to evaluate the potential protective efficacy of onion extract against tartrazine induce damages.

MATERIALS AND METHODS

Chemicals: Tartrazine (86.7% purity) was obtained from Food Net Chemical Company, Pakistan. Onions (*Allium cepa* L.), were purchased from Local Market of Lahore. Other chemicals were acquired from Merck & Co. and Sigma - Aldrich Company.

Onion Extract Preparation and estimation of total phenolic content: Onions were peeled and 100grams of onions were blended with 100ml of water in a blender (Jaiswal *et al.,* 2013). The mixture was sieved with fine muslin cloth and aqueous extract was stored at 4°C. Total phenolic content of onion extracts was estimated using folin-ciolcalteu method modified by Dutta and Ray (2020) and expressed as mg of gallic acid equivalents /g fresh weight (mgGAE/g FW). Total antioxidant capacity was determined by using procedure of Benzie and Devaki (2018).

Mice model breeding and maintenance: Albino mice (Swiss Webster strain) were used in current experiment and were kept at Animal House of Zoology Department, University of the Punjab. During the experimental period, mice were fed with basal mice diet and water *ad libitum*. The atmospheric temperature was maintained at (24 ± 2°C) and humidity level was 35- 60% while the photoperiod was 12:12 throughout the experiment. Experiment was conducted according to the guideline issued by IACUC University of Menoufia, Egypt, Approval No: MNSP155.

Preparation of dose and treatments: A stock solution of tartrazine was prepared in distilled water and each experimental mice received a dose of 10µg/g body weight of mice.

Five weeks old, eighty healthy male and female mice (weighing 14 ± 1g) were selected and assigned four groups with 10 males and 10 females (n=20). Group I is treated as control group administrated with water. Group II (treatment group) was given tartrazine 10µg/g BW of mice. Group- III received 10µg/g of tartrazine + 0.1ml of onion extracts while Group- IV received only 0.1ml of onion extract. The experiment was conducted for 30 days and dose quantity was regulated on weekly basis depending upon experimental animal's weight variations. During the experimental period, all mice were carefully observed for any behavioral change.

Sample's Collection: After 30-days, mice were weighed individually and euthanized after giving 5% Isoflurane anesthesia. Blood was drawn by cardiac puncture and stored in serum separating tubes. Serum was separated and stored at -20 C until the hormonal analysis. After taking blood samples, mice were dissected and gonads were removed. Gonads (testes and ovaries) were observed for gross morphological changes, weighed and stored in (10%) buffered formalin for histological studies.

Evaluation of Estrous phase: All male mice were sacrificed at a time after 30 days' treatments but for female mice, females in the estrous phase were euthanized at the same day, while rest of the female mice were dissected within 3 days after end treatment to avoid phase dependent hormonal fluctuations. To follow-up estrous cycle, vaginal discharge was collected by inserting saline solution (0.2 ml) into female vagina using a micropipette. A smear was made using the vaginal secretions and viewed under microscope. Presence of cornified squamous epithelial cells confirms that the animal was in the estrous phase. Females in estrous phase were selected for experimentation.

Hormone assay: Serum samples from male mice were used for testosterone measurement while serum sample from female mice were used to estimation of estradiol, follicle stimulating and luteinizing hormone. Enzyme linked immunosorbent assay (ELISA) was used to estimate the serum level of male and female hormones using commercially available kits (Invitrogen by Thermo Fisher Scientific, USA).

Sperm count and morphology: Male sperm count was assessed by method provided by Val and Robledano (2013). Epididymides of male mice were separated, minced with scissors and dispersed in phosphate buffer saline at 37°C. Count was done in Neubauer's chamber (Yawson Emmanuel *et al*., 2018).. Sperm morphological assessment was made by shifting 1ml of suspension with addition of few hematoxylins drops in test tube and incubated for 45 minutes. Smear was assembled, air-dried and mounted by a cover slip on same day. 100 sperms were observed per section from each group using light microscope for morphology (Jakubik-Uljasz *et al.,* 2020).

