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Protective efficacy of Aphanizomenon flos-aquae against Histopathological and Immunohistochemical alterations in rat's testis and liver exposed to a mixture of food additives

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Abstract: Background : Substances added to food in order to improve or maintain its freshness, flavour, consistency, appearance, or safety is known as food additives. For thousands of years, food additives such as sulphur dioxide (found in wine), sugar (found in marmalade), and salt (found in dried fish or meats like bacon) have been utilised for preservation. **This work aims** to investigate the histopathological alterations in the testes and liver in rats eating food additive combinations (FAM) with fast green, glycine, and sodium nitrate, as well as Aphanizomenon flos-aquae's possible protective effects. **Material and methods** Group I served as control; Group II included rats exposed to food additives; Group III included rats exposed to food additives and treated with Aphanizomenon flos-aquae. Each group contained ten rats and the exposure period was thirty days. **Results**, the histological studies of the testes and liver revealed many alterations. The testes revealed focal degeneration, apoptosis, and necrosis of spermatogonia, spermatocytes and spermatids with the formation of syncytial cells in some affected tubules and complete arrest of spermatogenesis in testes cells. Meanwhile, apoptosis and focal hepatocyte vacuolation were displayed by liver cells. The hepatic sinusoids seemed to be considerably dilated, and vascular endothelia exhibited degenerative and necrotic changes. According to the current findings, Aphanizomenon flos-aquae can shield the liver and testes' histological structures from the damaging effects of food additive mixtures. **Conclusion** The administration of Aphanizomenon flos-aquae with the food additive mixture (FAM) attenuated the significant changes in the testicular and hepatic histological structures that resulted from the FAM treatment.

Keywords: *Aphanizomenon flos-aquae*, Food additives, histopathological and immuno-histochemical changes, FAM

1.Introduction

Chemicals added to food to preserve its freshness or improve its texture, colour, or flavours are known as food additives. They may consist of various preservatives, flavour enhancers like monosodium glutamate (MSG) and food colourings like cochineal or tartrazine. Food additives are frequently employed to improve a food product's flavour, texture, or consistency in order to maintain food quality and facilitate large-scale production **(Mahindru 2004)**. A widely used flavor enhancer in food preparation worldwide is (MSG), a sodium salt of naturally occurring (non-essential) L-form glutamic acid **(Obaseiki *et al,* 2003).** When large amounts of MSG

are consumed, brain conditions such as Parkinson's and Alzheimer's manifest **(Arruda *et al,* 2003).** Excessive glutamate intake causes oxidative damage, neuroendocrine problems, and neuronal degeneration in several organs **(Moreno et *al.,* 2005).**

Although there are many benefits to utilize food additives, growth retardation, allergies, indigestion, anemia, neurotoxicity, issues with kidneys, liver, and spleen, as well as malignant disorders, can all result from long-term usage of items containing food additives **(Elbanna *et al*., 2017; Amin & Al-Shehri, 2018)**. Intestinal irritation, immune system problems, oxidative stress, and hypothalamic-pituitary-testis axis suppression, which results in reproductive problems, are other potential side effects **(Soltan & Shehata, 2012; Alhamadawi & Alsudani, 2020).**

Sodium nitrate is a food preservative that is particularly useful in processed meat, fish, and poultry products because it fixes color **(Cockburn *et al*., 2014)** .Its antibacterial qualities prevent the growth of microorganisms that pose a hazard to human health by releasing neurotoxins from food **(Marianski *et al*., 2009; Ziarati & Arbabi-Bidgoli, 2014).** However, some of the sodium nitrate in food is changed into nitrite after consumption, and in the stomach's acidic environment, nitrite mixes with amines and amides to generate nitroso compounds, which raise intracellular reactive oxygen species (ROS) **(Erkekoglu & Baydar, 2010).** There have been reports of MSG's harmful effects on kidneys and liver **(Nwaopara *et al.*, 2008 a).** Testis, liver, lung, kidney, and spleen all contain glutamate receptors. Most frequently, sodium metabisulfite (SMB) is employed as a preservative in food items like biscuits, chocolate, jam, sausage and salami; in several alcoholic drinks like champagne, wine and beer; and in medications like parenteral amino acid solutions **(Rencuzogˇullari *et al.,* 2001a).**

A synthetic organic food dye called "fast green" is used to color a wide range of food items, including candy, drinks, ice cream, dairy products, and baked goods. Through the production of hydroperoxide isomers, consuming such compounds may have carcinogenic and hepatic nephron consequences. Furthermore, it could irritate the digestive system, respiratory system, eyes, and skin **(Ashour & Abdelaziz 2009 ; Helal *et al*., 2017).** It might also cause youngsters to have attention deficit disorder and hyperactivity disorder **(Lau *et al*., 2006; Tripathi *et al*., 2007).**

Furthermore, A. flos-aquae has strong probiotic substances that improve human health **(Wu *et al*., 2012).** Numerous investigations recorded A. flos-aquae's anti-inflammatory, hypolipidemic, radioprotective, and antioxidant properties **(Yang *et al*., 2011; Venkatesan *et al*., 2012; Eid *et al*., 2016).**

2- Materials and Methods

2.1 Substances

The German Egyptian Pharmaceutical Company provided *Aphanizomenon flos-aquae* (A. flos-aquae-Klamath 350 mg/each capsule, STEM Technology Health Sciences, San Clemente, CA, USA). Fast green, glycine, and sodium nitrate were purchased as food additives from Sigma-Aldrich Co. in the United States. Centronic Chemicals Co., Germany, offered the enzymatic colorimetric kits. The supplier of ELISA kits was Glory Science Co. (Ltd. Del Rio-TX-USA)

2.2 Animals

Thirty male albino rats, weighing between 150 and 180 g, were acquired from Al-Azhar University's Animal Unit, Cairo, Egypt.

2.3 Experimental Design

After the adaptation week, rats were divided into three groups (each with ten rats) and given standard chew and drinking water ad libitum. Group 1 (Control) received phosphate-buffered saline orally. Group 2 received food additive mixtures (FAM) (10 mg/kg sodium nitrate, 12.5 mg/kg fast green, and 12.5 mg/kg glycine). Group 3 received food additive mixtures (FAM) + A. flos-aquae (94.5 mg/kg) **(Abu-Amara *et al*., 2016).** The duration of the experiment lasted for 30 days.

