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The Effect of Some Food Preservatives in Dairy Products on Some Histological and Biochemical Changes in White Mice

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By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

{ وَيَرْزُقُهُ مِنْ حَيْثُ لَا يَحْتَسِبُ وَمَنْ يَتَوَكَّلْ عَلَى اللَّهِ فَهُوَ
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سُورَةُ الطَّلَقِ

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Supervisor Certification

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To my heart's beloved Imam Al Mahdi

To my father and soft hearted my mother

Who dedicate their lives to illuminate my steps in this world

To whom has filled me by their kindness and

blessing: My brothers, my sisters and my

friend Hussein Ali Hussein.

I present my modest effort and deepest and sincere

gratitude to their support

Yasmin

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Abstract

The current study focused on the histological and biochemical changes in some organs of males and females white mice after treated with formalin and citric acid as food preservatives in dairy products.

The present study noted the important changes after treated with formalin (0.2%) and citric acid (3%) for 30 days of oral administration. The experimental animals were divided into two main groups (A females and B males) and control groups. Each one of A and B main groups were divided into two subgroups (A₁, B₁, A₂ and B₂), A₁ and B₁ group that orally administrated with 0.5ml formalin, while A₂, B₂ with 1 ml citric acid, finally two groups as control groups. The histological parameters which included tissue sections from kidney, liver, and duodenum, while the biochemical tests determined the level of AST (Aspartate Transaminase), ALT (Alanine Transaminase), creatinine, and urea in the serum of animals under study.

The results of kidney for both A₁ and B₁ after treating with formalin revealed increase in the diameter of all part of nephron (hypertrophy), intra glomerular vascular congestion between glomeruli capillaries and renal tubules, and renal corpuscle were shrinkages, while the histological changes in the kidney after treating with citric acid were also an increasing in the diameter of the renal corpuscle and clear hemorrhage between the renal corpuscle, aggregation of inflammatory cells and exfoliated of the epithelial layer of tubules.

The histological changes of the liver sections for both A₁ and B₁ after oral administration with formalin have been noted significantly increase in both the thickness of capsule and diameter of hepatocyte nuclei, also the occurrence of the cystic dilations filled with blood in different location of liver parenchyma and wide necrosis region, while the histological results after administrating of citric acid showed also a slightly increased in both thickness of capsule and diameter of hepatocyte nuclei, enlargement hepatocyte with prominent vacuolation in their cytoplasm with dilation in the sinusoids with hemorrhage.

The current results of histological structure in the wall of duodenum for both A₁ and B₁ after treating with formalin noted a significantly increasing in the thickness of the epithelial layer, several damages in mucosa layer and blood congestion, while the histological results after administration of citric acid showed a significant increase in the thickness of the epithelial layer, exfoliated the epithelial layer that covering the intestinal villi to the intestinal lumen and separated between layers.

The main biochemical results for males and females for both treated (formalin and citric acid) showed a significant increase in the levels of ALT, AST, also noted a significant increase in the level of creatinine and urea compared with control groups. However, several results differed slightly between females and males. Furthermore, formalin has more effects than citric acid for the most results.

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List of Abbreviations

Abbr.	Meaning
ADI	Acceptable Daily Intake
AST	Aspartate Transaminase
ALT	Alanine Transaminase
BHA	Butylated hydroxyl anisole
BHT	Butylated hydroxyl toluene
D.C.T	Distal Convolute Tubule
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
EEC	European Economic Community
EU	European Union
FA	Formaldehyde
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FDH	Formaldehyde Dehydrogenase
P.C.T	Proximal Convolute Tubule
PPM	Part Per Million
ROS	Reactive oxygen species
WHO	World Health Organization

Chapter One

Introduction

1.1 Introduction

Dairy products as an important nutritional recommended consuming in daily and appropriate amounts. Cheese and yogurt are two kinds of dairy products that are used worldwide (Mazdeh *et al.*, 2017). The safety of dairy products should be considered in the presence of additives (WHO and FAO, 2011). The use of additives in dairy products varies in different continental regions. Though the addition is regulated by the European Union, for which the use of additives is not permitted or is restricted. The main categories of food additives are coloring agents, preservatives, flavor, emulsifiers and stabilizers (Herr, 2011)

Humans are exposed to complex mixtures of chemical compounds in their food, one of these substances are food preservatives (Aktaç *et al.*, 2003) because every living organism needs food to live. The food has limited shelf life, in order to increase the shelf life and maintain the quality sure preservatives are added, these preservatives may have some dangerous effects so if possible, and foods without preservatives may be used (Sharma, 2015).

In over the time, consumers have placed increased stress on food safety due to they need nutritious, healthy pure and safe food (Upadhyay, *et al.*, 2014). The use of preservatives in dairy products is subject to the restriction (Herr, 2011). The safety of dairy products must be considered in the presence of preservatives. According to ADI (Acceptable Daily Intake), the maximum permitted limit for food preservative is based on mg/kg of body weight (Mazdeh *et al.*, 2017). Foods contain food additives when consumption might lead to cancer in human not direct but gradually (Gultekin *et al.*, 2015).

Milk and milk products donate a very significant proportion in our daily diet, which is exposed to contamination and to combat this danger food preservatives are added. Most of these preservatives are formalin, but either involve a health danger or environmental danger or are inappropriate for long-term storage. Formalin is the only legally allowed preservative for milk and milk products (Upadhyay, Goyal and Kumar, 2014). Formalin is a 37% aqueous solution of formaldehyde (Sasseville, 2004).

Another preservative is citric acid, a type of food additives, used as a natural preservative (Grigor *et al.*, 2002), which is used for preventing antimicrobial growing in processed foods, also has an antioxidant effect is widely used in many prepared foods, particularly in cheese is known to have a flavor enhancer effect (Sacan *et al.*, 2007). However, they were reported to have carcinogenetic effects when used in high doses (Yılmaz S *et al.*, 2008)

Although preservatives play an important role in food safety, many studies have revealed that they have genotoxic and mutagenic effects when used for a long time (BAKAR and AKTAÇ, 2014). Therefore, studies investigating the potentially toxic effects of preservatives gain great importance today. Some food preservatives were previously reported to be able to be toxic on the living (Aktac *et al.*, 2003).

1.2 Aims of The study

To investigate the main effects of some food preservatives in dairy products on the white mice, following objectives are considered:

- 1- To identify the lethal dose.
- 2- The present study aim to show the histological changes in some organs of white mice after treating with formalin and citric acid and show the role of gender on the responses to the effects of the food preservatives.
- 3- The current study aim to evaluate the side effects of formalin and citric acid on some biochemical parameters (ALT,AST,Creatinine and Urea) in the male and female white mice.

Chapter Two

Literatures Review

2.1 Food Additives

Food can be fresh or processed materials that uptake by animals and humans to generate energy, keep good health and promote growth. The chemical structure of food products consists of water, fat, protein, carbohydrates, minerals and small amounts of organic compounds. All these compounds are the main source of energy for microbial growth because it will help the growth of microbial (Dwivedi *et al.*, 2017). Because the number of reported cases of food spoilage continues to increase, therefore food safety is a fundamental concern of both consumers and the food industry (Smith-palmer *et al.*, 2001).

The spoilage to food products occurred to chemical, enzymatic or microbial activities from the surrounding environment and also from the same food (Dwivedi *et al.*, 2017). Nutrition is responsible for the increased rate of cancer. Cancer might occur due to the additive contents and consumed a portion of food products, for example, the repeat foods may stimulate colon cancer in the form of carbohydrates (Gultekin *et al.*, 2015).

Milk and several dairy products such as yogurt, cream, and cheese have been produced and consumed worldwide for times (Visioli and Strata, 2014). Milk is a highly unstable food as it is a very good medium for the growth of many microorganisms (Dzigbordi *et al.*, 2013). Dairy products in our diet supply nutrients like proteins, fat, carbohydrates, vitamins, and minerals in a significant amount than any other single foods (Afzal *et al.*, 2011). Despite its many health benefits, yogurt (Dairy products) generally has a short shelf life (Dzigbordi *et al.*, 2013) and thus to improve their freshness, safety, texture, taste and preserve their nutritional value, a diversity of food preservatives (food additives) are being added during processing (Banipal *et al.*, 2015).

Additives are defined by the United States Food and Drug Administration (FDA) any substances purposely added to food (Dar *et al.*, 2017) that is synthetic or natural, these added to food products in small amounts to extend shelf life or added to sensory characteristics such as appearance, taste, consistency namely color and sweetness (Mirza *et al.*, 2017). The type and amount of a particular additive are necessary to sure by food

manufacturers to use and do not increase the acceptable safety limits especially in children who consume high amounts of a particular food product (Al-Harthy *et al.*, 2017).

In late 1988, the European Economic Community (EEC) accepted the laws and labeling as numbering for use of additives in products for human consumption because food additives become essential to use in the food industry (Haen, 2014). In the European Union, an E number refers to all food additives. The E-number expression was used in the food ingredient label whereas "E" is for European safety approval. The approval of a substrate as a food additive is intended to both ensure the protection of public health regarding the use of a food additive and inform consumers about the presence of food additive in food products (Robin and Sankhla, 2013).

Foods before use must subject to toxicological analytic studies to ensuring safety evaluation (Gultekin *et al.*, 2015). The assessment of food additives worldwide is supported by the control system of the Acceptable Daily Intake (ADI), developed by the Joint Food and Agriculture Organization (FAO) of the United Nations and World Health Organization (WHO) Expert Committee on Food Additives (Linke *et al.*, 2018).

The ADI represents amount of daily consumption of substance without any risk even for a lifetime. The provisions of the ADI level are legally established that allow the food additives are placed in the market. The most commonly used preservatives in dairy products such as cheese and yogurt are benzoate, sorbate, and natamycin (Mazdeh *et al.*, 2017).

The FDA maintains a list of over 3000 ingredients in its list "Everything Added to Food in the United States", many of which we use at home every day (e.g., sugar, baking soda, salt, vanilla, yeast, spices, and colors. When people consume foods containing these additives in large amounts, they can experience toxic effects. The risk associated with the ever increasing use of the abundant amount of food additives in our daily life is posing a huge risk to our immune system leading to various disorders and diseases (Dar *et al.*, 2017).

Foods containing food additives causes increased carcinogenicity risk occurs because some factors, reaction of additives with food ingredients, and change occur in the chemical formula of food additive that results from food processing might lead to the carcinogenic compound. So, food additives in some food processed when consumption might increase the risk of cancer in a human (Gultekin *et al.*, 2015), other cause problems, such as allergies, asthmatic reaction and hyperactivity, (Lee *et al.*, 2019) and carcinogenicity (Linke *et al.*, 2018).

The effects of food additives may be immediate or may be harmful in the long run on constant exposure. Long term effects of food additives had also been linked to an increased risk of cancer (Dar *et al.*, 2017). Many common additives have been associated with migraines, kidney and liver damage, birth defects and brain damage (Arora *et al.*, 2009). There is a relationship between dairy product consumption and breast cancer risk (Javanmardi *et al.*, 2019).