Histopathological preparations of gonadal tissues: For histopathological study, tissue samples (testes & ovaries) were dehydrated in ascending grades of alcohol (30-100%). The tissue samples were clarified with xylene and impregnated with paraffin wax (mp-50ºC) following Faheem *et al*. (2016). Sections of testes (6 µm thick) and ovaries (10 µm thick) were cut using rotary microtome. Tissue sections were stained with hematoxylin and eosin (Faheem and Lone, 2017) and observed under microscope (M4000-D Swift, Japan). The images of prepared slides were clicked by digital Panasonic camera (DMC-TZ15).

Statistical analysis: Numerical results are expressed as mean ± S.E.M. Statistical difference among groups were calculated through ANOVA following Tukey's post hoc test. The mean differences and interpretation were done at 90 % confidence level. P value ≤ 0.05 was considered as significant for all experimental groups.

RESULTS

During the experimental period, no clinical symptoms such as general behavioral signs of inactiveness, sickness, mortality, visually observable toxicity, and effected health were observed. Phenolic content analysis and FRAP assay showed that onion extract possesses high concentration of total phenolic content and significant anti-oxidant capacity (Table:1). Exposure to tartrazine resulted in significant decrease in anti-oxidant capacity within testes and ovaries homogenates. However, administration of tartrazine along with onion extract helped in increasing the total anti-oxidant capacity in gonadal tissues. Mice given only onion extract, didn't differ significantly from control with respect to total anti-oxidant capacity (Table-2).

The mice body weight, organs weight and gonado-somatic index included in morphometric measurements are described in Table 3 & 4. Body weights of male and female mice in tartrazine exposed groups was not increased in the manner of control and onion-tartrazine co-administrated group. However, this decrease was not statistically significant. Likewise, the body weights, gonadal weight and gonadosomatic index of male and female mice showed a significant decrease ($P<0.05$) in tartrazine exposed groups. Administration of onion extract alone and in combination with tartrazine did not affect gonadal weight and gonado-somatic index (Table 4).

In male mice, exposure to tartrazine resulted in significant ($p<0.05$) decrease in testosterone level. Mice co-treated with onion extract and tartrazine has maintained testosterone level comparable to control (Table 5). A significant ($P<0.05$) decrease in the level of follicle stimulating hormone, luteinizing hormone and estradiol was reported in female mice exposed to tartrazine. Co-administration of onion extract along tartrazine remained helpful to restore the level of these hormones near to control levels (Table 5).

Sperm count (million/ml) and abnormal sperm percentage is shown in Table 6. Sperm number as well as morphologically normal sperms decreased in tartrazine group in comparison with control group. Administration of onion extracts along with tartrazine improved sperm number as compared to tartrazine exposed group.

Histopathological studies after administration of tartrazine revealed some anatomical lesions in male gonads. Testis sections from control group showed regular and normal pattern of spermatogonia, spermatozoa, elongated and round spermatids. seminiferous tubules lumens were filled with Sperms. Sertoli and Leydig cells are also clearly observed in intra and inter tubular spaces respectively. However, in group exposed to tartrazine, vacant lumens of seminiferous tubules, interstitial tissue hypoplasia, atrophy of seminiferous tubules and azoospermia were more prominent. These defects were reduced to a great extent, when mice were given onion extract along with tartrazine (Fig. 1).

Table 1: Total phenolic content and anti-oxidant capacity of onion extract

Onion extract TPC	265±14.7 µg (GAE/ml of onion extract)
Onion extract FRAP	137±12.3 (µM ferrous sulphate / ml of onion extract)

Table 2: Total anti-oxidant potential of testes and ovaries

Groups	Antioxidant capacity of Testis tissue	Antioxidant capacity of ovary tissue
Control	$70.16^{d\pm}0.19$	$126.9^{c\pm}0.91$
Tartrazine	$21.14^{a\pm}0.78$	$99.3^{a\pm}0.54$
Tartrazine + Onion extract	$35.54^{b\pm}0.56$	$102.5^{a\pm}0.75$
Aqueous onion extract	$61.12^{c}\pm0.43$	$113.7^{b}\pm0.39$

Data expressed as mean±S.E.M. Different superscripts indicates significant difference among groups.