2.4 Histopathological and Histochemical studies

At the completion of the trial, all rats were slaughtered, and tiny samples of their testicles and liver were selected for histological and histochemical analyses. Tissue specimens were fixed in 10% buffered formalin for 48 hours, and processed according to the method of **Suvarna et al., (2013).**

Total protein was detected by using the Mercury Bromophenol blue method (**Pears, 1972) .** However, collagen fibres and total carbohydrates content were detected by Mallory trichrome stain **(Pears,1977)** and PAS **(Drury &Willingten,1980)** respectively. Stained sections were examined for any pathological changes in the examined tissues.

2.5 **Quantitative histochemical analysis**

The optical density of histochemically stained sections in liver and testes for carbohydrates and total protein of the experimental groups was recorded using IPWIN 32 image analysis software

2.6 Immunohistochemical study

2.6.1: Immunoreactivity of BCl_2

For immunohistochemical analyses, 5 µ thick sections were used for Bcl-2 [1:100]) immunoreactivity. In brief, deparaffinization was accomplished in Xylene for 1 h. Rehydration was done in descending alcohol series for 2 min each. After immersion in distilled water for 5 min, sections were washed in PBS for 10 min and exposed to microwave radiation at 500 W for 10 min in citrate buffer (10 mM, pH 6.0) for antigen retrieval. Then application of the primary antibody was done in an incubator at 4°C overnight then washed in PBS. The biotinylated secondary antibody was applied, washed with PBS before incubating with the enzyme conjugate and 3,3-diaminobenzidine tetrahydrochloride. Then sections were stained with Mayer's hematoxylin **(Hashish and Kama, 2015).**

.C Morphometric analysis

Image Interpretation the Olympus digital camera (model number: LC20-Japan) was used to digitize the slides. It was mounted on an Olympus microscope (model number: BX-50, Tokyo, Japan) with a 1/2X picture adaptor and a 40X objective. Using Video Test Morphology 5.2 software (Russia) with a dedicated built-in process for immunohistostaining analysis and stain quantification, the resultant images were examined on an Intel® Core I3® based computer. The area percentage of positive expression for Caspase-3 was measured by the system. Three slices of each tissue were imaged at 200 µm intervals. To analyze positive cells, three visions per slice were selected at random and subjected to image analysis software (JID801D). The grayscale average of the affirmative cells was computed automatically **(Hashish and Kamal, 2015).**

.2.7 Statistical analysis

The mean ±SE was used to express the current results. Utilising SPSS application, data were examined. The student T-test was used to identify significant differences between treatment means. The results were shown as mean ± SE, and statistical significance was determined by P<-0.05. Using image pro-program, the optical density of the testes and liver in the current study was analysed visually.

3- Results

3.1 **The histopathological observations**

3.1.1 **Testes**

Group 1 control (rats were orally ingested phosphate-buffered saline)

The testes' sections under examination revealed intact seminiferous tubules lined by normal spermatogonia, spermatocytes, spermatids, and Sertoli cells. Sections also revealed varying amounts of mature spermatozoa within their Lumina. Vascular structures, interstitial tissue, and dying cells were all normal. The dimensions of the dividing cells, which include the secondary spermatocytes, the size of the main spermatocyte, and the total thickness of the tubules' cellular contents, including spermatozoa, are measured (654,340.78 um, respectively). Estimates were also made of the proportion of edematous testicular interstitial tissue, the quantity of epididymal tubules completely or partially filled with mature spermatozoa, and the type of epithelium that lined those tubules.(11%, 98%, and normal epithelium, in that order) (Fig.1).

Group 2 (rats were ingested with food additive mixtures (FAM)

On other hand, sections from the **testis of treated group** revealed focal degeneration, apoptosis, and necrosis of spermatogonia, spermatocytes, and spermatids with the formation of syncytial cells in some affected tubules and complete arrest of spermatogenesis. About 25-30% of the tubules appeared affected, the remaining were

normal. The interstitial tissue appeared mildly thickened by edematous fluid. The vascular structures and Leydig cells were free from apparent pathologic changes compared to control group (Fig.2).

Group 3 (rats were ingested with food additive mixtures (FAM) + *A. flos-aquae*)

Serial sections from the **testis** showed normal parenchymal seminiferous tubules while keeping normal features of germinal cells, spermatocytes, spermatids, and normal spermiogenesis. The interstitial vascular, stromal and Leydig cells were in a proper morphological appearance (Fig.3).

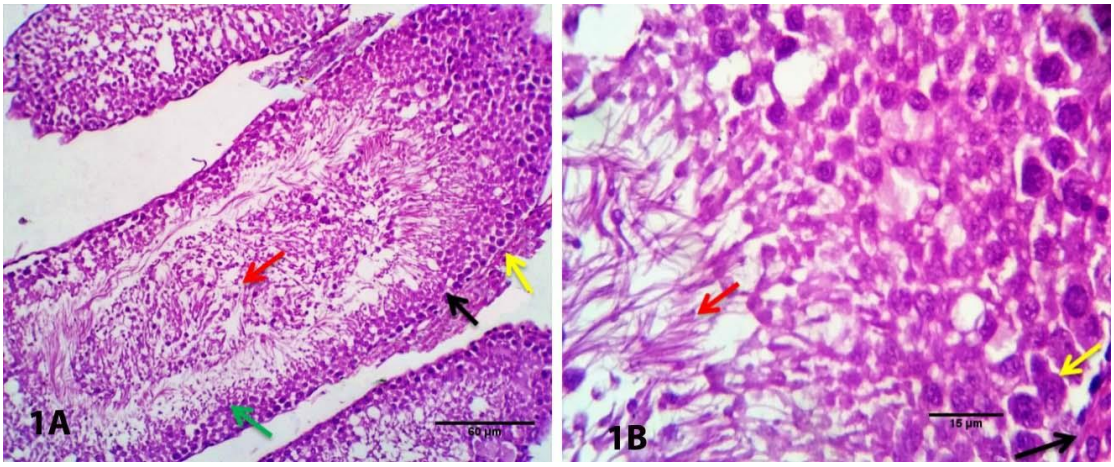


Fig.1 (A&B). photomicrograph of normal rat's testis (control rat) showing normal seminiferous tubules with preserved spermatogonia(black arrows),Leydig cells (A, yellow arrow) spermatogenesis (primary and secondary spermatocytes((A, green arrow ,B , yellow arrow) and spermgenesis (sperm formation) (Red arrows) (HX&E X400). Scale bars 60, 15 um