2.2 Classes of Food Additives

There are four general classes of food additives, according to a different function (Chaudhary, 2010; Robin and Sankhla, 2013):

- Nutritional additives are used to restore nutrients lost or degraded during production and enriching sure foods (ex. vitamins).
- Processing agents a some of agents are added to foods to support in processing or to maintain the desired consistency of the product (Stabilizers).
- Sensory agents are a very significant sensory characteristic of foods, including (Colorants, Flavorings, and Sweeteners).
- Preservatives.

2.3 Preservatives

Preservatives are food additives that prevent damage or spoilage to food by inhibiting microbiological growth, extend shelf-life of food products, and maintaining the texture, quality, consistency, color, taste, acidity, or alkalinity (Samal *et al.*, 2017). During transport, food products may begin to spoilage, lose their appearance and lowering in nutrition values. Spoilage caused by the action of microorganisms. Food preservatives used to inhibit the growth of the microbial in the food product or to prevent food spoilage (Sharif *et al.*, 2017).

Food preservation is one of the ancient diversities of technology used by humans, that may be natural (salt and sugar) or chemical, and this is the most effective type in preservation for lengthier periods (Martyn *et al.*, 2013; Pongsavee, 2015).

The preservative is widely used in foods and many researchers have found it to be effective against microbial activities of yeasts and molds. Yeasts are a major cause of spoilage of yogurt. The preservative acts by disrupting cell membranes of yeasts causing leakage and eventual lysis (Dzibordi *et al.*, 2013), preservative inhibition the growth by the number of activities including, accumulation of toxic anions, inhibition of essential metabolic reaction and stress on intracellular pH homeostasis (Brul and Coote, 1999), a diversity of food preservatives are added during processing dairy products (Banipal *et al.*, 2015)

There are forty-five substances used as preservatives, being their application and purity regulated in Europe United (EU). Although some of these substances are harmless when used in small amounts, the use of others is the hazard to human health (Silva and Lidon, 2016). Studies note it is safe to eat foods contain preservatives within ADI (Shim *et al.*, 2011).

Preservatives must meet standards for their use, similarly to other classes of food additives, they are chemicals and may cause opposite health effects. The excessive intake of these preservatives might be potentially dangerous to the consumers (Khoshnoud *et al.*, 2018). Current toxicological studies show that the concentrations of preservatives and their continuous use may be potential as cancer causing or agents

carcinogens (Mirza *et al.*, 2017). The side effects of preservatives are skin rashes and itching, breathing difficulty, sneezing or gastrointestinal upsets can be found (Silva and Lidon, 2016). It also cause hypersensitivity, allergy, asthma, neurological damage and cancer (Hossain *et al.*, 2016).

Some preservatives are used now in food and beverage industries such as benzoate, sorbates, sodium salts (Samal, Gouda and Patra, 2017), acetic acid, benzoic acid, propanoic acid, and sorbic acid (Silva and Lidon, 2016). Also, nitrates, nitrites, nisin and natamycin (Chaudhary, 2010) .

Nisin is applied worldwide in dairy products especially in making cheese (Gharsallaoui *et al.*, 2015) to control spoilage that found to action of microorganisms (Joo *et al.*, 2012). E234 is referred to a nisin (Zacharof and Lovitt, 2012). Nisin is used in controlling growth of microbes in yogurt (Penney *et al.*, 2004). Natamycin E235 is an antibiotic used to inhibit the growth of bacteria and fungi in various foods like cheeses (EFSA, 2009).

Sodium benzoate is a salt of benzoic acid, which is used as an important preservative in the food industry against bacteria, fungi and yeast. Sodium benzoate is the first preservative allowed by the FDA for use in food products. Sodium benzoate has been reported to be used in various cheeses (Shahmohammadi *et al.*, 2016). It has been significantly dangerous to the DNA (Linke *et al.*, 2018). Sodium nitrite is added to cheese as a preservative (Helal, 2001).

2.3.1 Types of Food Preservatives:

Food preservatives may be classified as Natural, Artificial, and Microbial preservatives (Seetaramaiah *et al.*, 2011; Dar *et al.*, 2017) :

A: Natural Preservatives:

Natural Preservatives are good not harm to our health. Natural preservatives are salts, sugar, vinegar, and rosemary extracts. Sugar and salt are the earliest natural food preservatives that very efficiently prevent the growth of bacteria in food.

B: Artificial Preservatives:

Artificial preservatives are the chemical substances that stop or delay the growth of bacteria thereby preventing spoilage and its discoloration without affecting its natural features when added to the food.

C: Microbial Preservatives:

Microbial preservatives are the preservatives that inhibit the growth of bacteria and fungi, or antioxidants, for example oxygen absorbers, which inhibit the oxidation of food constituents.

2.3.2 Classification of Food Preservatives:

Preservatives can mainly divide into two groups based on their purpose of practices, including preservatives as antioxidants and preservatives as antimicrobial (Samal *et al.*,2017).

2.3.2.1 Antioxidants

Antioxidants are chemicals that prevent oxidation of other molecules caused by free radicals. Antioxidant activity of dairy food is important both for the shelf life of the product as well as for protection from oxidative damage in the human body. Natural antioxidants used to extend the shelf life of the products in addition to have fewer side

effects than synthetic antioxidants to the human body (Alenisan *et al.*, 2017). Antioxidants including, BHA (butylated hydroxyl anisole) and BHT (butylated hydroxyl toluene), ethanol and formaldehyde (Seetaramaiah *et al.*, 2011).

2.3.2.2 Antimicrobials

Antimicrobial preservatives decrease the microbial spoilage of foods by inhibiting the growth and proliferation of bacteria, yeasts, and molds, for example, benzoates (E210 - E219), nitrates (E240 – E259), and sulfites (E220 – E229) (Samal *et al.*, 2017).

2.4: Formalin as Food Preservative

Formalin is a 40% aqueous solution of formaldehyde (FA) which is a colorless gas having a strong, pungent and irritating odor (Zararsiz, *et al.*, 2006), its highly toxic to all animals, any way of intake (Kamruzzaman, 2016). FA is highly soluble in water, it can change into the gaseous form at room temperature. FA is produced by the oxidation of methanol (Zararsiz *et al.*, 2006). Formalin is essentially pH-neutral and thus cannot be classified as either an acid or alkaline substance (Hawley and Harsch, 1999).

Formalin or FA is the simplest organic compound of aldehyde or alkane group. The contamination through the food and feeding diet with high doses level of FA is very dangerous for the body especially for an organ like liver and kidney because formaldehyde is source of reactive oxygen species (ROS) and free radicals substances for the body (Mahdi and Aulaniam, 2012). FA is classified as a probable human carcinogen according to The International Agency for Research on Cancer (Songur *et al.*, 2008).

FA, when taken into organisms, is metabolized into formic acid in the liver and blood by several enzyme systems, including the formaldehyde dehydrogenase (FDH) complex (Pandey *et al.*, 2000) and is then expelled either with the feces and urine or by the respiratory system (Davarci *et al.*, 2013). Formaldehyde ingestion has proved to have

fatal complications in the past. Once absorbed into the bloodstream, when changed to formic acid, which can rapidly necrosis cells in the liver, kidneys, heart, and brain. Formic acid levels can accumulate in high concentrations as rapidly as 30 minutes after ingestion. High blood levels of formic acid can lead to metabolic acidosis, central nervous system depression and renal damage (Hawley and Harsch, 1999).

FA is added to food because of its antiseptic and preservation properties, improving the appearance of the product, and keeping it moist and odorless (Xu *et al.*, 2011). Varge limited for formaldehyde in milk was not recognized by regulatory agencies, but when the World Health Organization appears (WHO) stated that the concentration in food can vary from 3 to 27 mg/kg (Nascimento *et al.*, 2015). The lethal oral dose of formalin is 30-90 ml and lethal period ranges from 1-2 days (Verma *et al.*, 2016).

In recent years, formaldehyde is reported to be widely used as a food preservative to increase the shelf life of food; it's harmful to human health. However, formaldehyde is abundant in the environment and is present in many animal and plant species as a product of their normal metabolism (Nowshad *et al.*, 2018).

Formalin its high reactive with several substances, its stability over time, but in the air, its broken down by sunlight in the half-life of about 30-50 minutes (WHO and IARC, 2008). Formalin is character expansive and effective preserve that rapidly penetrates tissue its use to preserve fish (Haque and Mohsin, 2009). The widespread formalin applying in fishes, vegetables, meats, fruits, and milk for long tern preservation poses a massive threat to nutrition and public health at disturbing level (Soffritti *et al.*, 2002).

FA used to preservatives fish after fishing, when humans eat these fish containinf FA may cause uncontrolled cell growth or cancer in any part of the body like the stomach, lung and respiratory system (Haque and Mohsin, 2009). Ingestion can lead to immediate deleterious effects on almost all systems of the body, causing gastrointestinal hemorrhage, cardiovascular collapse, unconsciousness or convulsions, severe metabolic acidosis, and acute respiratory distress syndrome (Pandey *et al.*, 2000).

FA it's a dangerous chemical to use as preservatives because it's rapidly absorbed from the gastrointestinal tract after ingestion and from the respiratory tract after inhalation (Mamun *et al.*, 2014). FA has toxic effects on humans, including irritation of the respiratory tract and eyes, nausea, headaches, allergic skin reactions, and drowsiness (Xu *et al.*, 2011). Many studies suggest there is relationship between formaldehyde exposure and nasopharyngeal cancer (Soffritti *et al.*, 2002).

FA which causes an increase in cytotoxic effects by compromising the intracellular balance has a strong tendency to combine with proteins, nucleic acids (DNA and RNA), and unsaturated fatty acids by non-enzymatic processes. These combinations result in allergic reactions, inflammatory reactions, cytotoxicity, necrosis, and mutagenic and carcinogenic effects by developing denaturation in proteins. Also, free oxygen radicals are increased in the tissues affected by FA, and this increase accelerates the processes (apoptosis or necrosis) that cause cell death (Davarci *et al.*, 2013).

Formalin is highly toxic causes liver and kidney damages (Mabood *et al.*, 2017), when it is ingestion can directly cause severe injury to the upper digestive system. This may show up as symptoms of vomiting blood, nausea, and diarrhea with bloody stool, blood from the urine, vertigo, breathlessness and circulation failure, then death. Also, it causes severe pain with inflammation, ulceration, and necrosis of the mucous membranes, which lines nearly every internal organ of the body. Also, Formalin's effect on pregnancy and the reproductive system has been studied in both humans and laboratory animals. Formaldehyde has been shown to decrease fertility and increase the risk of spontaneous abortion in humans (Kamruzzaman, 2016).

