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Effect different levels of safflower seed powder to the diets on some histological traits to the diets of common carp *Cyprinus carpio* L.

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Abstract

The study was conducted at special cages in mud pond, Agricultural Research and Experiment Station, Animal Production Department, Agriculture College, Al-Muthanna University, to study the partial replacement of safflower seed powder by levels of 0, 5, 10 and 15%, in the diets, instead barley and yellow corn, and the effects on the growth and blood parameters of common carp fish. The experiment was designed as four treatments with four replicates for each treatment (five fish/ replicate). The study included the effect of seeds on the histological parameters of carp fish, where the results showed histological examination of the organs of the liver, kidneys and gills, treatments of safflower seed powder fed at 5%, 10%, and 15% improved compared to T1 treatment. We conclude from this study the possibility of adding raw safflower seed powder by 10% to the diets of common carp fish *Cyprinus carpio* L.

Keywords: safflower seed, histological traits, common carp *Cyprinus carpio* L.

Introduction

Common carp belongs to the family Cyprindae and is one of the best mined fish in the world (Ahmed, 1987). One of the most common species of freshwater fish of commercial importance in the world, native to Eastern Europe and Central Asia (FAO, 2013). This fish can change its behavior and feeding habits according to the environmental conditions that surround it (Mohammad, 2015).

Carps are also classified as omnivorous, which feed mainly on aquatic insects, crustaceans, ringworms, herbs, aquatic plants and algae (Al-Ani, 2011), distinguishes it also is that the size and quality of meat can be increased according to the feeding provided (Kottelat, 2007).

Sulit (1953) explained that the protein content of common carp is evenly distributed in the abdomen, back and tail area. Fish meat has also been shown to be beneficial for preventing vascular obstruction (Calder) and Yaqoob, (2009). Vladau (2008) explained that its beneficial effect on eyesight and nervous system health. As well as the prevention of cancer and diseases related to the colon, breast and prostate (Connor, 2000).

The study aims to determine the effect of adding different percentages of raw safflower seed powder in the diets of common carp fish and their effect on some tissue structures.

Material and methods

The study was conducted at special cages in mud pond, Agricultural Research and Experiment Station, Animal Production Department, Agriculture College, Al-Muthanna University, from 25/9/2021 to 10/12/2021 (75 days period), 7-day acclimation period include. A total of 500 common carp *Cyprinus carp L.* fish were brought, to select 80 homogeneous fish, an average weight of 85 ± 5 g.

The experiment was conducted by stocking fish using plastic floating cages, dimensions: 90×50, with a length of 95×2.17 m, equipped with a 4-inch pipe for the purpose of a flood in a mud pond with dimensions of 20×15 m. The cages were covered with an iron clip from the top for the purpose of preserving the fish to prevent their escape. The fish were placed in a 3% saline solution, for 3-5 minutes until signs of fatigue appear on them, for eliminating external parasites.

Eighty fish were selected and distributed equally to four treatments, for each treatment 20 fish, with 5 fish per replicate, were feeding 3 times a day; the first meal was served at 7 am, the second meal was served at 11 am, the third meal was served at 3 pm. The percentages were ad-justed every 10 days after weighing the

fish to monitor growth and performance efficiency.

The samples were subjected to histological examination after being prepared and the unimportant plankton were removed and then placed in formalin at a concentration of (10%) for 24 hours. Transferred to Al-Rikabi Specialized Laboratory / Al-Muthanna). According to the modus operandi of what Drury and his group (1967) and Luna (1968) referred to as follows:

1. Fixation: The tissue samples were placed in plastic bottles containing neutral (10%) formalin for the purpose of fixing them completely.

2. Washing & Dehydration: Then wash the samples with running water to get rid of formalin residues. Then the samples were passed with rising concentrations of ethyl alcohol (30, 50, 70, 80, 95, 100, 100%) from one hour to two hours for each A pass for the purpose of drawing water from the samples (dehydration).

3. Clearing: the samples were cleared using the xylol solution, as the samples are passed twice in the xylol for an hour each time.

4. Embedding: The samples were passed in molten wax at a temperature of 58-60 degrees Celsius and in two stages with two hours for each stage, after which the samples were placed in special molds and wax was poured over them and left to solidify.