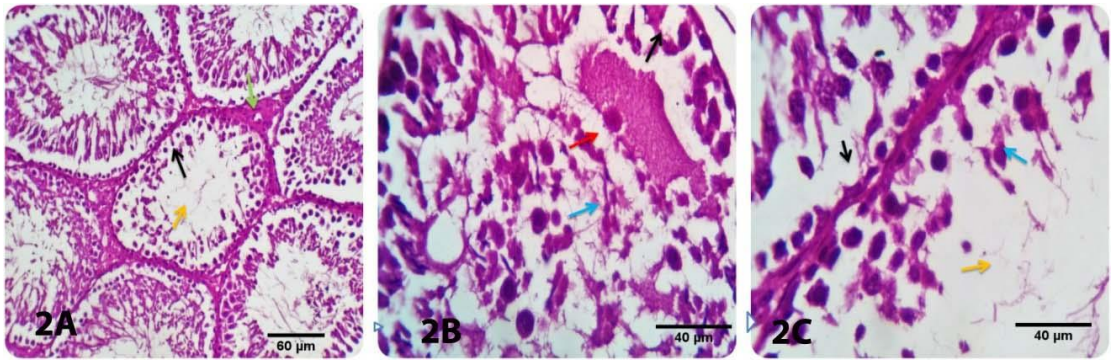


Fig.2 (A,B&C) photomicrograph of rat's testis (T-group) showing focal degeneration, apoptosis and necrosis of spermatogonia , spermatocytes and spermatids (black and blue arrows) with the formation of syncytial cells in some affected tubules (red arrow)and complete arrest of spermatogenesis(yellow arrows).The interstitial tissue appeared mildly thickened by edematous fluid (star) . The vascular structures and Leydig cells are free from apparent pathologic changes (green arrow). Scale bars ,60,40,40 um.

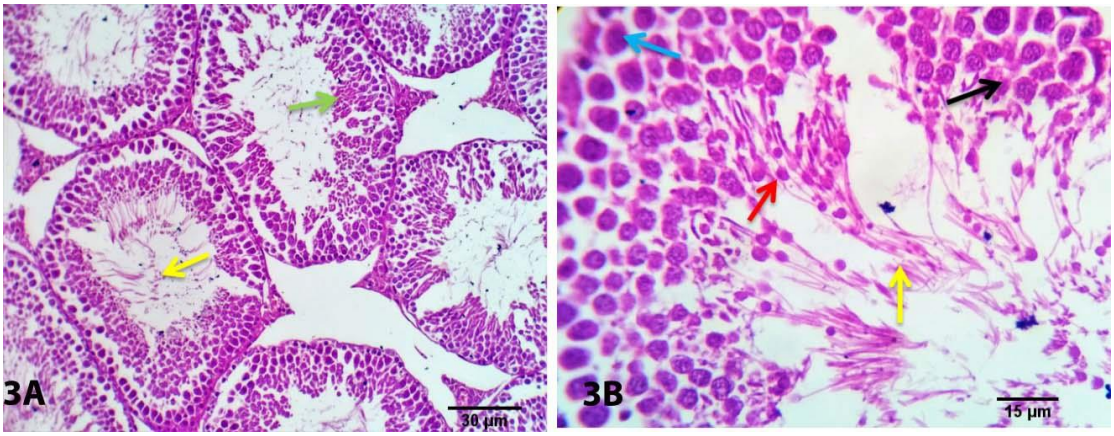


Fig.3(A&B). photomicrograph of rat's testis after exposure to **(mixtures(FAM) + *A. flos-aquae*) treatment** showing apparently normal parenchymal seminiferous tubules with a keeping normal features of germinal cells (yellow star), spermatocytes , spermatids and normal spermiogenesis (black , red , green and yellow arrows) .The interstitial vascular , stromal, and Leydig cells (red star)are in a proper morphological appearance. Scale bars 30, 20 um.

3.1.2 Liver

1- Control group (rats were orally ingested phosphate-buffered saline.)

Serial **liver s**ections revealed normal parenchyma with the preserved lobular pattern, portal area (hepatic arteriole, portal venule, bile ductulus and lymphatics), sinusoids (irregular blood spaces lined by endothelial and Von-Kuffer cells), hepatic cords arrangement (double layer Polygonal hepatocytes separated by bile canaliculi) and stroma (fine fibrous and reticular strands support the hepatic parenchyma) (Fig.4).

2- Group 2 (rats were ingested with food additives mixtures (FAM)

On contrast, examined **liver sections treated group** represented focal hepatocytes vacuolation and apoptosis. The vascular endothelium showed degenerative and necrotic changes and the hepatic sinusoids appeared moderately dilated as compared to the control group (Fig.5).

Group 3 (rats were ingested with food additives mixtures (FAM) + *A. flos-aquae*)

Liver sections pointed out normal parenchymal and stromal structures with preserved features of hepatic lobules, cords arrangement, central veins, portal triads structures, sinusoids and Von-Kuffer cells (Fig.6) .

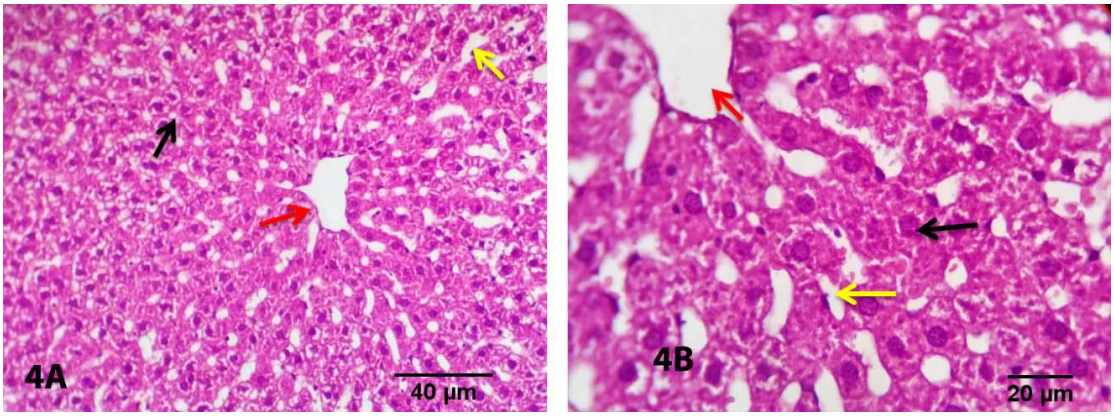


Fig.4 (A&B) photomicrograph of normal rat's liver (control group)showing hepatic parenchyma with preserved central veins (red arrows) , hepatic cords (black arrows) , hepatic sinusoids with Von- Kupffer cells(yellow arrows) . Scale bars 40,20 um.