FA or formalin added as a preservative in milk to increase its shelf life due to the antiseptic nature and decreases the bacteria count. In a recently reported show that some cases of milk adulteration by formaldehyde (Singh and Gandhi, 2015; Mabood *et al.*, 2017). Moreover, adulteration of milk is also done to increase its volume and then starch and other reconstituted milk powders are added to increase its viscosity (Hossain *et al.*, 2016).

Formaldehyde is added to milk as an antimicrobial agent, the accepted amount of free formaldehyde (less than 0.5 ppm) that remains in the cheese after maturing does not cause for concern. The FA must be lower than 0.5 ppm, the concentration of 0.5 ppm of FA present in cheese would represent a danger for human health. The potential hazard of FA caused by the reaction of milk proteins and products of formaldehyde when added, it reacts very rapidly with caseins of milk (Alkhalif and Mirghani, 2017).

2.4.1 The Side Effect of Formalin on Some Organs

Effect of formalin on the kidney, the kidney is a typical bean-shaped, smooth, reddish-brown color it is covered by a thin connective tissue capsule. The kidneys lay along with the vertebral column in the abdominal cavity. The nephron is the functional unit of the kidney, each nephron consists of corpuscle which consists of glomerulus and Bowman's capsule, proximal convoluted tubules (P.C.T), a loop of Henle which consists of ascending and descending limb of a loop of Henle, distal convoluted tubules (D.C.T), and collecting tubules, the kidney consists of two regions, the outer cortex and the inner medulla (Al-Samawy, 2012).

The kidney function is to regulate the fluid and electrolyte balance of the body and the site of production hormones as rennin and erythropoietin. Also to maintain the composition of body fluids at a constant level and to remove excretory wastes (Rayner *et al.*, 2016)

(Davarci *et al.*, 2013) observes clear histological changes in the kidney due to the toxic effects of formaldehyde in several systems of the body. In a study in alcoholic patients, it was showed that methanol, which metabolizes into FA and formic acid, causes tubular necrosis of renal tissue, then resulting in renal failure. National Toxicology Program reported that the higher levels of formaldehyde exposure caused kidney cancers.

High degenerative changes were found in the kidney rats when treated with formalin in which formalin caused high inflammation grade and degenerative effect on kidney

tubule cells (Abdulqader and Mustafa, 2014). In the study on rats, it was observed several results indicating histopathological and biochemical changes in renal tissue, which occurred due to FA exposure. It was shown that systemic application of FA impaired glomerular patterns and thickened tubular and glomerular basal membranes, congestion of the intratubular vessels, dilatation in distal tubules, and vacuolization (Davarci *et al.*, 2013).

Urea, commonly referred to as blood urea nitrogen when measured in the blood, is a product of protein metabolism. An increase in urea can be the result of a diet that is high in protein content or decreased renal excretion. Creatinine, also a waste product, is produced from the breakdown of creatine and phosphocreatine and can also serve as an indicator of renal function. Creatine is synthesized in the liver, pancreas, and kidneys (Salazar, 2014). The urea and creatinine levels were increased, which are significant indicators of renal function due to toxic effect formaldehyde (Boj *et al.*, 2003).

Effect of formalin on the duodenum, the small intestine is a long convoluted tube extends from the junction with stomach to join with the large intestine. The small intestine is divided into three parts: the duodenum, jejunum, and ileum. The duodenum composed of four-layer, mucosa, submucosa, muscular and serous layer. The mucosa layer consists of the epithelium (simple columnar epithelium), Lamina propria (loose connective tissue) and muscular mucosa consist of an inner circular and an outer longitudinal layer of smooth muscle. The duodenum submucosa contains clusters of glands. The muscular layer is composed of usual inner circular and outer longitudinal layers of smooth. The serosa layer is formed by mesothelium with a vascularized connective subserous tissue (Kong *et al.*, 2018).

FA ingestion can lead to late gastrointestinal complications similar to those seen in acid and alkali ingestion (Hawley and Harsch, 1999). A study showed mice exposed to formalin with a concentration of 6 to 15 ppm for 2 years developed squamous-cell carcinoma (a type of cancer in mucous membrane) in the nose, stomach (Kamruzzaman, 2016). In the study that was done on rats the treated with formaldehyde shown

oncological lesions of the intestine, particularly at the highest dose (Soffritti *et al.*, 2002).

Formalin is significantly associated with cancer in human particularly gastrointestinal cancer when administered of drinking water with high a concentration of it and nasopharyngeal cancer when inhalation it during occupational exposure (Mamun *et al.*, 2014). It was found that FA administered orally to rats caused mucosal ulcers, hemorrhage, necrosis and perforation in the gastrointestinal system, then the development of metabolic acidosis, hematuria, circulatory failure, anuria, and renal papillary necrosis (Zararsiza *et al.*, 2006).

Effect of formalin on the liver, the surface of the liver is smooth and dome-shaped, where it is related to the concavity of the inferior surface of the diaphragm. The liver lies mainly in the right upper quadrant of the abdomen where it is hidden and protected by the thoracic cage and diaphragm. The normal liver lies deep to the ribs 7 – 11 on the right side. The liver is divided into 4 lobes. The basic functional unit of the liver is the liver lobule, at the center of the lobule is the central vein (Allen, 2002).

Hepatocytes are parenchymal cells, which organized into plates separated by vascular channels called sinusoids, which appeared discontinuous, fenestrated endothelial cell lining (Ramadori *et al.*, 2008). The primary function of the liver is a center for the metabolism of nutrients and excretion of waste metabolites, secretion of bile, and storage of minerals and vitamins (Allen, 2002).

The changes occurs in the liver after absorption of formalin including, appeared of inflammatory cells, degeneration of the hepatocytes that included shrunken, and swelled appearance. The shrunken cells appeared with hypereosinophilic cytoplasm, condensed nucleus and blebbed plasma membrane. These changes are similar to the apoptotic cell death (Abdulqader and Mustafa, 2014) .

(Mamun *et al.*, 2014) has shown that the section of liver mice treated with formalin showed severe histopathological changes including, congestion in the central vein, deleterious degeneration in tissues, enlargement in the sinusoids, hemorrhages inside the tissue and reduce compactness of the cells by the formation of intracellular spaces.

The measurement of the ALT and AST can provide specific indicators of hepatocellular damage, these enzymes are normally located in the cytoplasm and released into the circulation after cellular damage (Hall and Johnny, 2012).

In a study of the effect of FA in the liver, showed raised activities of ALT and AST are a sign of loss of hepatocellular integrity. The type of enzyme released is dependent on the timing and severity of the damage. In acute disease condition high ALT activity is observed but in chronic disease condition high AST activity is observed (Olooto, 2010).

2.5 Citric Acid as Food Preservative

Citric acid is a weak organic acid found in the greatest amounts in citrus fruits, such as lemon, grapefruit, tangerine, and orange (Abdel-Salam *et al.*, 2014). Citric acid is the most common type of food additives, acidity regular and an antioxidant (Al-Harthy *et al.*, 2017) that used as a natural preservative (Abd-ALGadir *et al.*, 2010), commonly used in the drink and food industry as a food preservative (Chen *et al.*, 2015).

The citric acid (E330) also increases the acidity of the food product. High acid foods such as fruit formulations require less heat treatment for their stability and therefore citric acid is added to maintain their pH at < 4.6 (Musyoka *et al.*, 2018).

Citric acid is used as a pH control agent, preservatives to prevent microbial growth, also used as flavor assistant to get a good taste (Lawrence *et al.*, 2004). Also used as a flavoring agent and preservative in food, especially in biscuit, prepared soup, cake, cheese and cheese products, baby food, chewing gum, margarine, fizzy lemonade, fish, meat, and beverages, especially soft drinks (Aktac *et al.*, 2008).

Organic acids and their salts are generally regarded as safe and have been accepted by most member states of the EU to be used as the feed additives in animal production. The organic acids undissociated organic acids can easily penetrate the lipid membrane of bacteria and disrupt the normal physiology of certain types of bacteria (Adil *et al.*, 2010). In a cell, the organic acids release the protons in the alkaline cytoplasm, resulting in the decrease of intracellular pH. This alters enzymatic reactions and the nutrient

transport system, forcing the bacterial cell to use energy to release protons and cause an intracellular acid anion accumulation (Hernández *et al.*, 2006). When ingested, is rapidly and completely metabolized to carbon dioxide and water (EFSA, 2015).

2.5.1 The Side Effect of Citric Acid on Some Organs

The primary cause of histopathological changes in renal structure when using citric acid due to the changes in membrane permeability and related enzyme activities caused by covalent attachment of citric acid to bioactive proteins (Chen *et al.*, 2015). The study on the effect of citric acid on kidney cells of mice showed shrinkage of glomeruli, Bowman's spaces were lobulated and contracted tufts, some tubules demonstration desquamated cells (Abd-ALGadir *et al.*, 2010).

The results of citric acid-treated orally to rats showed distinct renal capsule spaces, lobulated tufts, and desquamation or moderate dilatation of kidney tubules (Chen *et al.*, 2015). The study of oral administration of citric acids to a rat resulted in the significant gradual increase in the level of serum creatinine, increase in the level of serum urea nitrogen. Also showed causes damage to renal function (Abd-ALGadir *et al.*, 2010).

(Aktaş *et al.*, 2003) showed the effect of treatment with citric acid on liver mice caused loosening and combining of hepatocyte membranes, pyknotic nuclei, mononuclear cell infiltration in small areas in the liver can be expressed as a protective response that happened against the degenerative effects of citric acid. Also, the results noted cytoplasmic vacuolization in hepatocytes, degeneration of blood vessel endothelium in the liver.