5. Sectioning: The wax molds containing the samples are cut by a rotary microtom, as the cuts are initially on a thickness of 20 microns until the tissue of the sample appears, and then cut on a thickness of 5 microns, as the tissue begins to appear through the wax strip, which is taken and then placed in a water bath at a temperature of 40-50 ° C in order for the tissue to expand, then spread it on a glass slide, leave to dry in a dry place or put in the oven for 15 minutes at a temperature of 35 degrees Celsius, then the slide is ready for staining.

Slide pigmentation

The process of dyeing depends on the use of hematoxylin and eosin dyes, as mentioned by Luna (1968) and according to the following method:

1. Removing the wax using warm xylol for 5-10 minutes and in two stages so that the tissue is clear of the remnants of wax.

2. Adding water (Hydration) to the tissue by passing the tissue with decreasing concentrations of alcohol (100, 90, 70%) for 5 minutes for each pass.

3. Place the tissue section in hematoxylin stain for 2-3 minutes.

4. Wash the section with tap water for 5-10 minutes.

5. Dipping the section in alcoholic acid (1% HCL in 70% alcohol) for 5-15 seconds.

6. Wash the slide with tap water 3-5 minutes.

7. Putting the slide in Eosin dye (1% Eosin) for one minute.

8. Infusion (water withdrawal): The water was withdrawn by passing the slide with rising concentrations of alcohol (70, 90, 100%) at a rate of two minutes for each pass.

9. The shedding is done through three passes in the xylol for a period of (10, 15, 30) seconds in succession.

10. Putting Distrene plasticizer, Xylene (DPX) on the tissue section to preserve the tissue for a long time, then put the cover slide, and leave it to dry, then examine the slide under the light microscope.

The tissue was read in the laboratory of the College of General Medicine at Al-Muthanna University using a Leica Microscopic

Results and Discussion

Histological findings of the kidney:

Histological results of the kidney in the control treatment:

Histological results in the T1 control treatment of carp fish showed that the permanent, functional and functional kidney is the mesonephros, medium kidney. The excretory functions and regulation of the water-salt balance in fish are carried out by the kidneys and gills, although the gills are an organ that returns to the respiratory system, however, most nitrogenous substances and wastes are excreted and excreted through the gills, and therefore the kidneys play the largest role in regulating the water-salt balance. The kidney of carp fish is a mixed organ consisting of two parts, the hematopietic reticuloendothelial endocrine part. The other part is the Executive Elements, it is located at the site of the retroperitonal reflux against the ventral side of the spine. The kidney is divided into the anterior vertical kidney, which is the largest part and is composed of hematopoietic elements, and the posterior exocrine kidney, the histological structure of the kidney consists of the renal units Nephron or nephrons and renal tubes Renal tubes of the kidneys.

The results of histological sections showed that the renal unit consists of two parts, the first is the renal corpuscle, or called Malpighi's body, which is histologically composed of glomerulus and Bowman's capsule that surrounds it. The glomerulus enters a small afferent venous vessel that is divided into several coiled capillary vessels that form the glomerulus, these

vessels increase by coiling and leave the capsule of the glomerulus in the form of an efferent vessel. The spaces between the capillaries are filled with mesangium cells and podocytes. The glomerulus consists of two layers, an inner and an outer layer of single flat epithelial cells, the second part is the renal tubules and consists of the proximal coiled tubule, which is in two pieces and is lined with cuboidal epithelial cells with fine cilia and villi, the distal coiled tubule lined with columnar epithelial cells containing the Brush Border. Finally, the collecting tubules whose tissue structure does not have a brush edge, contains elongated columnar epithelial cells and connective tissue consisting of blood vessels and layers of smooth muscle, Fig. (1) and (2). These results are in agreement with Al-Hamali italics (2016), who described the phenotypic and histological structure of the esophagus, stomach, intestines, and kidney of Kawally fish (Scombers combus) from the Mediterranean Sea overlooking the city of Misurata in Libya, with Soliman italics (2018), who explained when studying some of the blood and biochemical parameters and the anatomical and histological structure of some vital organs such as the liver, kidneys and gills as indicators of vitality and activity of tuna fish (Euthynnus alletteratus) in the waters of the Mediterranean Sea, which indicated a histological structure similar to what appeared in the current results.