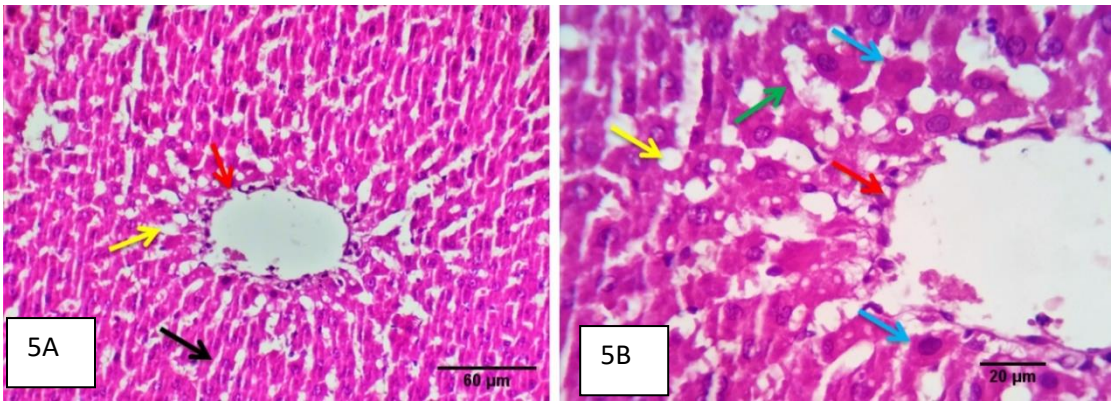


Fig.5 (A&B) photomicrograph of rat's liver (exposed- group) showing focal hepatocytes vacuolation and apoptosis (yellow and blue arrows).The vascular endothelium appears degenerative and necrotic (red arrow), the hepatic sinusoids appears moderately dilated (black and green arrows). Scale bars 40,20 um.

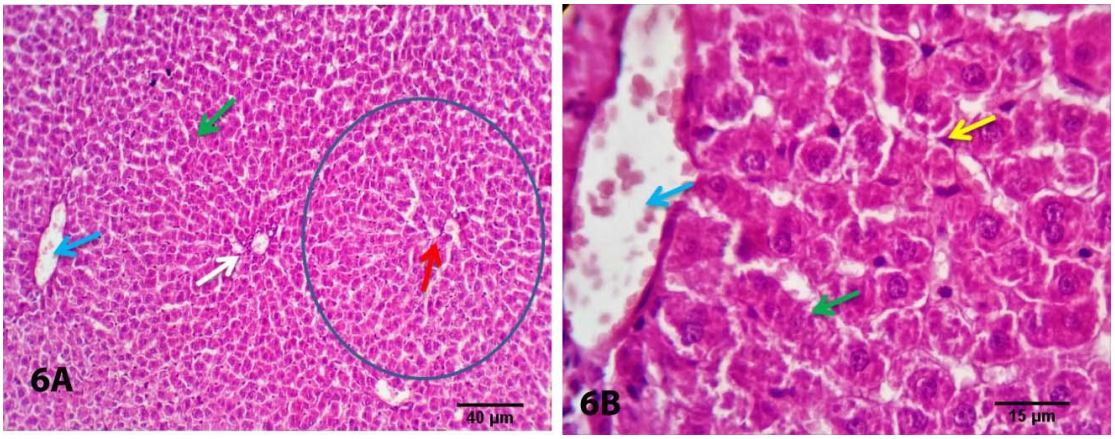


Fig.6 (A&B).photomicrograph of rat's liver **(mixtures(FAM) + *A. flos-aquae*) treatment** showing normal parenchymal and stromal structures with preserved features of hepatic lobules (circle), cords arrangement (green arrows), central veins (white and blue arrows), portal triads structures, sinusoids and Von- Kupffer cells (yellow arrow). Scale bars 40,15 um...

The Mallory staining method makes use of three different dyes. This method has the advantage of visualizing a larger number of tissue structures. Mallory stain combines **aniline blue**, which stains connective tissue, extracellular matrix, glycoproteins, and mucus, **orange G**, which stains proteins, and the dark red **fuchsin**, which stains RNA and DNA

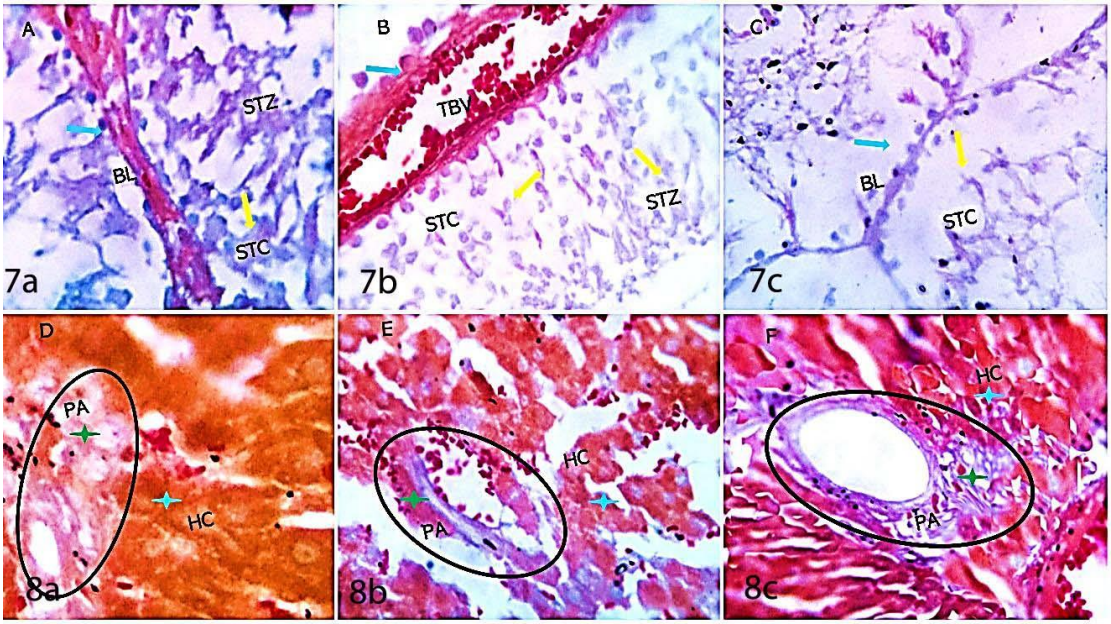
Stained sections of tests showed a moderately positive staining reaction in the basal lamina of seminiferous tubules of the control-free group (fig. 7a). The Spermatocytes and spermatozoa appeared mildly to moderately react.