Table 3: Weekly Body weight calculations of mice in all treated groups

Group		Week 1 Body weight (g)	Week 2 Body weight (g)	Week 3 Body weight (g)	Week 4 Body weight (g)
	Male	$13.94^{a}\pm0.21$	$18.07^{c}\pm0.20$	$20.67^{d}\pm0.16$	$22.85^{d}\pm0.18$
Control	female	$13.87^{a}\pm0.21$	$18.02^{c}\pm0.19$	$20.71^{d}\pm0.20$	$22.80^{d}\pm0.18$
	Male	$14.11^{a}\pm0.20$	$15.67^{a}\pm0.13$	$16.84^{a}\pm0.15$	$18.06^{a}\pm0.10$
Tartrazine	female	$13.91^{a}\pm0.20$	$15.64^{a}\pm0.13$	$16.88^{a}\pm0.19$	$18.00^{a}\pm0.10$
Tartrazine +	Male	$13.88^{a}\pm0.12$	$17.04^{b}\pm0.15$	$19.20^{c}\pm0.07$	$21.32^{c}\pm0.09$
Onion	female	$13.85^{a}\pm0.12$	$16.96^{b}\pm0.16$	$19.16^{c}\pm0.08$	$21.27^{c}\pm0.09$
Aqueous onion	Male	$14.43^{a}\pm0.19$	$17.02^{b}\pm0.12$	$18.74^{b}\pm0.08$	$19.77^{b}\pm0.10$
extract	female	$14.29^{a}\pm0.27$	$16.99^{b}\pm0.12$	$18.67^{b}\pm0.09$	$19.71^{b}\pm0.10$

Data expressed as mean±S.E Different superscript shows statistically difference among groups P<0.05.

Table: 4: Organ weight and gonado-somatic index of male and female mice

Groups	Weight of Testes (mg)	Gonado-somatic index of testes	Weight of ovaries (mg)	Gonado-somatic index of ovaries
Control	$66.6^{c}\pm0.43$	$0.29^{b}\pm0.003$	$6.96^{a}\pm0.42$	$0.03^{bc\pm}0.002$
Tartrazine	$54.4^{a\pm}0.56$	$0.26^{a}\pm0.004$	$6.85^{a\pm}0.36$	$0.02^{a\pm}0.001$
Tartrazine + Onion extract	$63.7^{c\pm}0.73$	$0.30^{bc\pm}0.006$	$7.46^{ab\pm}0.24$	$0.04^{c\pm}0.002$
Aqueous onion extract	$59.8^{b}\pm1.8$	$0.31^{c}\pm0.01$	$8.46^{b\pm}0.61$	$0.03^{b}\pm0.001$

Data expressed as mean±S.E.M. Different superscript shows statistically difference among groups P<0.05.

Table: 5: Hormonal analysis of male and female mice after 4 weeks of tartrazine exposure

Parameters	Testosterone (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)	Estradiol (mIU/ml)
Control	1.37^{c}±0.10	0.97^{d}±0.01	2.15^{d}±0.007	$44.50^{d\pm}$0.71
Tartrazine	0.70^{a}±0.007	0.42^{a}±0.008	$1.36^{a\pm}$0.008	21.00^{a} ±0.71
Tartrazine + Onion extract	1.20^{b}± 0.006	0.88^{c}±0.01	2.11^{c}±0.007	38.30^{c} ±0.78
Aqueous onion extract	1.18^{b}± 0.10	0.65^{b}±0.03	1.63^{b}±0.02	31.50^{b}±1.77

Data expressed as mean±S.E.M. Different superscript shows statistically difference among groups P<0.05.

Table 6: Sperm count and percentage of normal and abnormal sperms in male mice after 4 weeks of tartrazine exposure

Groups	Sperm Count (Mean ± S.E.M)	Normal Sperm (Mean ± S.E.M)	Abnormal Sperm (Mean ± S.E.M)
Control	25.16^{c} ±0.83	48.09^{c} ±0.72	23.92^{a} ±0.78
Tartrazine	19.10^{a} ±0.70	36.18^{a} ±0.67	30.89^{b} ±0.95
Tartrazine + Onion extract	23.39^{bc} ±0.69	51.88^{d} ±0.87	22.41^{a} ±0.75
Aqueous onion extract	21.83^{b} ± 0.79	41.62^{b} ± 0.75	29.85^{b} ± 1.44

Data expressed as mean ± S.E.M. Different superscripts indicates significant difference among groups P<0.05.