Histological results of the kidney in the experiment parameters:

The histological results of the kidneys in carp fish for the T2, T3 and T4 treatments showed the same histological structure and histological layers of the kidney represented by the two parts of the renal unit, were the renal corpuscle, the glomerulus, Bowman's capsule, which surrounds it, and the numerous coiled renal tubules, it was noted from the tissue sections that the tissue structure of the kidney in treatment T2 was not affected by light and moderate concentrations of Safflower (Carthmus tinctorius), with an increase in the number of coiled capillaries entering the glomerulus, an increase in the number of coils in the renal tubules, and an increase in the number of mesangium cells and podocytes (Fig. 3), as for the high concentrations of Safflower in treatments T3 and T4, some changes were observed on the kidney tissue, such as the appearance of glomerular necrosis, swelling and glomerular congestion with the presence of edema, inflammatory areas, and hypertrophy areas in the glomerulus and kidney tissue, as for the renal tubules, it is noted that they have some ruptures in the epithelial lining of the tubules, irregular proliferation of the

proximal and distal coiled tubules, a decrease in the presence of micro villi with hemorrhages and congestion of blood vessels in the kidney parenchyma and smooth muscle layers. Figure (4). These results agree with Araujo et al., (2019), who explained in his study the effect of using different concentrations of safflower seed powder in feeding catfish (Pangasius bocourti) and indicated that there were no significant and apparent differences on the composition of the kidney tissue in the low concentrations of the plant, while it appeared.

Second: Histological findings of the liver:

Histological findings of the liver in the control treatment:

Histomorphological results in the T1 control treatment of carp showed that the liver is located in the apical region of the abdominal cavity in front of the dorsal surface of the stomach and intestines, so that it appears reddish-brown in color, divided into three lobes of different sizes, the ends of which are directed towards the back of the fish, and the right lobe forms the largest lobe compared to the left and middle lobes. The histological sections of the current study showed the parenchyma tissue composed of hepatocytes, which were large in size and polygonal in shape, with homogeneous cytoplasm, basal pigment, and centrally located dark nuclei. The liver tissue is surrounded by a capsule of connective tissue lined with squamous epithelial cells. It does not send sacs separating the hepatic lobules, and there is no portal area or triads, as in the liver of other mammals (Fig. 5).

The cross-sections of the fish liver tissue also contain a number of separated blood sinuses, by the spread of many hepatic cords that are arranged radially around the central vein and are in the form of plates with the thickness of one or two cells. The histological sections of the liver tissue also showed that the hepatic vein surrounds it and is permeated by two types of pancreatic tissue, internal and external in the form of secretory vines for endocrine glands surrounded by connective tissue and surrounded by assemblies of the centrioles of pigmented macrophages, it spreads outside the hepatic vascular supply area and the bile ducts in the liver (Fig. 6) and (Fig. 7). These results are in agreement with Maria et al., (2018) who described the normal structure of liver tissue in fish (Odontesthes bonariensis in the control treatment, and with Ahmed et al. (2013), who explained the histological structure of liver tissue in fish (Oreochromis mossambicus) similar to what was described in our current results. , and with Avigliano italics (2015) who indicated the histological structure of the normal liver in fish Tilapia nilotica, Oreochromis niloticus

and similar to what is described in the current results.

Histological results of the liver in the experimental parameters:

The histological results of the liver in carp fish for the second treatment T2 showed the spread of hepatocytes in the liver tissue with clear central nuclei and the spread of the two types of pancreatic tissue, vessels, blood sinusoids and connective tissue areas surrounding the liver tissue. It also notes an increase in the density of cells and nuclei in hepatocytes, hyperplasia, an abundance of fat droplets and bile materials, and cellular degeneration in the internal pancreatic tissue, which indicates the activity of the liver tissue due to the average concentrations of the safflower plant, which stimulates the liver tissue to increase activity and the discharge of fatty substances through the bile ducts and the storage of carbohydrates in an optimal manner (Fig. 8) and (Fig. 9).