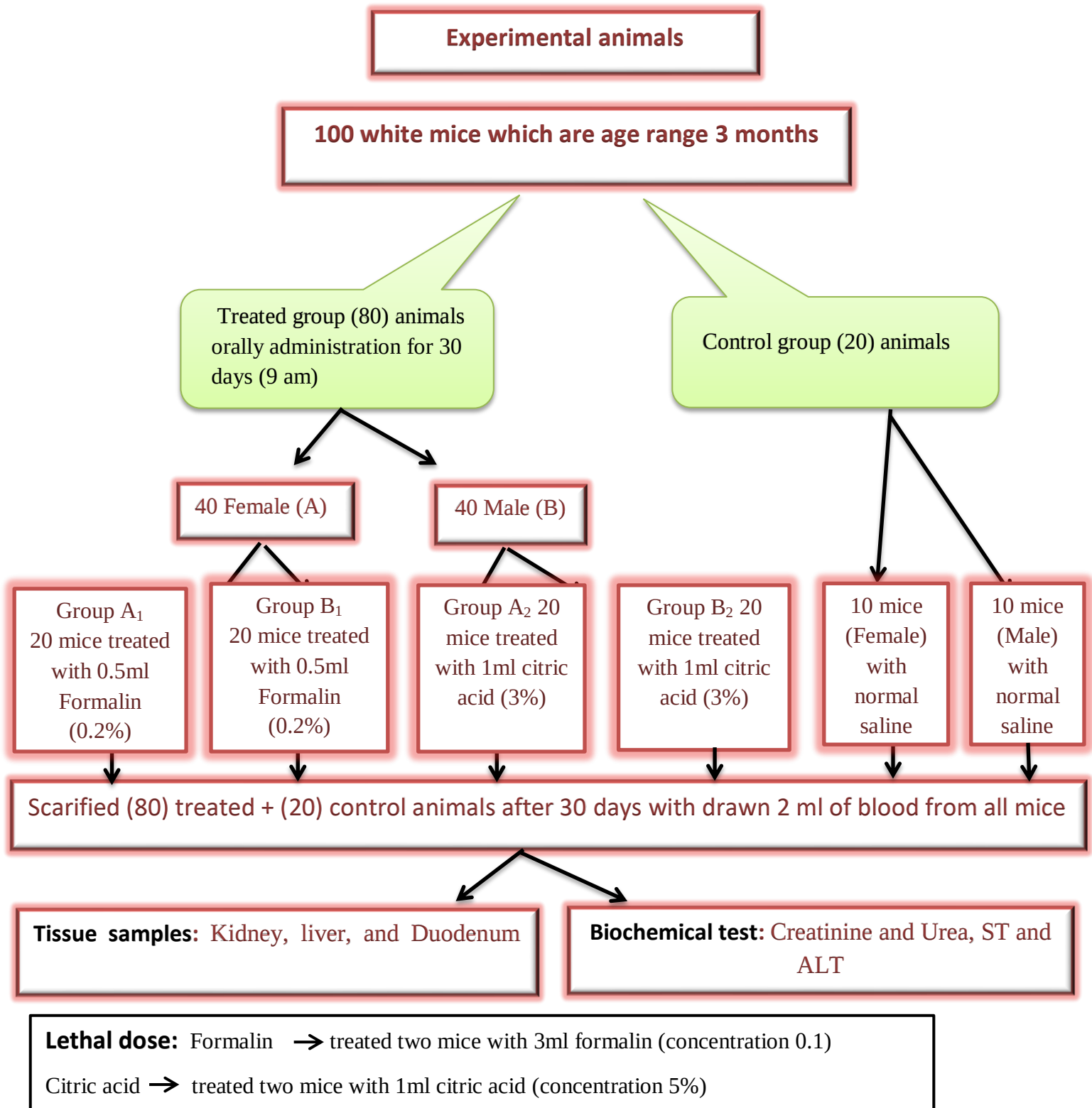
While the effects of citric acid on duodenum caused significantly decreased thickness of epithelial (Nourmohammadi and Afzali, 2013) and muscular layer in the duodenum which contribute to the reduced digestive efficiency and nutrient absorption (Adil *et al.*, 2010).

Chapter Three

Materials and Methods

3. Materials and Methods

3.1 Experimental Design



3.2 Materials

3.2.1 Experimental Animals

The present work applied to laboratory female and male white mice. The experimental animals were collected from The Drug and Health center in Baghdad province. Eighty adult white mice (40 females and 40 males) which consider as treated groups that treated with two types of food preservatives. The forty female (A) and forty male (B) treated animals were divided into two subgroups, which included (A₁, A₂) and (B₁, B₂). The A₁ and B₁ treated groups which treated with formalin for 30 days. The A₂ and B₂ were treated with citric acid for 30 days.

The experimental study started at (2018-2019) of experimental time. The experimental animals living in laboratory plastic cages, all cages put in the animal house of the college of science at Al Muthanna University and the mice were left for at least two weeks for adaptation. The main weight of experimental animals were (25-35) gm. living in a lab house under controlling temperature 25-28°C with feeding by using standard pellets.

3.2.2: Chemicals:

Table 3-1. Chemicals with Their Productions

NO.	Chemicals	productions
1	Formalin 37%	Linear chemical, Barcelona -Spain
2	Citric acid	India
3	Glycerin	England
4	Paraffin wax	Egypt
5	Hematoxylin crystals	England
6	Eosin	England
7	Oil emersion	China
8	Xylene	India
9	Absolute alcohol	Japan
10	Chloroform	India

Table 3-2. Mayer's Hematoxylin and Eosin stain

Hematoxylin and eosin stain	1.0 gm
Distilled water	1000 ml
Sodium iodate	0.2 gm
Ammonium or potassium alum	50.0 gm
Citric acid	1.0 gm
Chloral hydrate	50.0gm

Table 3-3. The Biochemical Kits and Their Production

NO	Test Kits	Productions
1	ALT kit	Randox-United Kingdom
2	AST kit	Randox-United Kingdom
3	Urea kit	Randox-United Kingdom
4	Creatinine kit	Randox-United Kingdom

Table 3-4. Instruments with Their Productions

NO	Tools	Productions
1	Surgical set	England
2	Slides	China
3	Cover slide	China
4	Ependroff tube	Germany
5	Flasks	China
6	Gel tube	Cellco- Jordon
7	Electrical balance	Shimadu company- Japan

8	Centrifuge	GMBH-Germany
9	Bulking	Thermo-England
10	Microtome	Micron-Germany
11	Water path	Sakura-Japan
12	Oven	Sakura-Japan
13	Micropipette	Huawei-China
14	Light compound microscope	GENEX-USA
15	Microscope camera	OMAX-China
16	Deep frizz	Japan

3.2.3 Prepared of Stock Solution and Lethal Dose of Formalin:

According to (El-Gendy *et al.*, 1980; Mamum *et al.*, 2014; Kamruzzaman, 2016) the dose of formalin was 2ppm that measured as follows:

Prepared stock solution with volume 37 ml (concentration 0.2%)

$$C1 \times V1 \text{ (concentration)} = C2 \times V2 \text{ (diluted)}$$

$$37\% \times V1 = 0.2 \text{ \% (2ppm)} \times 37\text{ml}$$

$$V1 = 0.2 \text{ ml of formalin stock solution (0.5ml of formalin for each mouse)}$$

The prepared lethal dose of formalin with volume 10 ml (concentration 0.1)

$$C1 \times V1 \text{ (concentration)} = C2 \times V2 \text{ (diluted)}$$

$$37\% \times V1 = 0.1 \times 10\text{ml}$$

$$V1 = 2.7 \text{ ml of formalin stock solution (3ml of formalin for each mouse).}$$

3.2.4. Prepared of Stock Solution and Lethal Dose of Citric Acid:

According to (Abd-ALGadir *et al.*, 2010) the dose of citric acid were (100 - 1250 mg kg-1 Body weight that measured as follows:

Dilution 3 gm. of citric acid in 100 ml of distal water to getting diluted citric acid (3%) and 1 ml give for each mouse. The prepared lethal dose of citric acid with volume 10 ml (concentration 5%). Added 0.5 gm of citric acid to 10 ml of distal water for prepare 5% of citric acid.

3.3 Methods**3.3.1 The Tissue and Blood Samples Collection**

The blood samples collected from each experimental group after 30 days of study time. Two ml of blood was attained by cardiac puncture after anesthetics the mouse by using chloroform, transferred the blood samples to gel tubes. The blood was left at room temperature for 20 minutes after that centrifuged with (3000 rpm) for 5 minutes the serum was collected in Eppendorf tubes and divided into equal amount (50µl) and frozen (20-35°C under 0) until used for laboratory assessments, to purpose examine ALT, AST, creatinine and urea.

The tissue samples were taken after sacrificed experimental animal, and taken kidney, duodenum, and liver. The tissue samples were passed through the histological technique which including many steps (Jensen, 2008).

3.3.2 Histological Technique

1- Fixation: The tissue samples (kidney, liver and duodenum) were placed in labeled container contain 10% formalin for 48 hours, and shakely the container gently several times to ensure that the fluid reached all surface and that pieces were not sticky to the

bottom or sides (A shank of glass wool placed in the container will aid in keeping the tissue free of the bottom) (Bancroft and Gamble, 2008).

2- Washing: The samples were washed by using the tap water for (1 hr.) after fixation to remove the excess of fixative out of the tissue to prevent intervention with subsequent processes, washing is often was done with running water (Wilson *et al.*, 1994).

3- Dehydration: The samples were done to remove all extractable water by dehydrated diffusing through the tissue, Ethanol was used. The dehydration procedure was continued by upgrading alcohol from 50% to absolute alcohol (50%, 60%, 70%, 80%, 90%, 90%, 100%, and 100%) (Bancroft and Gamble, 2008).

4- Clearing: This stage is considered as an intermediate step, it was essential because the used alcohol for dehydration will not dissolve or mix with molten paraffin. Thus tissue must be submerged in some fluid miscible with both alcohol and paraffin before infiltration can take place. The clearing was too removed or clear opacity from dehydration tissues and making them transparent. Therefore, blocks of tissue seemed almost crystalline and never milky. Xylene was reagents used for clearing (Bancroft and Gamble, 2008).

5- Infiltration and Embedding (Blocking): Paraffin wax was considered hard (melting point 60-68°C). Hot weather will power the use of harder paraffin. Paraffin must be fully molten to infiltrate the tissue effectively. Embedding with paraffin, paraffin was permitted to solidify around and within the tissue. The tissue was sited in a small container or a paper box was already filled with melted paraffin. The orientation of the tissue is important to limit the proper surface for sectioning.

6- Cutting: Cutting was done by using the rotary microtome. The thickness is (5 µm). The sample was put in a hot water path with (52°C) for extending tissue. Then the samples were carried on the slides which had a thin layer of Mayer egg albumin, the slide was put on the hot plate with (40°C) for overnight.

7- Staining: Haematoxyline and eosin yellow solution was used to stain the tissue for preparing a permanent slide for demonstrating the general structure of the tissue (Bancroft and Gamble, 2008).

3.4 Statistical Analysis

The experimental results were statistically analyzed using Minitab V.16 to assess the significance of changes between the control and treated mice group results. We used the two in depended t-test to test the hypothesis of the study. The results were expressed as Means with a p. value equals 0.05 as statistical significance.

3.5: The Measurement

- 1- Measuring diameters of renal corpuscles, proximal and distal convoluted tubules and the Henley loop.
- 2- Measuring diameter of hepatocyte nuclei and the thickness of capsule.
- 3- Measuring the thickness of mucosa layer hepatocyte nuclei and of capsule.
- 5- Measuring the levels of Creatinine, Urea, AST and ALT

Chapter Four

Results & Discussion

4.1 The Histological Results

4.1.1 The Histological Results of Kidney

4.1.1.1 The Histological Result of Female Treated Group with Formalin after 30 Days Orally Administration

The histological result of the kidney in female mice after oral administration with 0.5ml formalin showed the renal corpuscle has prominent diameter with $(24.81 \pm 0.49 \mu\text{m})$ (Table 4-1), and (Appendix-1) which has significantly increased compared with the control group. In control group noted the tissue section of the cortical region showed renal corpuscle, proximal convoluted tubules and distal convoluted tubules Fig (4-1, 2).