The ovaries sections of control group revealed regular ovaries texture having mature oocytes with antrum surrounded by zona pellucida, granulosa cells, primary, secondary, atretic follicles and corpus luteum in regular fashion. Ovarian structure after treatment of tartrazine showed disorganizations in follicular cells. Follicular atresia with mild vacuolations can also be seen in this group. Female ovaries from tartrazine along with onion administration resumed granulosa cells pattern to a certain extent. Oocyte, zona pellucida, antrum and corpus lutea seems to be normal comparative to tartrazine group. After treating female mice with aqueous extract showed reduction of vacuolations, normal zona pellucida, granulosa cells, primodial follicles, and corpus luteum, probably due to the antioxidant activity of aqueous onion extract against reproductive deteriorations and altered steroidogenesis (Fig. 2).

DISCUSSION

Current study investigates the toxic effects of tartrazine on reproductive system of male and female mice using biochemical and histopathological biomarkers. The potential protective role of onion against tartrazine induce reproductive toxicity is examined.

Synthetic azo compounds are still routinely consumed in daily dietary products of human food industries and pharmaceutics on large scale irrespective of its toxicological effects (Elhkim *et al.,* 2007). Body weight is one of basic factor used for assessment of proper growth and animal's body weight retardation is considered a definitive sign of toxicity caused by chemical or dye (Helal *et al.,* 2000; Ara *et al*., 2022). In present study, group of mice exposed to tartrazine showed a significant decrease in body weight of both mice genders. Sperm count and percentage of healthy sperm decreased significantly in mice exposed to tartrazine. We suggest that the reduced amount of healthy sperm and increased percentage of abnormal sperms may be due to the failure of spermatogenesis or due to apoptosis of spermatozoa. The alterations in the function of Sertoli cells may also be a contributing factor in decline of sperm count. The histopathological alterations observed in the present study clearly revealed that exposure of tartrazine caused histopathological damage leading to lower sperm count. The concentration of testosterone in tartrazine exposed group had a significant lower level which can be associated with infertility problems in males (Du Plessis *et al.,* 2010). A normal testosterone level is necessary for spermatogenesis and release of healthy sperms (Boussada *et al.,* 2017). Decrease in testosterone level is directly related with increase in percentage of abnormal sperms as observed in the present study. Khaki *et al.* (2017) reported that *Allium cepa* juice increased sperm number via its antioxidant capability.

Gonadotrophic hormones (Follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) are released by pituitary. These hormones act on Sertoli and Leydig cells and stimulate the production of healthy sperms and on ovaries to produce and release estrogen. Testosterone (in males) and estrogen (in females) are important to maintain healthy reproductive stage. These reproductive hormones are synthesized and released under the complex neuro-endocrine control. Exposure of tartrazine resulted in decreased level of gonadotrophic hormones and subsequent decrease in male and female sex hormone. Similar findings were reported with other synthetic food dyes. Female rats when given 10& 20mg/kg of azorubine powder also resulted in decreased body weight and lower serum estrogen concentration (Amin, 2018). Consistent with our results, male rats have decreased testosterone levels when given tartrazine (Gautam *et al.,* 2010). Decrease in level of testosterone may be due to reduced level of acid phosphatases in serum (Mahmoud, 2006) and also due to atrophy of leyding cells, as observed in the present study. Exposure of food dyes and preservatives effects adenohypophysis which ultimately disturb the release of LH and FSH (Kumar *et al.,* 2016). Lower level of these hormones in our study may be due to the direct effect of tartrazine on adenohypophysis.

Another important criterion for determination of toxic effects of any anthropogenic agent is examination of histological changes in tissues. Testis of mice exposed to tartrazine showed histopathological abnormalities. Interstitial spaces were wide and seminiferous tubules become less circumscribed. A marked decrease in number of Leydig cells along with reduced number of sertoli cells and spermatozoa were observed in testis of mice exposed to tartrazine exposed group. These all above results is in line of recorded literature of (Dixit and Goyal, 2013). Similarly, female ovarian tissue from tartrazine exposed group showed vacuolization of follicular cells. the basement membrane of ovarian tissue was broken and granulosa cells were disorganized. Similar vacuolation of ovarian granulosa was reported by Sharma (2015) in mice after exposure of azo dye.