As for the treatments of the fourth experiment, T3 and T4, with the highest concentrations of safflower, it was histological sections showed that the liver tissue was affected by the active and active substances of the plant, being one of the tissues with a high blood supply, represented by a large number of blood sinusoids, spread within the liver cells and its impact on any high concentration may be positive for the body, however, it shows some negative effects on the tissue, which are represented by an expansion of the blood sinusoids, congestion of blood vessels, and a slight cellular degeneration of the liver tissue, swelling of the cells, slight disorientation in the hepatocyte membranes, with Shrinkage and necrosis in some hepatocytes, exocrine pancreatic hypertrophy with slight infiltration of inflammatory cells (Fig. 10) and (Fig. 11).

Third: Histological findings of the gills:

Histological findings of the gills in the control treatment:

Histological results in the control treatment showed that the gills in fish contain the gill epithelium, which is thin with a large surface area for the purpose of providing a high level of exposure between the capillaries and water, allowing effective gas exchange to absorb O2 and release CO2. Histological sections also showed that the gill arch is a hyaline cartilage structure surrounded by a mixed bone that carries in its front and inner side two rows of gill rakers, and in its posterior and outer side are two rows of Gill filaments. Primary lamellas radiate from the gill arch a number of paired rows of primary lamellas or filament pairs, which increases and expands and spreads in each of them to contain another series of secondary lamellas that are central to the

primary lamellas. The gill arch is covered with typical epidermal tissue, which at the origin of the primary plates are thicker, containing a large number of mucous cells and rows of lymphoid tissue. The primary plates are covered with a mucous epidermal tissue that may contain chloride salt-secreting cells, which are abundant in the basal part of the plates, and their function is ion transport and detoxification.

Secondary lamellae appear in the tissue sections in which gaseous exchange occurs through the countercurrent exchange of blood flowing in the opposite direction to the external water, the surface of the secondary lamina consists of a single layer of overlapping squamous epithelial cells supported and separated by pilar cells with a defensive function, when these cells are in contact with the basement membrane, they spread to form lips and combine with neighboring Beller cells to completely line the blood channels, Beller cells may contain contractile proteins, and platelets enter the blood directly from the abdominal aorta with high pressure and the presence of contraction factors work to resist swelling under normal conditions and water currents. The surface of the lamellar epithelium contains microvilli that protect the epithelium from harmful contact, friction and infection and facilitate gas exchange. The epithelium of the gills also contains goblet cells (Figs. 12 and 13). These results were in agreement with the results of Maria et al., (2018) who described the normal structure of gill tissue in Odontesthes bonariensis fish in the control treatment, with Mansour (2008), who described in his study the gill arcs and their locomotor muscles in Otolith ruber fish in southern Iraq. The results do not agree with Al-Luhaibi and Al-Mukhtar (2012), who described in his anatomical and histological study of the gills in adult mosquito fish (Gambusia affinis), where he indicated that the histological structure of the gill arch contains an irregular black line dashed along its length. The existence of this line has been explained by the fact that it consists of dense gatherings of melanocytes, and that the presence of variation and heterogeneity in the histological structure, it may be due to the difference in the different types and sexes of fish, or to the difference in the environments in which the fish live, the type of water that may be fresh or salty, the nature of nutrition, the type of food and other organisms present in the water, which impose many variations and modifications suitable for the performance of its natural functions in fresh or salt water.

Histological results of the gills in the experimental parameters:

Histological results showed that the gills of carp fish for the experimental

treatments T2, T3 and T4 had the same histological structure as in the control treatment, represented by the structure of the gill arch, primary lamellae and secondary lamella, their histological structure, and their epithelium containing microvilli, saline chloride cells and Beller cells. Histological sections also showed an increase in the number of double rows of primary lamella pairs, which radiate from the gill arch, as well as an increase in the number of secondary lamellas that appear in these transactions perpendicular to the primary lamellae, also, an increase in the thickness of the epidermal tissue covering the gill arch, an increase in the mucous cells and the rows of lymphatic tissue surrounding the skin, is noted, an increase in the number of salt-secreting chloride cells is also noted, especially in the basal part of the primary plates.