The histological result showed wide spot of hemorrhage randomly distributed through the kidney parenchyma exactly between renal tubules and wide abnormal degeneration Fig (4-7). This histological change in the tissue section of the kidney may be due to increase the rate of excretion or elimination the toxic material through the kidney which increased the blood congestion in the renal tissue which lead to these changes these results were agreement with (Verma *et al.*, 2016) which noted the kidney of rats when treated with formalin caused hemorrhages in the kidney.

In control group the Bowman's capsule is formed from two thin cellular layers, the outer layer (parietal) and an inner layer (visceral). The podocytes and the mesangial cells were located among the glomerular capillaries to support the glomerular capillaries, between the two layers of the capsule was limited space called Bowman's space (Fig.4-3) while in treated group the tissue sections showed the glomeruli capillaries were shrinkages, the renal corpuscle have very progressive bowman's space and the weak parietal layer of Bowman's capsule. The mesangial cells between the glomerular capillaries have dark spherical nuclei may be due to high stress on nuclei. Also noted wide dilation in renal tubules may be because of some renal tubules degeneration due to the toxicity of formalin after the treated time Fig (4-8).

The histological result of kidney noted very wide cystic dilations may be due to destruction in the junction between renal tubules, also noted high aggregation of inflammatory cells beside abnormal cystic dilations which referred to immune response. Also the result showed prominent damage in Bowman capsule and progressive of glomerulus capillaries may be due to high hydrostatic pressure on capillaries Fig (4-9) that different from the tissue of control group Fig (4-2, 3).

The histological result showed the diameter of proximal convoluted tubules (P.C.T) was $(10.44 \pm 0.21 \mu\text{m})$, (Table 4-1), and (Appendix-2) which have significantly increased compared with the control group and have degeneration in some regions of its wall. The epithelial layer that lining the internal lumen of the P.C.T. has acute degeneration, some tubular cells lost nuclei and exfoliated epithelia nuclei into the lumen may be due to effect of formalin on these cells Fig (4-10) that different from the control group Fig (4-2). These results were consonant with (Dorairajan, 2010) which noted formalin caused degeneration and necrosis of the tubules.

The histological results of distal convoluted tubules (D.C.T) noted an abnormal wall, and have significantly increased in diameter with $(15.34 \pm 0.20 \mu\text{m})$, (Table 4-1) and (Appendix-3) compared with the control group. The tissue sections of D.C.T appeared have acute degeneration in the walls, and destruction the epithelial layer. The tissue sections showed the D.C.T filled with infiltration that affected on the high of the epithelial layer that lining the inner lumen of D.C.T the result noted large aggregations of inflammatory cells located in the interstitial spaces between renal tubules Fig (4-11).

The histological result of kidney showed the medulla contained on descending, ascending branches of the Henley loop, and collecting ducts. The (Table 4-1) noted the descending branch has a prominent diameter with $(10.72 \pm 0.17 \mu\text{m})$, (Appendix-4). Also the diameter of ascending branched was $(13.68 \pm 0.18 \mu\text{m})$, (Appendix-5). Both diameters of descending and ascending branches of the Henley loop have significantly increased compared with the control group Fig (4-4). The tissue section demonstrated thick spots of infiltration in their lumen and damage in some tubules that appeared as cystic dilation Fig (4-12).

The histological result noticed in some regions the abnormal branched of the Henley loop surrounded by high aggregation of inflammatory cells and the both branched of Henley loop has prominent thick fluid in their lumen this aggregation may be due to blockage in urinary tubules Fig 4-13). These histological changes of kidney after treated with formalin were agreement with (Zararsiz *et al.*, 2007) which noted the kidney of rats when treated with formaldehyde caused damage glomerular and thickened tubular, hemorrhage and dilatation in renal tubules may be to high filtrate in renal corpuscular then pressure on the vessels blood lead to hemorrhage.

4.1.1.2 The Histological Result of Male Treated Group with Formalin after 30 Days Orally Administration

The current study of the kidney in male mice after oral administration with 0.5ml formalin demonstrated the diameter of the renal corpuscle was ($24.65 \pm 0.38 \mu\text{m}$), (Table 4-1) and (Appendix-6) which have significantly increased compared with the control group. All tissue sections of kidney in male control group have histological structures similar to tissue structure of female control group Fig (4-5, 6). In The microscopic examinations of the histological sections of kidney noted vascular congestion, necrosis and aggregation of inflammatory cells Fig (4-14). The histological section showed damage in the renal corpuscle including dark nuclei of mesangial cells; hemorrhage between glomerular capillaries may be due to high hydrostatic pressure due to the formalin toxicity. Also, the result noted degeneration in the partial layer of Bowman's capsule Fig (4-15).

The present study demonstrated the wall of P.C.T have significantly increased in diameter with ($10.57 \pm 0.17 \mu\text{m}$) compared with control group, (Table 4-1) and (Appendix-7). The histological section noted acute degeneration between renal tubules with abnormal aggregation of inflammatory cells around the wall of the renal tubules. Also, the result of P.C.T showed damage in the wall and exfoliated of the epithelial layer Fig (4-16).

The (Table 4-1) showed the D.C.T abnormal wall and have significantly increased in diameter ($15.40 \pm 0.21 \mu\text{m}$), (Appendix-8) compared with the control group. The histological slide result showed severe damage to the epithelial layer of D.C.T, and epithelia exfoliated from an inner surface and hemorrhage between renal tubules Fig (4-17). Some D.C.T showed wide abnormal enlargement Fig (4-18). The incidence of hemorrhage may be due to the high toxicity of formalin.

The current study in the medulla of kidney noted it's contained descending, ascending branched Henley of the loop and collecting ducts. The (Table 4-1) showed significantly increased in diameter of descending and ascending branches of the Henley loop were ($10.24 \pm 0.17 \mu\text{m}$), (Appendix-9) and ($13.63 \pm 0.16 \mu\text{m}$), (Appendix-10) respectively.

In (Table 4-1) non-significant differences between the female and male treated groups in diameters of the following tubular structures (Renal corpuscle, P.C.T, D.C.T, Ascending and descending branches of the Henley loop (Appendix-11, 12, 13, 14, 15) respectively.

These findings indicated that formalin causes damage in kidney in both male and female treated group. These result were consonant with (Mamun *et al.*, 2014) which showed the kidney of male mice after treated with quantity of formalin as 30 μl , 3 μl and 0.3 μl have shrieked in glomeruli, aggregation of inflammatory cells may be due to immune response, degenerated tissue, and congestion of renal glomerulus along with hemorrhage due to high toxicity of formalin.

All these changes in nephron kidney that including increased the diameter of all part of nephron may be due to nephron hypertrophy, this state occurred because the high damage in nephron structure, there for the kidney tries to repair damage occurred in other nephrons by this stat by accumulation the largest quantity of infiltrating.

4.1.1.3 The Histological Result of Female Treated Group with Citric acid after 30 Days Orally Administration

The histological result of kidney in a female treated group that treated with 1 ml of citric acid showed significantly increase in the diameter of the renal corpuscle which become ($24.48 \pm 0.65 \mu\text{m}$) in the treated group compared with control group, (Table 4-1) and (Appendix-16). This result referred to acute progressive in the renal corpuscle after treated with citric acid which leads to shrinkage of the renal corpuscle. This histological change in the renal corpuscle may be due to the high toxicity of citric acid on the renal corpuscle.

The tissue section of the cortical region in the kidney showed aggregation of inflammatory cells, blood congestion and wide abnormal cystic dilution filled with blood. Also, the result noted the renal corpuscle has wide Bowman's space and shrinkage of glomeruli Fig (4-19,20). These histological changes of kidney after treated with citric acid were similar to (Chen *et al.*, 2015) which noted the kidney of male mice when treated with citric acid caused shrinkage of glomeruli, wider renal capsule spaces and severe dilation of renal tubules and some tubule lumens were irregular.

The (Table 4-1) demonstrated that the wall of P.C.T. has no significantly increase in diameter with ($10.06 \pm 0.18 \mu\text{m}$) compared with a control group (Appendix-17). The histological section noted acute degeneration in the wall of P.C.T and exfoliated the epithelia to the lumen of P.C.T. Fig (4-21). The tissue section of the D.C.T showed increased in diameter ($16.54 \pm 0.27 \mu\text{m}$) compared with a control group (Appendix-18). Also, the result noted severe damage in the wall of D.C.T and exfoliated of epithelial layer Fig (4-21).

In (Table 4-1) the tissue section of descending limb of the Henley loop has no significant increase in diameter ($10.07 \pm 0.15 \mu\text{m}$), (Appendix-19) while ascending Henley loop have wide diameter ($12.8 \pm 0.16 \mu\text{m}$), (Appendix-20), both branches ascending and descending arms of the Henley loop characterized by have acute lumen with very prominent basement membrane in the wall of the Henley loop. Mostly have acute degeneration in the wall and exfoliated the epithelia into the lumen Fig (4-22).

These result of kidney after treated with citric acid were coincided with (Aktac *et al.*, 2008) which noted the kidney of mice when treated with (480 mg/kg) citric acid caused degenerations of tubules and fusing of tubules may due to materials toxicity of citric acid metabolism.

4.1.1.4 The Histological Result of Treated Group (Male) with Citric acid after 30 Days Orally Administration

The current study of the kidney in male mice after oral administration with 1ml citric acid noted the diameter of the renal corpuscle was ($24.45 \pm 0.48 \mu\text{m}$) which have significantly increased compared with the control group in (Table 4-1) and (Appendix-21). The histological result showed blood congestion, damage in the renal corpuscle and aggregation of inflammatory cells randomly distributed through the kidney parenchyma Fig (4-23). This histological change of kidney after treated with citric acid were coincided with (Ortiz *et al.*, 2006) which noted degeneration convoluted tubules and disruption of the cells in the kidney when treated with food additives (flavor enhancer).

The (Table 4-1) and (Appendix-22) showed the diameter of the proximal convoluted tubules was ($10.08 \pm 0.19 \mu\text{m}$) which has no significant increase compared with the control group. Also the table showed significantly increase in diameter of D.C.T with (16.82 ± 0.24) compared with the control group (Appendix-23). The tissue section in the cortical region noticed prominent hemorrhage, cystic dilation, wide Bowmans space, and noted sever damage in the P.C.T. and D.C.T. wall Fig (4-24).

The (Table 4-1) and (Appendix-24) noted significantly increase in the diameter of descending limp of Henley loop was ($10.171 \pm 0.13 \mu\text{m}$), while ascending limp was ($12.59 \pm 0.16 \mu\text{m}$) which have no significant increased compared with control group (Appendix-25). In (Table 4-6) appeared no significantly differences between male and female in the diameter of renal corpuscle as shown (Appendix-26), proximal convoluted tubules (Appendix-27), distal convoluted tubules (Appendix-28), descending limp (Appendix-29) and ascending limp (Appendix-30).