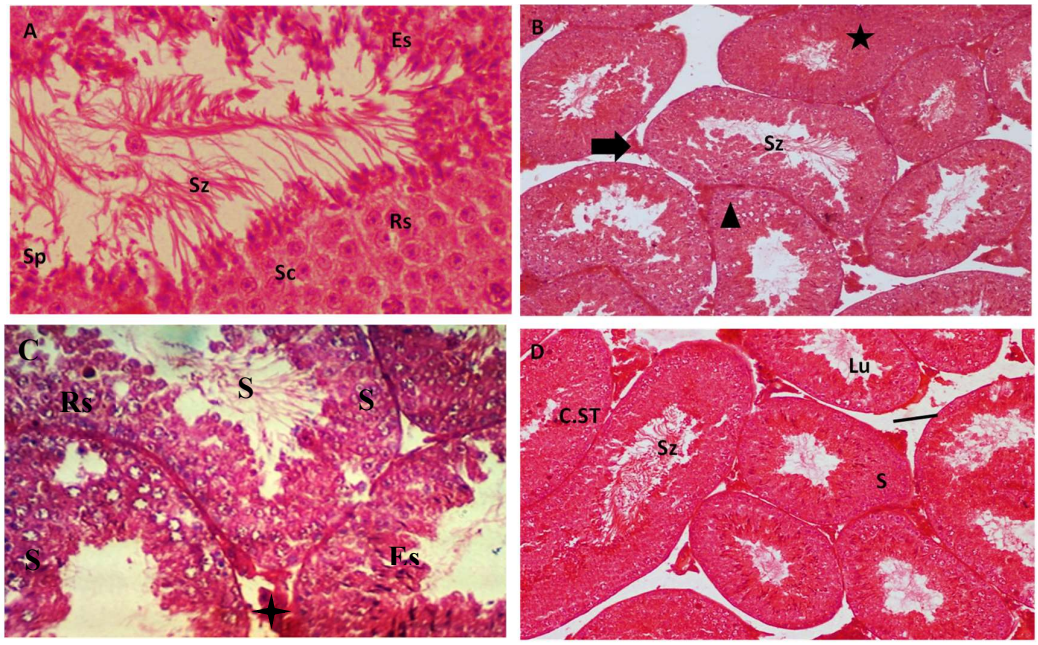

Fig. 1: Photomicrographs of testes administrated with different dose treatments (A) Control group (B) tartrazine; (C) tartrazine + onion extract; (D) onion extract group. Labelled as Sz, Spermatozoa; Sp, Spermatocytes; Sc, Sertoli cells; Rs, Round Spermatids; Es, Elongated Spermatids; Sg, Spermatogonia; S, Sperms; Lu, Properly assembled lumen, C.ST, convoluted seminiferous tubules (C.ST), black headed arrow, degenerative pattern of Leydig cells; five point star, reduction in sperms; triangle, aspermia, four pointed star, leydig cells reduction. Stain used H& E.