Negative staining reactivity is demonstrated in all structures of treated rats(fig.7b) .

Moderately positive staining reaction was seen in the basal lamina of seminiferous tubules and in a wall of testicular blood vessel (TBV) after exposure to mixtures (FAM) + *A. flos-aquae*) treatment rats.

The Spermatocytes and spermatozoa appeared mildly to moderately reacted (Fig.7c). The liver section showed a mild staining reaction in the portal area collagen contents and moderate reaction to the protein contents of the hepatocellular cytoplasmic contents in control-free rats (fig. 8a). A comparatively prominent collagen deposition in the portal area with moderate staining reaction was seen in the liver of treated rats. In this concern, we have to point out that as the liver cells of treated rats were comparatively atrophied, they falsely assumed a more intense staining reaction (fig.8b). mild staining reaction in the portal area collagen

contents and moderate reaction to the protein contents of the hepatocellular cytoplasmic contents were seen in the liver ***after exposure to (mixtures(FAM) + A. flos-aquae) treatment***.(Fig.8c)



Figs.7, 8. Photomicrographs of Mallory trichrome stained sections of testes and liver

Showing Fig. 7a moderately positive staining reaction in the basal lamina of seminiferous tubules (BL light blue arrows) of control control-free group.

Fig. 7b Negative staining reactivity is demonstrated in all structures of treated rats. The basal lamina of seminiferous tubules

In Fig. 7c, the wall of a testicular blood vessel (TBV) in testes after exposure to mixtures *(FAM) + A. flos-aquae treatment*. Spermatocytes and spermatozoa appear mildly to moderately be reacted. (yellow arrows).

Fig. 8a, Liver sections showing mild staining reaction in the portal area collagen contents (PA, green stars, black circles) moderate reaction to the protein contents of the hepatocellular cytoplasmic(HC, light blue stars) in control free group

In Fig. 8b, a comparatively prominent collagen deposition in the portal area (PA, green star, and black circle) with moderate staining reaction is seen in the liver of treated rats. Regarding the hepatocellular protein contents, as the liver cells of treated rats are comparatively atrophied, they falsely assumed a more intense staining reaction.

In Fig. 8c, Mild staining reaction in the portal area collagen contents (PA, green stars, black circles) and moderate reaction to the protein contents of the hepatocellular cytoplasmic(HC, light blue stars) are seen in. the liver ***after exposure to (mixtures(FAM) + A. flos-aquae) treatment*** **(Mallory stain) . Scale bars 50 um**

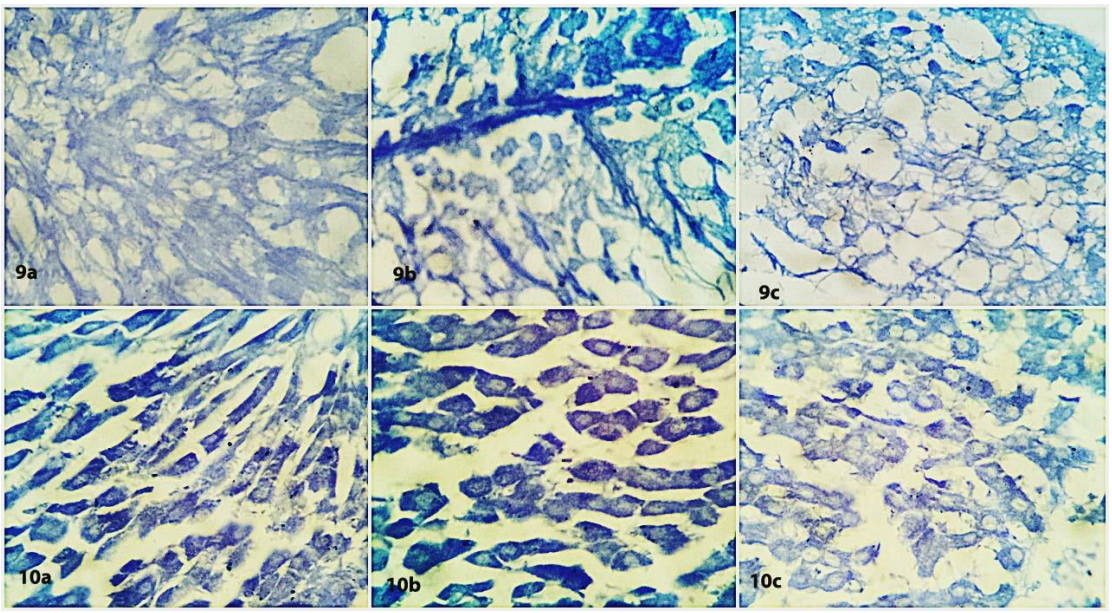
3.2 Histochemical Findings

1-Total protein (Bromophenol blue)

Testicular and hepatic protein distribution demonstrating normal cellular protein contents in the form of irregular particles of various sizes distributed equally in the cytoplasm of control-free rats 0.26±0.022 & 0.749±0.083 (Figs.9a&10a).

Sections from the testis and liver of treated rats showed a diminution in the cellular cytoplasmic protein content 0.202± 0.040 & 0.661±0.094 (Figs. 9b&810b).

In testicular and hepatic cellular nucleoli are intensely stained while the ground cytoplasm and the nucleoplasm display faint stainability 0.427± 0.052 & 0.391±0.057 after exposure to **(mixtures(FAM) + *A. flos-aquae*) treatment** (Figs.9c&10c).



Figs.9&10. Photomicrographs of bromophenol stained sections of testes and liver Figs (9a&10a.), Testicular and hepatic protein distribution demonstrating normal cellular protein contents in the form of irregular particles of various sizes distributing equally in the cytoplasm of control free rats.

Figs (9b&10b) Sections from the testis and liver of treated rats showing diminution in the cellular cytoplasmic protein content.

Figs (9c&10c), **after exposure to {mixtures(FAM) + *A. flos-aquae*) treatment** of testes (STG, STC, red arrows) and liver cells (HC, red stars) showing normal cellular protein contents in the form of irregular particles of various sizes distributing equally in the cytoplasm. The nucleoli are intensely stained while the ground cytoplasm and nucleoplasm display faint stain ability. **Bromophenol stain. Scale bars 50 um**

2- Total carbohydrate content (Periodic Acid Schiff).