Also shown in the histological sections of the experiment treatments T2, T3 and T4 an increase in the number of layers of overlapping squamous epithelial cells and an increase in the number of pilar cells with a defensive function, which was observed abundantly scattered on the surface of the epithelial cells of the secondary plates, also, in histological sections, a significant increase in the number of microvilli and goblet cells is observed on the epithelial surface of the primary and secondary plates, which all work to increase the ability to contractive and motor activity and increase the vital capacity of these plates that enable the installation of the tissue gills, to increase the capacity for gas exchange and increase the protection of platelets and the installation of the gill epithelium from diseases and bacterial invasion, it is exposed to from water currents as it is characterized by a large flow of blood to complete the gas exchange process of the fish in the fullest form and with high efficiency due to the active substances included in the composition of the safflower plant, worked to stimulate and activate the cells that make up the structure of the gills (Fig. (14) and (15). These results are in agreement with the results of Evans et al. (2010), who showed when studying some hematological and biochemical parameters and histological changes of aqueous extract of safflower seeds (Carthmus tinctorius) on tilapia fish (Oreochromis niloticus), indicated the presence of an increase in the number of villi, goblet glands and saline chloride cells in the histological structure of gills in fish, with Ayotunde italics (2011) who studied the effects of adding aqueous extract to safflower seed powder on Nile tilapia fingerlings (Oreochromis niloticus) Line 1779.

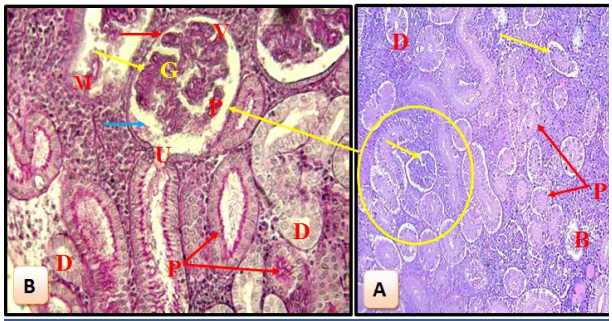

Figure (1) Cross-section of the kidney tissue in the control group of carp showing the renal corpuscle (ye arrow), (G) glomerulus, Bowman's capsule (red arrow), Bowman's space (blue arrow), (U) urinary po the glomerulus and its connection with the tubules Renal Urinary Poles, (V) Vegetal pole, (P) Prox coiled tubules, (D) Distal coiled tubules, (M) Mesengial cell, (P) Podocyte cells, (B) Blood vessels, (A)) I (H&E), (B) 400X (PAS)

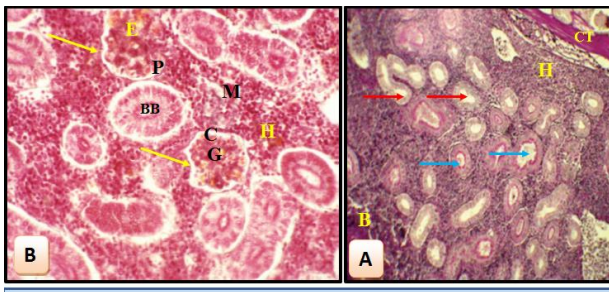

Figure (2) Cross-section of the kidney tissue in the control group of carp showing the renal corpuscle (ye arrow), (G) glomerulus, (C) Bowman's capsule, distal coiled tubules (red arrow), proximal coiled tub (blue arrow), (CT) Connective tissue of the kidney, (H) Hematopoietic tissue of the kidney, (E) Red bl cells within the glomerulus, (BB) Brush edge in the epithelium lining the distal tubule, (M) Mesengial cells, (P) Podocyte cells, (B) Blood vessels, (A) 100X (PAS), (B) 400X (H&E)

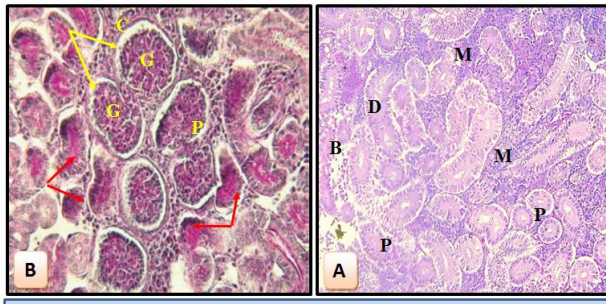

Figure (3) A cross-section of the kidney tissue in T2 of carp fish showing the renal corpusc (yellow arrow), (G) increased capillary blood vessels entering the glomerulus, making it large an small glomerular space, (C) Bowman's capsule, frequent involution of the renal tubules (Re arrow), (P) proximal coiled tubules, (D) distal coiled tubules, (M) increase in number of mesengi cell cells, (P) increase in proliferation of Podocyte cells, (B) increase in number of blood vessels, (A) 100X (H&E), (B) 400X (PAS)