These histological change in the tissue section of cortical region of the kidney after treated with citric acid were coincided with (Bakar and Aktac, 2014) they noted the kidney of male mice when treated with citric acid and sodium benzoate caused degeneration in glomerular structure, lost the nuclei of tubular cells and basal membrane injury due to toxicity of these food preservatives.

4.1.2 The Histological Results of Liver

4.1.2.1 The Histological Results of Female Treated Group after 30 Days Orally Administration with Formalin

The histological result of the liver oral administration with 0.5 ml formalin showed significantly increase in the thickness of the capsule with ($6.93 \pm 0.17 \mu\text{m}$) and significantly increase in the diameter of hepatocyte nuclei with ($9.646 \pm 0.13 \mu\text{m}$) compared with a control group that appeared in (Table 4-2), (Appendix-31) and (Appendix-32). In control group noted the liver parenchyma composed of hepatic cords that consist from hepatocytes that arranged as long cordial structures, between hepatic cords showed the prominent sinusoids. The kupffer cells were normal distribution through the hepatic parenchyma. The liver was surrounding by capsule Fig (4-25).

The results of the microscopic investigation showed the liver parenchyma have abnormal hepatic cord irregular arrangement, acute degeneration in the liver parenchyma including enlargement of hepatocytes, wide necrosis region in the liver treated group compared with the control group Fig (4-27) this may be due to materiales toxicity of formalin metabolism that accumulation in hepatocytes which failed to eliminted it caused damage hepatocyte and increased release of enzymes that lead to increased AST and ALT in blood.

Also, the tissue section of the liver noted prominent aggregation of kupffer cells around the necrosis region and hemorrhage Fig (4-27), this increase aggregation of kupffer cells in certain liver diseases may be due to an activated defense mechanism against formalin. This histological change of liver after treated with formalin was

agreement with (Sinha and D'Souza, 2010) which showed the liver of male rat when treated with sodium benzoate have enlargement of hepatocytes, and necrosis region.

The histological section of the liver showed hypertrophy in the hepatocytes that have very dark nuclei spherical, the hepatocyte cytoplasm has prominent vacuoles, some hepatocytes lost their nuclei and other cells have destruction nuclei Fig (4-28). Also, the result noted fatty aggregation and interlobular duct filled with secretion. These changes may be to swelling of hepatocyte due to the imbibition of fluid inside the cell. Subsequently, the cells lose their normal shape and become indistinct from each other. This may be because of increased the activity of hepatocyte of liver response to toxicity

These histological change of liver after treated with formalin was the agreement with (Khidr *et al.*, 2012) which showed the liver of male rat when treated with food preservatives caused a liver change, this changes represented by loss most architecture of a liver tissue, enlarged vacuolated cytoplasm, large spaces were detected in some areas due to degeneration of hepatocytes.

The result of the liver after treated with formalin showed many figures of mitotic divisions in the hepatocyte, the blood vessels in the liver parenchyma have congestion with blood, and aggregation of the inflammatory cells a rounded congested blood vessel (Fig.4-29). This histological change of liver after treated with formalin was agreement with (Mamun *et al.*, 2014) which noted the liver of mice when treated with formalin caused degenerations in tissues, congestion and dilatation in the central vein, enlargement in the sinusoids, hemorrhages inside the tissue and reduced compactness of the cells by the formation of intercellular spaces. These results may be due to materials toxicity of formalin metabolism caused damage hepatic cord and swelling the hepatocyte with pressure on the sinuses lead to the hemorrhage.

4.1.2.2 The Histological Results of Male Treated Group after 30 Days Orally Administration with Formalin

The present result of the liver after 30 days of oral administration with 0.5ml formalin noted there were significant increase in the thickness of the liver capsule with ($6.87 \pm 0.17 \mu\text{m}$) and the diameter of the hepatocyte nucleus with ($9.47 \pm 0.14 \mu\text{m}$) which showed in (Table 4-2) and (Appendix-33) (Appendix-34). The results of the male tissue control group similarly to female that showed hepatocytes, kupffer cells and sinusoids Fig (4-25, 26) which different from treated group that noted the liver parenchyma have cystic dilation filled with blood surround by aggregation of inflammatory cell showed clear shrinkage hepatocyte and extensive cytoplasmic vacuolization these may be due to damage of hepatocyte left cystic dilation while the aggregation of inflammatory cells referred to immune responses (Fig.4-30).

The tissue section of the liver showed aggregation of kupffer cells with different figures of mitotic division Fig (4-31). The hepatic cord noted abnormal in shape as short segments randomly distributed a rounded the central vein. The hepatocytes have prominent cytoplasm vacuoles and other have destruction nuclei Fig (4-32). The results showed the liver parenchyma have cellular degeneration with hemorrhage in different location of parenchyma. So, the sinusoid was filled with blood as blood congestion Fig (4-32). These histological change of liver after treated with formalin was the agreement with (Uçmakli *et al.*, 2013) which noted degeneration in hepatocytes and kupffer cell hyperplasia in the liver of male rat when treated with formaldehyde.

Most histological changes that seen in the tissue sections of the liver which included abnormal cellular structure, cytoplasmic vacuoles, hemorrhage, blood congestion were may be because of exposure to toxic material that effects on the liver tissue which lead to these changes. Our results were similar to (Verma *et al.*, 2016) which noted the liver of male rat when treated with formalin caused dilated and congested central vein, ballooning degeneration with fatty changes of cell cytoplasm.

The current result in both male and female treated groups administration with 0.5ml formalin showed non-significant differences in the thickness of liver capsule between

male and female treated groups (Table 4-2) and (Appendix-35) in addition to the diameter of hepatocytes nuclei didn't show any significant differences between both treated groups of study (Appendix-36).

4.1.2.3 The Histological Results of Female Treated Group after 30 Days Orally Administration with Citric acid

In (Table 4-2) noted the liver tissue after treated with 1ml citric acid showed the thickness of the liver capsule was ($6.22 \pm 0.20 \mu\text{m}$), which have significant differences with capsule thickness in a control group that noted in (Appendix-37).

The (Table 4-2) showed the measurement of diameter hepatocytes nuclei in the treated group was ($8.868 \pm 0.12 \mu\text{m}$). Also, the result noted didn't have any significant difference compared with diameter of hepatocytes nuclei in the control group, (Appendix-38). These results referred as the treated with citric acid have slight effects on the liver capsule of the treated group.

The histological result of the liver showed the liver parenchyma has prominent degeneration, damage in hepatic cords and large spaces were detected in some areas due to the degeneration of hepatocytes Fig (4-33). This result may be due to the lysosome action, which are very sensitive to any pathologic effect, are ruptured and then released their powerful enzymes, which cause degeneration and dissolution of various cellular compounds. This histological change of liver after treated with citric acid were agreement with (Aktaş *et al.*, 2003) which noted the liver of male mice when treated with citric acid have necrotic lesions. These changes were degeneration of tissue, cytoplasmic vacuolization, damage in hepatic cords and hemorrhage.

The tissue section of the liver showed dilation in the sinusoids with hemorrhage Fig (4-33). This histological change of liver after treated with citric acid was constant with (Abushofa *et al.*, 2019) which noted the liver sections after treated with preservatives showed dilation of blood sinusoids appear between hepatocytes with vacuoles in the cytoplasm.

The current result in the liver of the treated group with citric acid appeared enlargement or hypertrophy of hepatocytes in small segments of destruction hepatic cords (Fig.4-34, 35). The tissue figures showed wide space a rounded the central vein some of its filled with blood and surrounded by aggregation of inflammatory cells Fig (4-34). These findings were maybe to effects of citric acid toxicity vessels caused hepatic degeneration in the liver parenchyma.

The histological result of the liver showed some hepatocytes were devoid of nuclei this may due to cell has undergone lyses leaving wide empty space and hemorrhage may be due to rupture of the vessel wall because of the dose of citric acid Fig (4-35). These histological changes of liver after treated with citric acid were agreement with (Bakar and Aktac, 2014) which noted the liver of male rat when treated with food preservatives caused vacuolization and nucleus losses, hypertrophic hepatocyte, degeneration of hepatocytes, were seen in liver tissue these maybe to toxicity of citric acid lead to high damage in the liver.

4.1.2.4 The Histological Results of Male Treated Group after 30 Days Orally Administration with Citric acid

The tissue section of liver after 30 days of treated oral administration with 1ml citric acid showed no significance increased in the thickness of the capsule ($6.25 \pm 0.20 \mu\text{m}$) and the diameter of hepatocyte nuclei ($8.793 \pm 0.12 \mu\text{m}$) when compared with the control group that noted in (Table 4-2), (Appendix-39), and (Appendix-40).

The tissue section noted the main histological changes in the liver which were similar to histological changes in the female treated groups with citric acid that noted cystic dilation filled with blood, aggregation of inflammatory cells and damage in the hepatic cord Fig (4-36). The static analysis noted slightly significant changes in the measurement of liver capsule and diameter of hepatocyte nuclei between the treated and control groups.

These histological changes of the liver after treated with citric acid was an agreement with (Aktac *et al.*, 2008) which noted the liver of male mice when treated with citric acid caused necrotic changes, loosing and combining of hepatocyte membranes, cytoplasmic vacuolization in hepatocytes, and aggregation of inflammatory cells in small areas perhaps to the immune response.

In (Table 4-2) demonstrated the thickness of the liver capsule and diameter of hepatocytes nuclei didn't have significant differences between female and male (Appendix-41) and (Appendix-42). The histological result of the liver showed enlargement hepatocytes with prominent vacuoles in the cytoplasm of hepatocytes, and aggregation of kupffer cells Fig (4-37). This histological change of liver after treated with citric acid was an agreement with (Agarwal *et al.*, 2016) which noted the liver of rat when treated with food additives caused vacuolization in the cytoplasm, damage of hepatocytes, dilated central vein and hemorrhage this may be due to the materials toxicity of the citric acid metabolism lead to high stress on the hepatocyte caused swelling then rapture.

4.1.3 The Histological Result of the Duodenum

4.1.3.1 The Histological Result of Female Treated Group after 30 Days Orally Administration with Formalin

The histological result of the duodenum in the female treated group after oral administration of 0.5ml formalin showed the thickness of the epithelial layer was ($2.364 \pm 0.056 \mu\text{m}$) which have significantly increase compared with the control group as shown in (Table 4-3) and (Appendix-43). These change in the duodenum wall unlike the control groups that composed from four distinct layers (Mucosa, Submucosa, Muscular, and Serosa). The mucosal layer which projected into the lumen as leaf-like projections called villi covered by epithelial layer Fig (4-38). This increased in the thickness because of the enlargement of villi due to the founded prominent cytoplasmic vacuoles in the cytoplasm of epithelial cells Fig (4-40) These food additives may be increased

intestinal permeability because of the damage in intestinal epithelial layer, this result agreement with (Lerner and Matthias, 2015).