PAS-stained sections of testes and liver showed moderate PAS-positive reaction in the thin regular basal lamina surrounding the seminiferous tubules, the spermatogenic cells, spermatocytes, and spermatozoa of the control free rats 0.620±0.022 (fig. 11a).

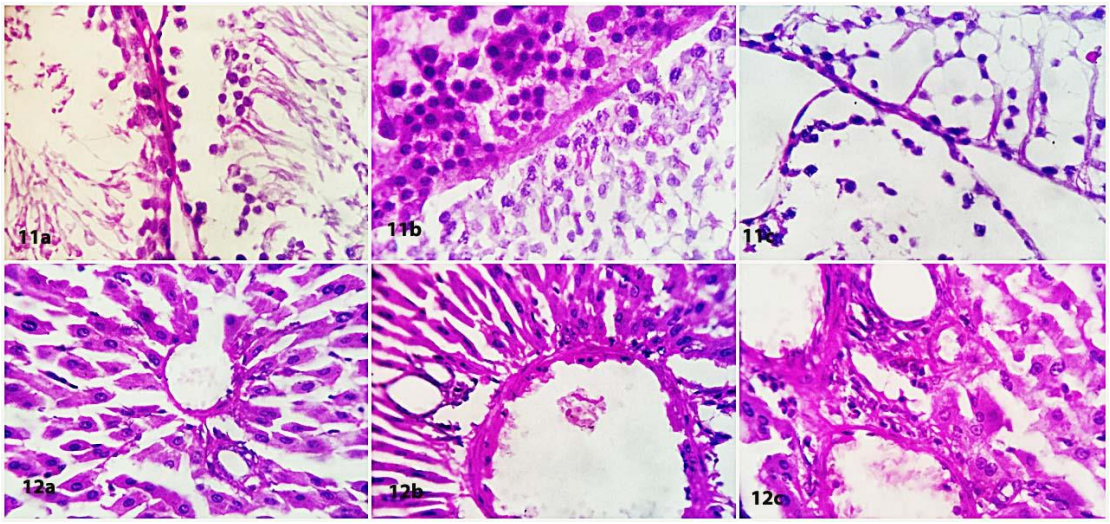
Moderate staining reaction was seen in the hepatocellular cytoplasmic contents and the portal area structures 0.165±0.0103 (fig. 12a).

The treated group showed an overall reduction in the PAS reaction in the basal lamina, the germinal epithelium, spermatocytes, spermatozoa, and in the cytoplasmic contents of hepatocytes and portal area structures 0.180± 0.013 (fig. 11b).

Also in treated liver showing a reduction in the PAS reaction in the cytoplasmic contents of hepatocytes and portal area structures 0.0127± 0.007 (fig. 12b.)

Moderate PAS-positive reaction was seen in the thin regular basal lamina surrounding the seminiferous tubules, the spermatogenic cells, spermatocytes, and spermatozoa of testes after exposure to **{mixtures of (FAM) + *A. flos-aquae*) treatment** with prominent cytoplasmic contents of Leydig cells 0.350± 0.029 (fig. 11c).

Moreover, a moderate staining reaction was seen in the hepatocellular cytoplasmic contents and the portal area structures 0.148±0.024 (Fig. 12c).



Figs. 11, 12. .Photomicrographs of periodic acid Schiff (PAS) stained sections of testes and liver

Fig.11a) in testes control group, showing moderate PAS-positive reaction in the thin regular basal lamina surrounding the seminiferous tubules (BL, black arrows), the spermatogenic cells (STG) , spermatocytes (STC) and spermatozoa(STZ) (yellow arrows)

Fig.12a in liver control rats, Moderate positive staining reaction is seen in the cytoplasmic contents of hepatocytes (HC, red stars) and in the portal area structures.

Fig. 11b in testes-treated groups showing an overall reduction in the PAS reaction in the basal lamina (BL, black arrow), the germinal epithelium, spermatocytes, spermatozoa (yellow arrow) and in **(fig. 12b)** the cytoplasmic contents of hepatocytes (HC, red stars) and portal area structures (PA, green stars).

Fig.11c in *rat* ***(FAM + A. flos-aquae),*** moderate PAS-positive reaction in the thin regular basal lamina surrounding the seminiferous tubules (BL, black arrows), the spermatogenic cells (STG), spermatocytes (STC) and spermatozoa (STZ) (yellow arrows) with prominent cytoplasmic contents of Leydig (LCD, green arrow)

Fig. 12c in *rat* ***after exposure to (mixtures of (FAM) + A. flos-aquae) treatment,*** moderate positive staining reaction is seen in the cytoplasmic contents of hepatocytes(HC, red stars) and in the portal area structures (PA, green stars)) **(PAS) . Scale bars 50 um.**

Table 1 showing the optical density of total proteins and polysaccharides (PAS) in the testes of rat between control, treated and mixtres of *(FAM + A. flos-aquae)* treatment

Parameter	**Control**	**Treated (FAM)**	**FAM + A. flos-aquae) treatment**
Protein	0.264 ±0.02298	0.228 ±0.04*	0.442 ±0.05*
PAS	0.592 ±0.093815	0.176 ±0.01*	0.352 ±0.03*