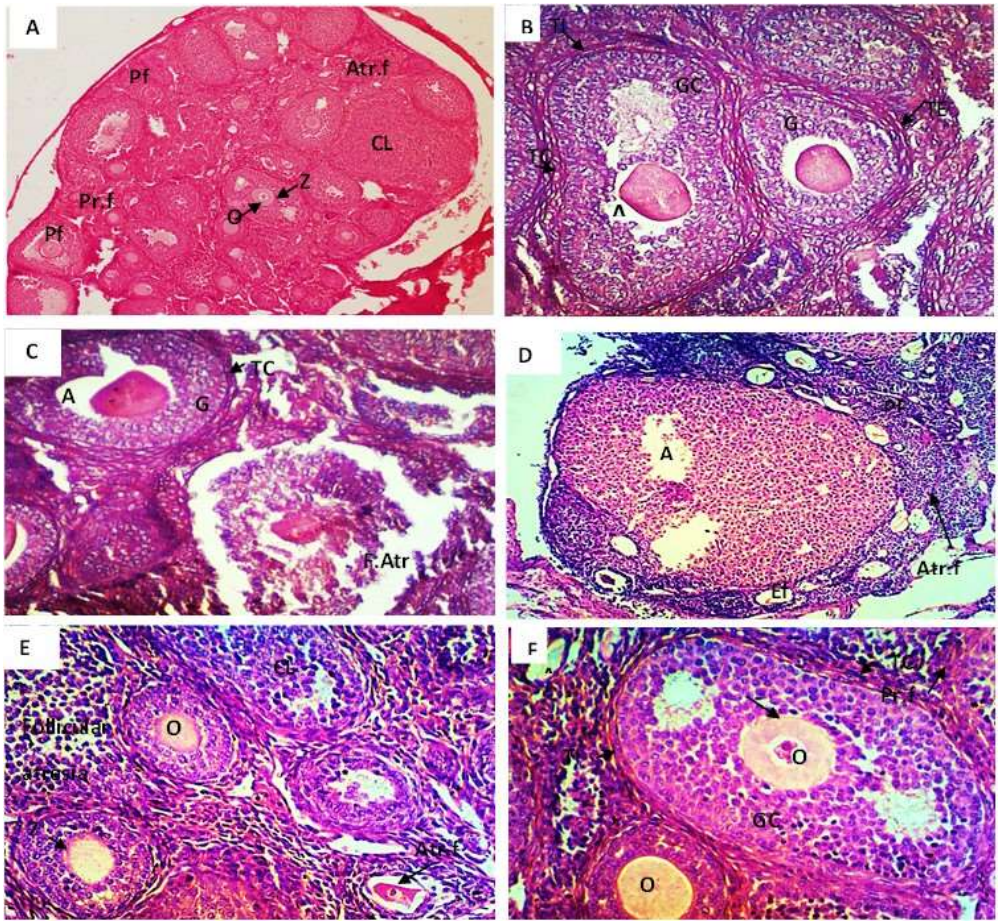

Fig 2: Microphotographic observations of female ovary histology sections (A,B) Control group (C) tartrazine group; (D,E) tartrazine + onion extract group; (F) onion extract group. Labelled as O, Oocyte; Atr.f, Atretic follicles; Pr.f, Primodial follicles; Pf, Primary follicles; CL, Corpus luteum; GC, Granulosa cells; TC, Theca cells; TE, Theca externa; TI, Theca interna; Z, Zona pellucida; A, Antrum; Ef, Empty follicle; stratified squamous epithelium; follicular atresia. H& E stain.

Administration of onion extracts along with tartrazine resulted in progressive changes in ovarian tissue. Oocytes development becomes normal and presence of healthy primary and secondary follicles were evident of protective efficacy of onion extracts. Moreover, decrease in vacuolation of granulosa was observed. Antrum and corpus luteum become organized after in ovarian tissue of female mice exposed to tartrazine and given onion extracts. This may be due to the rich phytochemical profile of *Allium cepa*. Bioactive compounds present in onion, especially quercetin, compound have ability to repair the deteriorative changes in tissues (Zhao *et al.,* 2021).

Present result findings for FRAP and TPC assays depicted highest co-relation between antioxidant potential and total phenolic contents in onion extract. Quercetin present in onion incapacitate the free radicals' effects, which is referred as an effective antioxidant characteristic of onion (Khaki *et al.,* 2010). Similarly, administration of vitamin C helped in mitigating the glyphosate induced oxidative stress in mice (Namratha *et al.,* 2021). Therefore, the strong antioxidants present in onion extract might be linked with its overall protective efficacy.

Present study findings clearly showed that exposure of tartrazine resulted in reproductive abnormalities both in male and female mice. Administration of onion extract is proved to be useful against tartrazine induced noxious effects in males and female mice.

Authors contributions: Conceptualization: CA and MF; Data curation: CA, AA, MK, HAS; Formal analysis: CA, MF, AA; Writing original draft: AA, MF; Review and editing: CA, MK, HAS; all authors have read and approved the manuscript.

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