Most tissue section of duodenum showed wide cystic dilation between submucosal and muscular layers which lead to separate the mucosa into the lumen of the duodenum Fig(4-40). This result referred to the acute effected of formalin on the mucosal layer which lead to exfoliated the epithelial in to the lumen of the duodenum was similar to (Feick *et al.*, 2006) which showed that exposure to formaldehyde caused disrupted of intestinal epithelial cells.

Also, the result noted vascular congestions in the submucosal and muscular layer of the duodenum Fig (4-41) these results may be due to the toxicity of formalin caused damage to epithelial layer lead to damage vessels blood, these result in accordance with the finding of (Pandey *et al.*, 2000) which showed formalin causing gastrointestinal hemorrhage.

4.1.3.2 The Histological Result of Male Treated Group after 30 days of formalin oral administration

The present study of the duodenum of male mice after treated with 0.5ml formalin oral administration showed an increase in the thickness of the epithelial layer ($2.415 \pm 0.052 \mu\text{m}$) compared with the control group in (Table 4-3) and (Appendix-44). The results noted there was found tissue change in male treated with formalin when compared with control group Fig (4-39). In (Table-4-3) this statistical analysis didn't note any significant differences between the thickness of the epithelia between male and female control groups.

The present study of the male and female treated groups noted similar histological changes in the wall of duodenum in both treated groups with formalin. So, didn't have any significant differences between statistical analysis in the epithelial layer thickness between male and female treated groups that noted (Appendix-45).

These findings referred to the formalin have a same effect on the histological structures of the duodenum wall in the male and female treated groups Fig (4- 42). These results in agreement with the finding of (Elbanna *et al.*, 2017) which showed desquamation of the surface epithelium and degeneration in the intestinal crypts when treated rats with food dyes that used in the dairy product.

4.1.3.3 The Histological Result of Female Treated Group after 30 Days Orally Administration of Citric acid

Light microscopically result of the duodenum after treated with 1ml citric acid oral administration demonstrated the thickness of the epithelial layer was ($2.123 \pm 0.054 \mu\text{m}$) which have slightly significant increased compared with the control group in (Table 4-3) and (Appendix-46). This result was disagreement with the earlier workers (Nourmohammadi and Afzali, 2013) who reported decreased the epithelial thickness of the duodenum after treated with citric acid.

The histological results of the duodenum showed exfoliated the epithelial layer that covering the intestinal villi, exodus the core of villi into the lumen of the duodenum, and separated the submucosa from the muscular layer and showed with cystic dilation. Also the muscular layer noted wide degeneration in the muscular layer Fig (4-43) when compared with control group Fig (4-38). These histological change of duodenum after treated with citric acid were coincide with (Eweka and Om'Iniabo, 2007) which showed the food preservatives effect on the small intestine of rat caused distortions in the epithelia of the small intestine.

4.1.3.4 The Histological Result of Male Treated Group after 30 Days Orally Administration of Citric acid

The histological result of duodenum after treated with 1ml citric acid oral administration showed the thickness of the epithelial layer was ($2.107 \pm 0.055 \mu\text{m}$) which

have no significant increased compared with the control group, also the results noted non-significant differences between male and female treated groups with citric acid (Table 4-3) and (Appendix-47).

The mucosa layers of the duodenum in the male treated group with citric acid have prominent destruction of the epithelial layer that lining the internal lumen of the duodenum. So, the result showed prominent blood congestion in different regions of the submucosal layer with hemorrhage as in a treated group with formalin this mucosa was separated from submucosa these results may be to damage to the mucosa layer because of acidity of citric acid caused ulcer these change damage blood vessels that found in these layers lead to hemorrhage Fig (4-44) when compared with control group Fig (4-39).

Also, the results noted empty cystic dilation maybe because of the high toxicity of the food preservatives on the histological structures of the duodenum. These results were in a deal with (Roca-saavedra *et al.*, 2016) which noted the dietary emulsifiers caused damage to the mucus layer thickness. The current result showed non-significant in the epithelial thickness between both male and female treated groups with citric acid (Table 4-3), and (Appendix-48).

Table 4-1. Diameter the part of the kidney (control and treated group)

Descriptive Statistics N=50				
	SEX	Treatment	Mean	Std.D
Renal	Female	Control	22.69±0.61	4.35
		Formalin	24.81±0.49	3.49
		Citric acid	24.48±0.65	4.62
	Male	Control	22.92±0.55	3.87
		Formalin	24.65±0.38	2.69
		Citric acid	24.45±0.48	3.39
Proximal convoluted tubules	Female	Control	9.78±0.22	1.55
		Formalin	10.44±0.21	1.5
		Citric acid	10.06±0.18	1.26
	Male	Control	9.63±0.19	1.33
		Formalin	10.57±0.17	1.22
		Citric acid	10.08±0.19	1.31
Distal convoluted tubules	Female	Control	18.19±0.28	1.97
		Formalin	15.34±0.20	1.42
		Citric acid	16.54±0.27	1.91
	Male	Control	18.06±0.30	2.10
		Formalin	15.40±0.21	1.52
		Citric acid	16.82±0.24	1.71
Descending limp of Henley loop	Female	Control	9.79±0.16	1.16
		Formalin	10.72±0.17	1.22
		Citric acid	10.07±0.15	1.10
	Male	Control	9.64±0.15	1.07
		Formalin	10.24±0.17	1.17
		Citric acid	10.171±0.13	0.923
Ascending limp of Henley loop	Female	Control	12.16±0.17	1.17
		Formalin	13.68±0.18	1.24
		Citric acid	12.8±0.16	1.15
	Male	Control	12.18±0.17	1.19
		Formalin	13.63±0.16	1.13
		Citric acid	12.59±	1.10

Table 4-2. Thickness of liver capsule and diameter of hepatocyte nuclei.

Descriptive Statistics N=50				
	SEX	Treatment	Mean	Std. D
Thickness of liver capsule	Female	Control	5.72±0.14	1.03
		Formalin	6.93±0.17	1.25
		Citric acid	6.22±0.20	1.46
	Male	Control	5.789±0.13	0.938
		Formalin	6.87±0.17	1.27
		Citric acid	6.25±0.20	1.47
Diameter of hepatocyte nuclei in liver	Female	Control	8.571±0.10	0.742
		Formalin	9.646±0.13	0.964
		Citric acid	8.868±0.12	0.847
	Male	Control	8.595±0.10	0.726
		Formalin	9.47±0.14	1.03
		Citric acid	8.793±0.12	0.870

Table 4-3. Thickness of epithelial layer

Descriptive Statistics N=50				
	SEX	Treatment	Mean	Std. D
Thickness of epithelial layer	Female	Control	1.960±0.053	0.372
		Formalin	2.364±0.056	0.399
		Citric acid	2.123±0.054	0.384
	Male	Control	1.975±0.050	0.356
		Formalin	2.415±0.052	0.369
		Citric acid	2.107±0.055	0.392

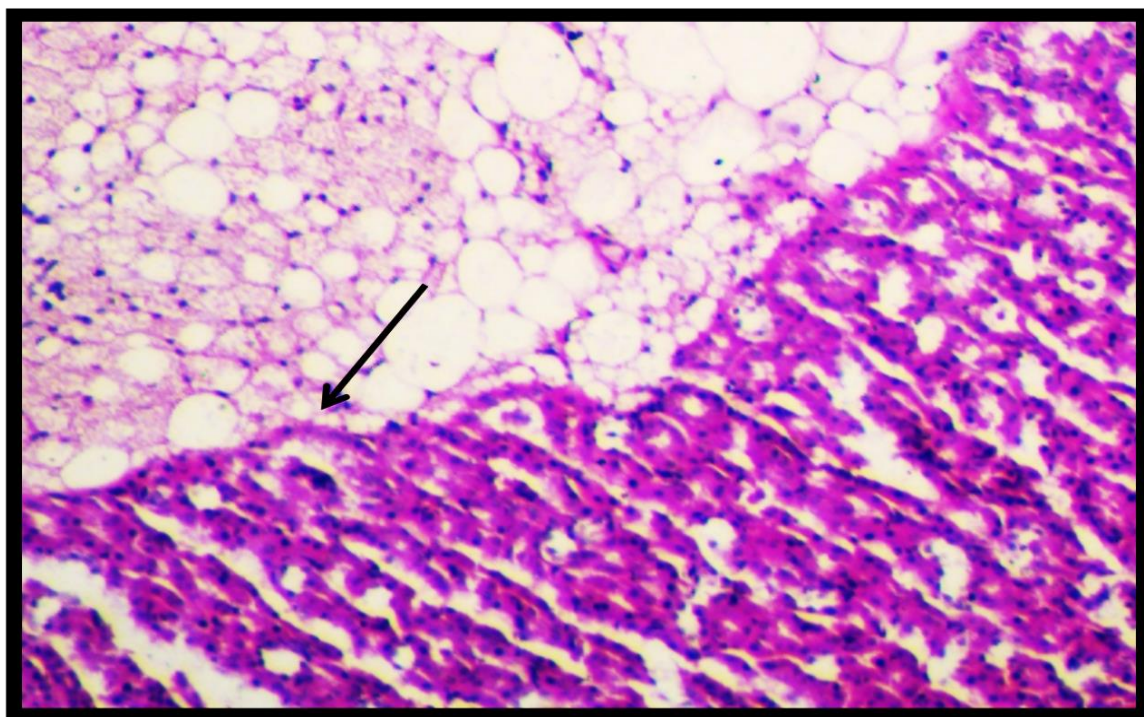


Fig 4-1. Transverse section of female kidney in control group which showed the capsule of kidney. H&E stain 20X.