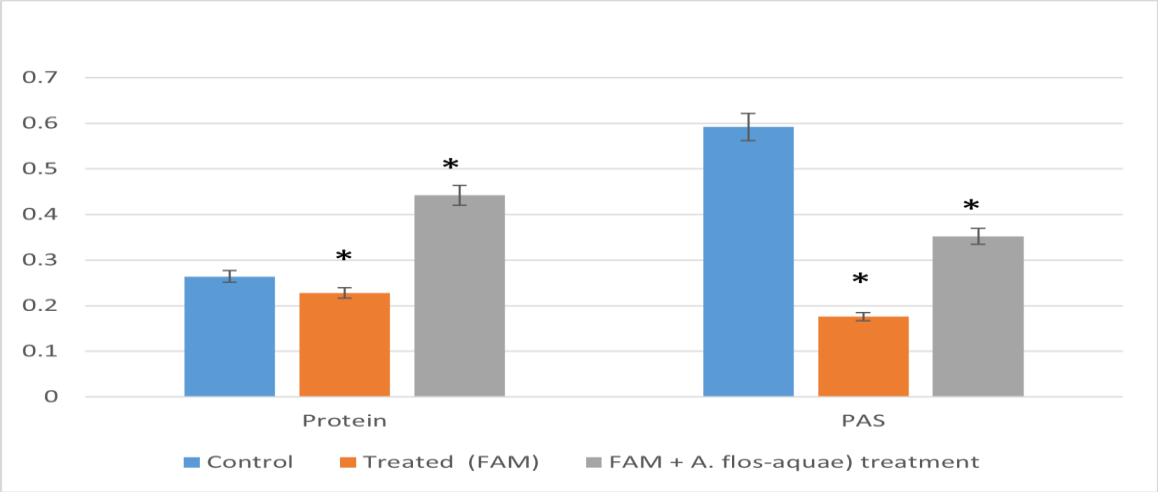
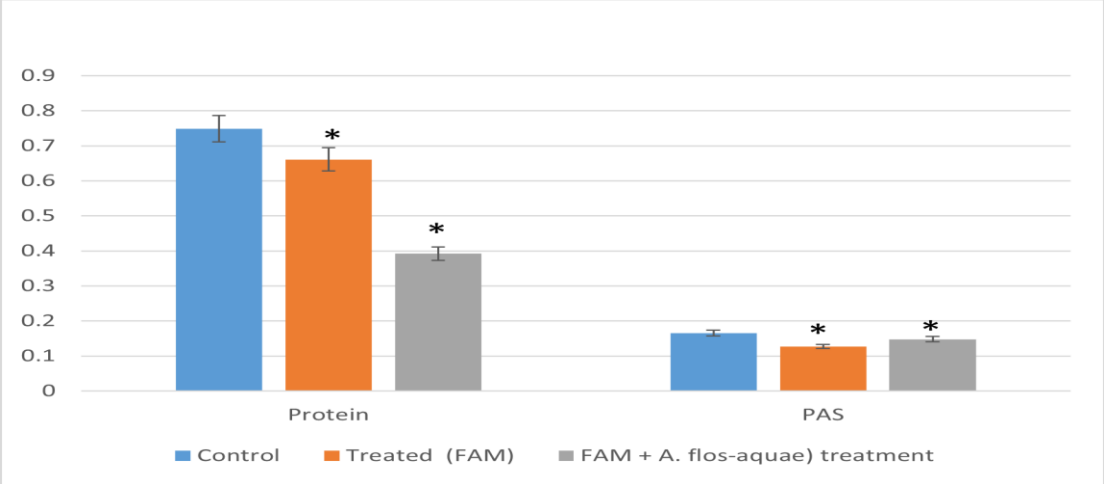


Table 2 *&* Histogram 2 show the optical density of total proteins and polysaccharides (PAS) in the liver of rats between control, treated, and mixtures of *(FAM + A. flos-aquae)* treatment

Parameter	**Control**	**Treated (FAM)**	**FAM + A. flos-aquae) treatment**
Protein	0.749 ±0.08	0.661 ±0.09*	0.392 ±0.06*
PAS	0.165±0.01	0.127±0.007*	0.148 ±**0.02***



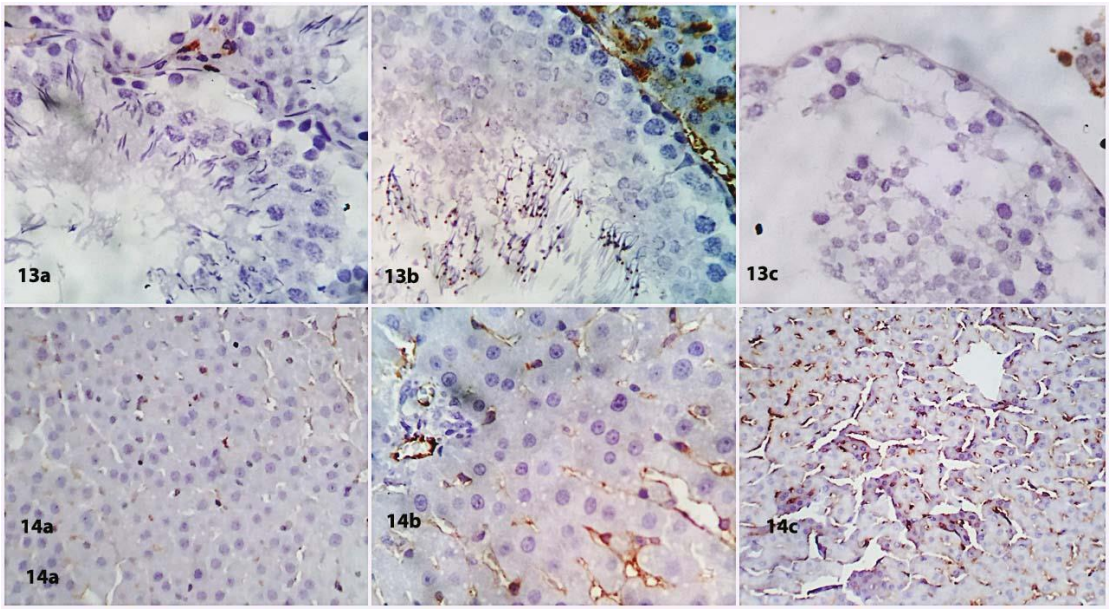
3.3 Immuno-histochemical findings

Examined sections from control negative group showed a strong immune reactivity of BCL2 in Leydig cells (LG, red arrows) and weak immune reactivity in spermatozoa, spermatids spermatocytes and spermatogonia (STG, yellow arrow) (fig.13a). Treated rats showed moderate immunoreactivity in Leydig cells and minimal immune reactivity of BCL2 in a few spermatogonia and spermatocytes (fig. 13b).

in *rats after exposure to a mixture of* ***(FAM+A. flos-aquae)*** treatment group, showed strong immune reactivity of BCL2 in Leydig cells and spermatids and moderate immune reactivity in primary spermatocytes and spermatogonia (fig. 13c).

Liver sections of the corresponding groups showed negative immune reactivity in the hepatocytes of the three experimental groups. Minimal reactive change was seen in the sinusoidal cells of control-free rats (fig 14a). Moderate reactivity to BCL2 is seen in the sinusoidal endothelium of treated rats (fig. 14b). A strong reactivity to BCL2 is seen in the biliary epithelium of the liver after exposure to Afros-*aquae* +***FAM*** treatment (fig. 14c).