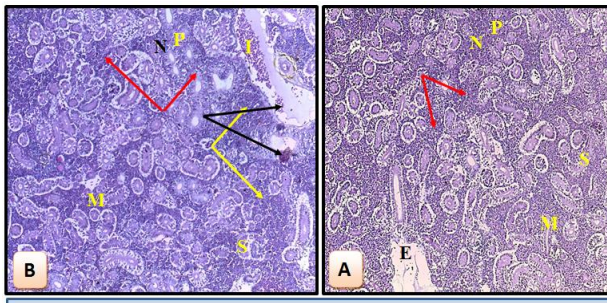

Figure (4) A cross-section of the kidney tissue in T3 and T4 of carp fish shows (N) glomerular necrosi hypertrophy areas (yellow arrow), irregular proliferation of renal tubules in the renal parenchyma (re arrow), hemorrhages and congestion of blood vessels In the kidney parenchyma (black arrow), (E edematous areas in the kidney tissue, (I) inflammatory areas in the kidney tissue, (S) necrosis in th epithelial lining of coiled tubules, (M) a decrease in the number of mesengial cell cells, (P)) Decrease Podocyte Count, (A) 100X (H&E), (B) 100X (H&E).

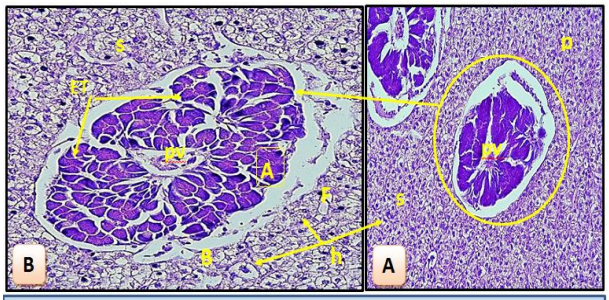

Figure (5) Cross-section of liver tissue in control carp group (P) Parenchyma liver tissue, (S) Sinusoids, (h) Hepatocytes, (py) hepatic portal vein, (F) Fat droplets, (B) Bile Ducts and Materials, (ET) Pancreatic Endogenous Tissue, (A) Exocrine Pancreatic Tissue (H&E), (A) 100X, (B) 400X

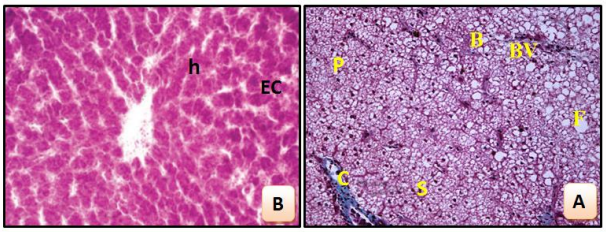

Figure (6) A cross-section of the liver tissue in the control group in carp showing (C) the capsule of the liver, (P) the parenchyma of the liver tissue, (S) the sinusoids of the liver, (hc) hepatic cells, (F) lipid droplets , (B) Blood Vessels, (EC) Endothelial cells (H & E), (A) 100X, (B) 400X

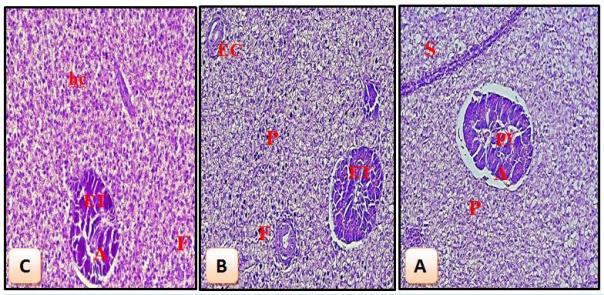

Figure (7) Cross-section of liver tissue in control group in carp showing (py) hepatic portal vein, (P) parenchyma of liver tissue, (S) sinusoids, (hc) hepatic cells, (ET) tissue Internal pancreas, (A) exocrine glands of pancreatic tissue, (F) lipid droplets, (B) blood vessels, (EC) endothelial cells (H&E), (A) 100X, (B) 100X, (C) 100X

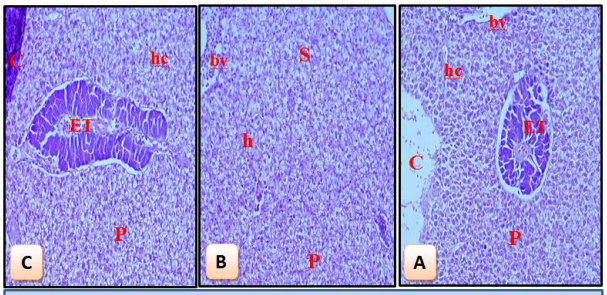

Figure (8) Cross-section of liver tissue in T2 and T3 groups in carp showing (C) Liver capsule, (P) Parenchyma liver tissue, (S) Sinusoids, (hc) Hepatic cells, (ET) Liver pancreatic internal tissue, (by) engorged blood vessels, (h) hepatocyte hyperplasia (H&E), (A) 100X, (B) 100X, (C) 100X

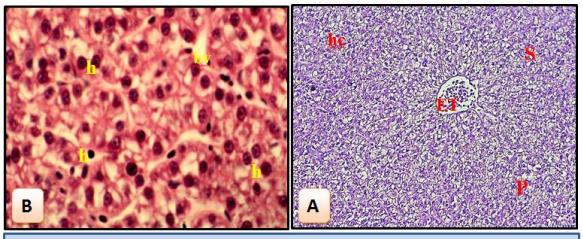

Figure (9) Cross-section of liver tissue in T2 and T3 groups in carp showing (P) Parenchyma liver tissue, (S) Sinusoids, (hc) hepatic cells, (ET) Degeneration in liver and pancreatic tissue Internal, (by) Congested Blood Vessels, (h) Hyperplasia (H&E), (A) 100X, (B) 400X

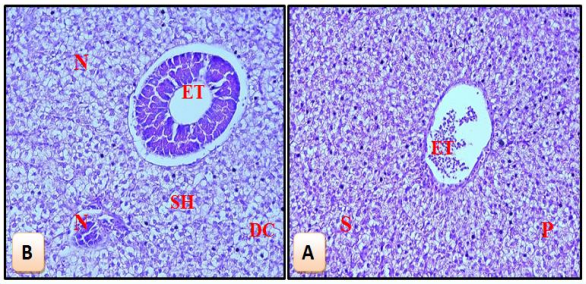

Figure (10) cross-section of liver tissue in T4 group in carp showing (P) Parenchyma liver tissue, (S) expansion of the sinusoids of the liver, (SH) Shrinkage hepatic cells, (ET) Degeneration in liver tissue Internal pancreas, (DC) Disorientation cell membrane loss, (N) Necrosis of hepatocytes and pancreatic liver tissue (H & E), (A) 100X, (B) 400X

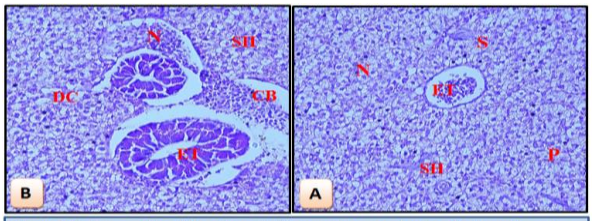

Figure (11) Cross-section of liver tissue in T4 group in carps showing (P) Parenchyma liver tissue, (S) Sinusoids expansion, (SH) Shrinkage hepatic cells, (ET) Degeneration in liver tissue Internal pancreas, (CB) engorged blood vessels, (DC) disorientation cell membrane loss, (N) hepatocyte and pancreatic necrosis (H&E), (A) 100X, (B) 400X