Morphometric analysis revealed that the estimated average percentages of positively reacted cells in the testes and liver of different experimental groups were 13.2, 1.3, 43.6 for testicular tissue of control-free, treated, and a mixtures of *Afros-aquae+* ***FAM*** treatment groups respectively, and were 4.88, 26.93, 27.55 for the corresponding liver groups respectively. Table 3 demonstrates the percentages of immunoreactive cells in different experimental groups. (13 a,b and c)



Figs.13, 14. Photomicrographs of immunohistochemical stained sections of testes and liver
(fig. 13a), the control group of testes showing strong immune reactivity of BCL2 in Leydig cells (LG, red arrows) and spermatids (STD, light blue arrows) and weak immune reactivity in spermatocytes (STC ,orange arrows).and spermatogonia (STG ,yellow arrow).
(fig. 13b) ,treated group showing moderate immunoreactivity in Leydig cells(LG, red arrows) and minimal immune reactivity of BCL2 in a few spermatogonia (STG, yellow arrow).and spermatocytes (STC, orange arrow) .
(fig. 13c) , in a mixtures of (*Afros-aquae+* ***FAM***) treatment group showing strong immune reactivity of BCL2 in Leydig cells (LG, red arrows) and spermatids (STD, light blue arrows) and moderate immune reactivity in primary spermatocytes (STC ,orange arrows).and spermatogonia (STG ,yellow arrow),.
(Fig. 14a) , Liver sections shows negative immune reactivity in hepatocytes of the three experimental groups (HC , red stars) Minimal reactive change is seen in the sinusoidal cells (SC, green star)of control free rats
(Fig. 14b) , Moderate reactivity to BCL2 is seen in the sinusoidal endothelium of treated rats(SC, green stars).. and in **(Fig 14c)** , A strong reactivity to BCL2 is seen in the biliary epithelium of *liver* (*a mixtures of(Afros-aquae+* ***FAM*** *) treatment group* (yellow star).
(Immunoperoxidase technique for bcl-2 X200,400). Scale bars 50 um

Table (3) demonstrate the percentages of immunoreactive cells in different experimental groups

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BCL2	Control	Treated	Mitures *Afros-aquae+ FAM treatment*
Testes	13.20333	1.23	43.66
Liver	4.88	26.93	27.55

3.4 Discussion

In the present study, rats treated with a mixture of food additives revealed many alterations in the testes represented in focal degeneration, apoptosis, and necrosis of spermatogonia, spermatocytes, and spermatids with the formation of syncytial cells in some affected tubules and complete arrest of spermatogenesis. These results agreed with (**Al-sharkawy *et al.*, 2017)**. However, rat livers treated with a mixture of food additives showed many alterations, focal hepatocyte vacuolation, and degenerative necrotic changes with moderately dilated hepatic sinusoids that agreed with **Al-sharkawy (2017), Al-Mosaibih[(2013) and Reddy *et al.*, (2015).**

Shrestha *et al.*, (2018) reported that rat liver treated with monosodium glutamate showed ruptured endothelial lining of the central vein with vacuolation of hepatocytes and decreased size of hepatocytes and nuclei. However, **Ewaka *et al. (2011)* and Hamad (2022)** stated that the treatment of liver rats with monoglutamate leads to the dilation of the central vein, hemorrhage, and necrosis.

In the current study, rats' livers were ingested with food additives mixtures and A.Flos-aquae showed normal parenchymal and stromal structures with preserved features of hepatic lobules, cords arrangement vein, portal trial structure, sinusoids and Von-Kupffer cells. These findings agreed with **Abdelhafez and Kandeal (2018) , who** reported that, treatment of rats with AFA showed a well-developed central vein with highly increased kupffer cells and a normal appearance of liver tissues.

In the present study, testes rats ingested with FAM in the second group showed many alterations, such as focal degeneration, apoptosis, and necrosis of spermatogonia, spermatocytes, and spermatids with the formation of syncytial cells in some affected tubules and complete arrest of spermatogenesis. The interstitial tissue appeared mildly thickened by edematous fluid. The vascular structures and Leydig cells were free from apparent pathologic changes. These findings agree with **Sakr &Badawy (2013) and Alalwani (2013)** who reported that, rat testes treated with MSG showed, the interstitial tissue appeared with different vacuoles, blood haemorrhage and Leydig cells had pyknotic nuclei. The seminiferous tubules showed deformed germ cells as well as Sertoli cells being detached from the irregular basal lamina. Many seminiferous tubules were severely damaged and had few Sertoli cells and spermatogonia with pyknotic nuclei. Spermatocytes and early spermatids were lost from most of the tubules. Animals treated with MSG and curcumin showed an improvement of seminiferous tubules and an increase in the number of germ cells.

In the current study, rat testes were ingested with food additives FAM+Afloes-aquae and showed normal seminiferous tubules, normal features of germinal cells, spermatocytes, spermatid, and normal Leyding cells. This finding agreed with **Sakr &Badawy (2013)** who reported that, the treatment of curcumin on rat testes after exposure to SMG, showed an improvement of seminiferous tubules and an increase in the number of the germ cells

In our study, a comparatively prominent collagen deposition in the portal area with moderate staining reaction is seen in the liver of treated rats

Waer *et al.*, (2006) found a moderate increase in the connective tissue around the portal area after giving a daily dose of Monosodium Glutamate (60mg/ 1000g) for one month Al- **Mosaibih (2013)** observed blood vessel fibrosis**. Nakanishi *et al.,* (2008)** also reported that , there was mild to moderate necrosis of the hepatocytes in the periportal areas and mild fibroplasia

The treated group showed an overall reduction in the PAS reaction in the basal lamina, the germinal epithelium, spermatocytes, spermatozoa, and in the cytoplasmic contents of hepatocytes and portal area structures

Bhattacharya *et al.,* (2011), reported similar findings**. Inuwa *et al*., (2011)** reported that there is a marked reduction in the carbohydrate content of tissue after the treatment with Monosodium Glutamate.

Conclusion: The administration of *Aphanizomenon flos-aquae* with the food additive mixture (FAM) attenuated the significant changes in the testicular and hepatic histological structures that resulted from the FAM treatment.

Ethics approval and consent to participate

This study was completed on albino rats and carried out according to Ain shams university ethical Research guidelines with Code Number in Experimental Animal Research Unit is [RE (345) 24].

Consent for publication

Not applicable

Availability of data and materials

Each author certifies that this paper contains the only source of data for the finding reported in our research

Competing interests

The authors declare that they have no conflict of interests.

Funding

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Authors contributions

HA carried out the data interpretation and research design, as well as the manuscript's drafting and revision. AM carried out the measurements, gathered the information, contributed to the research plan, and edited the paper. HA & AM edited the article, assessed the statistical analysis, and commented on slides showing immune histochemical and histological features. The final manuscript was read and approved by all writers

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