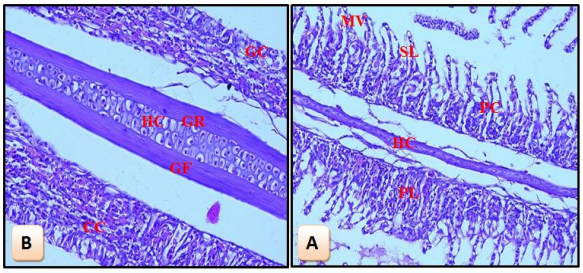

Figure (12) a longitudinal section of the gill tissue in the control group in carp showing (HC) the hyaline cartilage of the hyaline cartilage, (GR) inner rows of Gill Rakers, (GF) outer rows of Gill Filaments, (PL) Primary Lamella, (SL) Secondary Lamella, (CC) Chloride Cells, (PC) Piller cells, (MV) Microvilli, (GC) Goblet Cells (H&E) , (A) 100X, (B) 400X

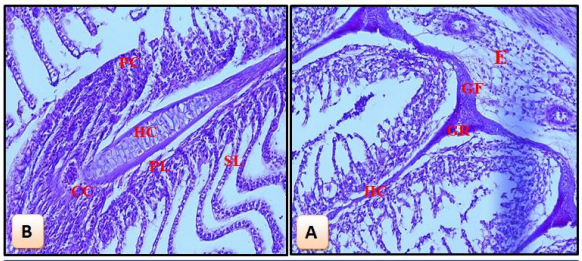

Figure (13) A longitudinal section of the gill tissue in the control group in carp showing (HC) the hyaline cartilage of the hyaline cartilage, (GR) inner rows of Gill Rakers, (GF) outer rows of Gill Filaments, (PL) Primary Lamella, (SL) Secondary Lamella, (CC) Chloride Cells, (PC) Piller cells, (E) Epidermis lining the gills containing lymphatic and vascular tissue (H&E) , (A) 100X, (B) 200X

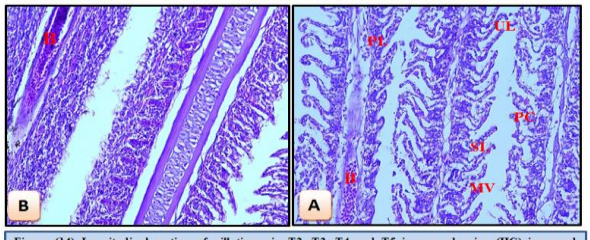

Figure (14) Longitudinal section of gill tissue in T2, T3, T4 and T5 in carp showing (HC) increased thickness of the hyaline cartilage of the hyaline cartilage, (B) the presence of bony tissue permeating the hyaline cartilage, (UL) union of Beller cells and secondary plates Contiguous, (PL) Increased number of primary lamella, (SL) increased number of secondary lamella, (U) increased proliferation of contiguous primary lamellae, (CC) increased proliferation of chloride cells, (PC) increased proliferation of Piller cells , (MV) Increased number and inflections of microvilli (H&E), (A) 100X, (B) 200X

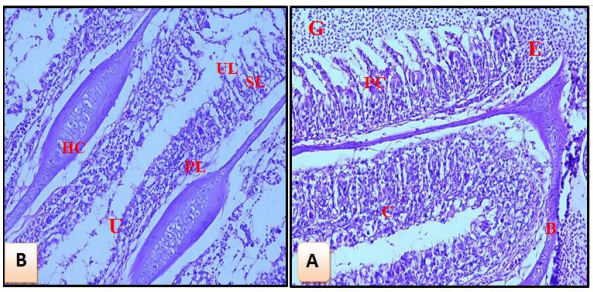

Figure (15) a longitudinal section of the gill tissue in T2, T3, T4 and T5 in carp showing (HC) increased thickness of the hyaline cartilage of the hyaline cartilage, (B) the presence of bony tissue permeating the hyaline cartilage, (UL) union of Beller cells and secondary plates Contiguous, (PL) Increased number of primary lamella, (SL) increased number of secondary lamella, (U) Confluence of contiguous primary lamellae, (CC) increased proliferation of chloride cells, (PC) increased proliferation and surrounding of Beller cells With connective tissue, (E) epidermis lining the gill tissue containing lymphatic tissue and blood vessels, (G) erected germinal tissue (H&E), (A) 200X, (B) 200X

Conclusion

We conclude from this study the possibility of adding raw safflower seed powder by 10% to the diets of common carp fish *Cyprinus carpio* L.

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