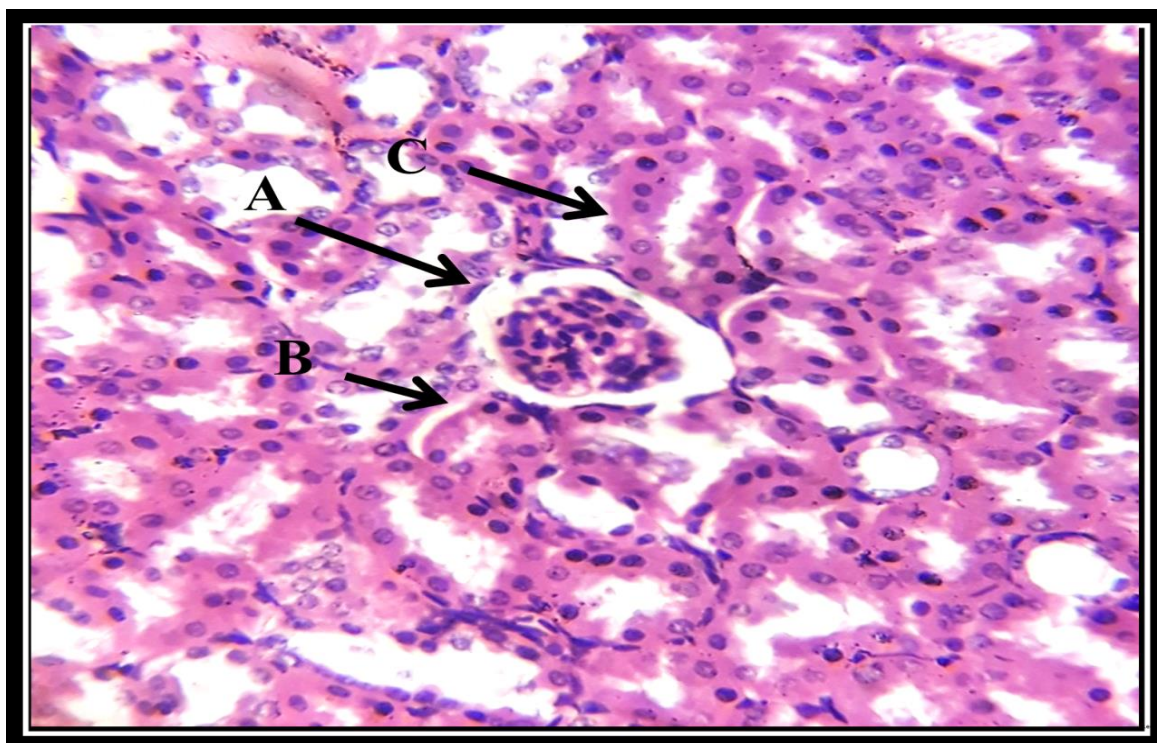


Fig 4-2. Transverse section of female kidney in control group which showed A-Renal corpuscle, B -Proximal convoluted tubule, C-Distal convoluted tubule. H&E stain 40X.

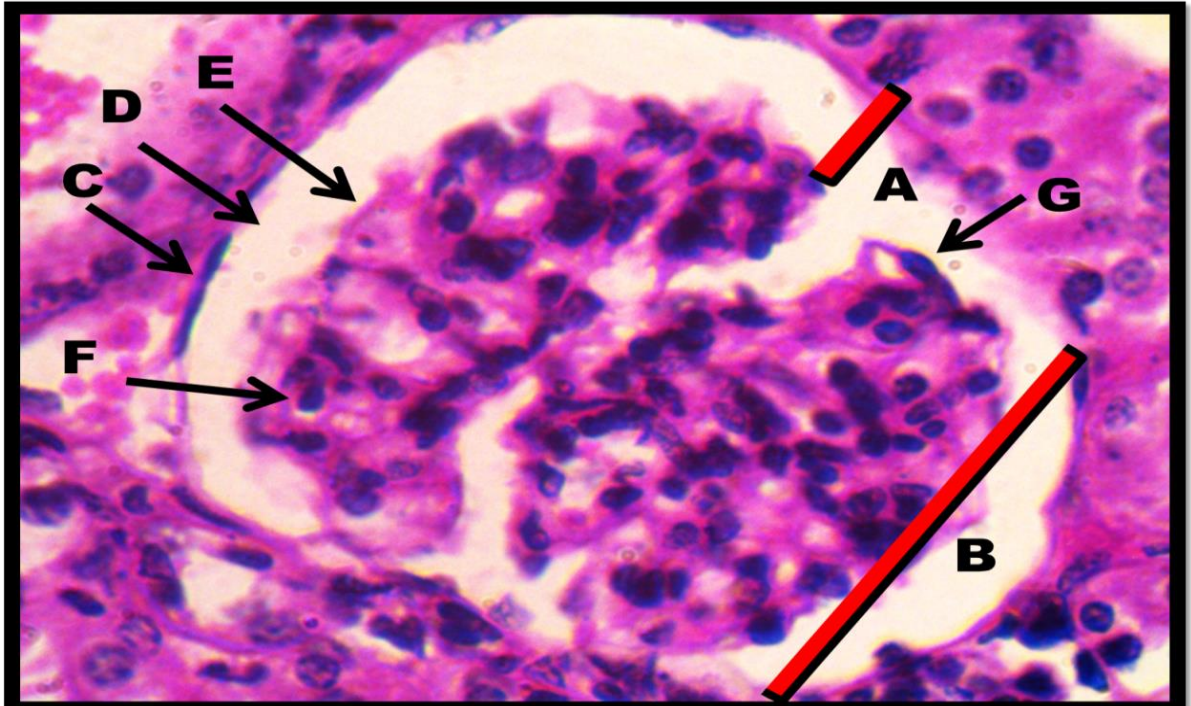


Fig.4-3. Transverse section of female kidney in control group which showed A- Bowman's capsule, B- Glomerulus, C-Parietal layer, D- Bowman's space E- visceral layer, F- Mesangial cells and G- Podocytes. H&E stain 100X.

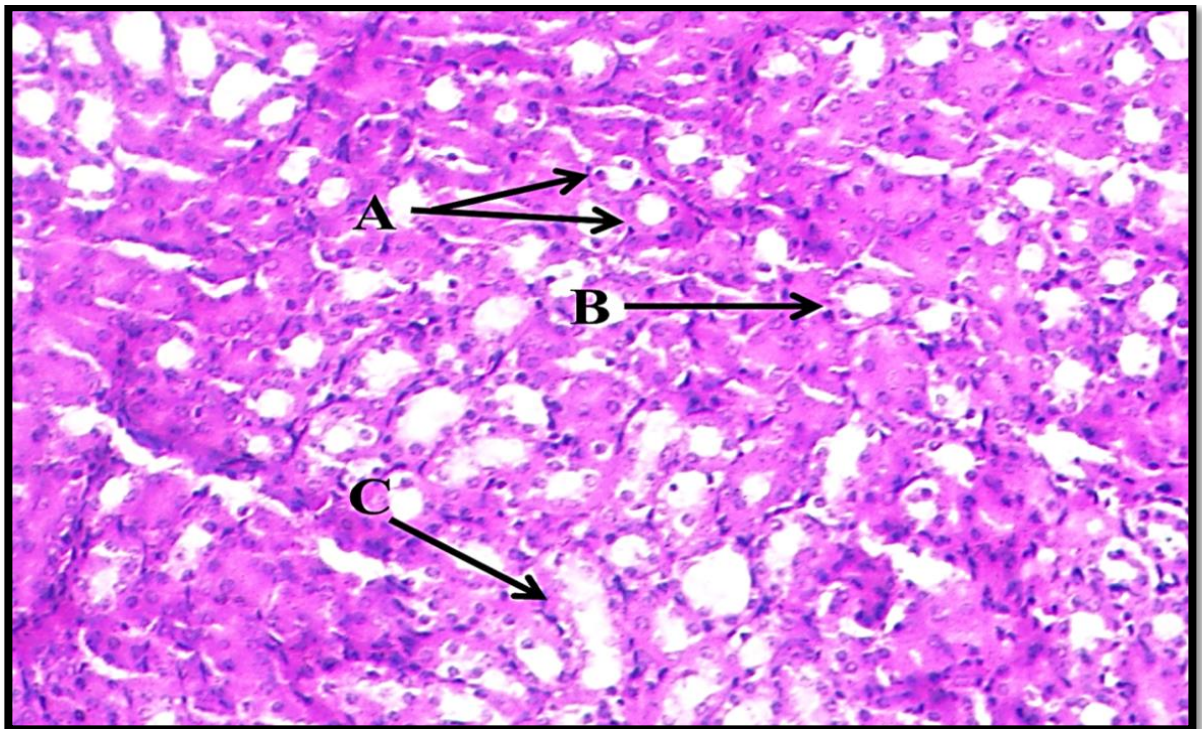


Fig.4-4. Transverse section of female kidney in control group which showed A- Descending branch of Henley loop, B- Ascending branch of Henley loop, C-Collecting duct. H&E stain 20X.

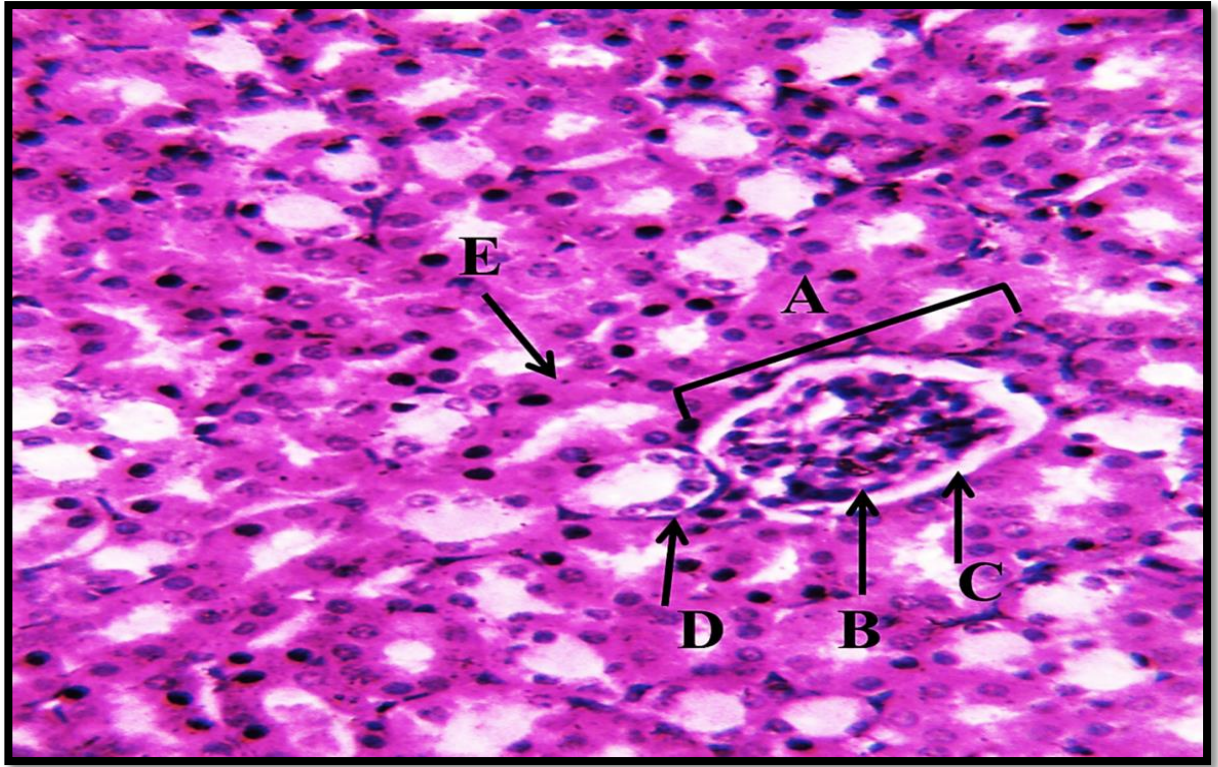


Fig 4-5. Transverse section of male kidney in control group which showed A-Renal corpuscle, B-Glomerules C-Bowman's space D-Proximal convoluted tubule, E-Distal

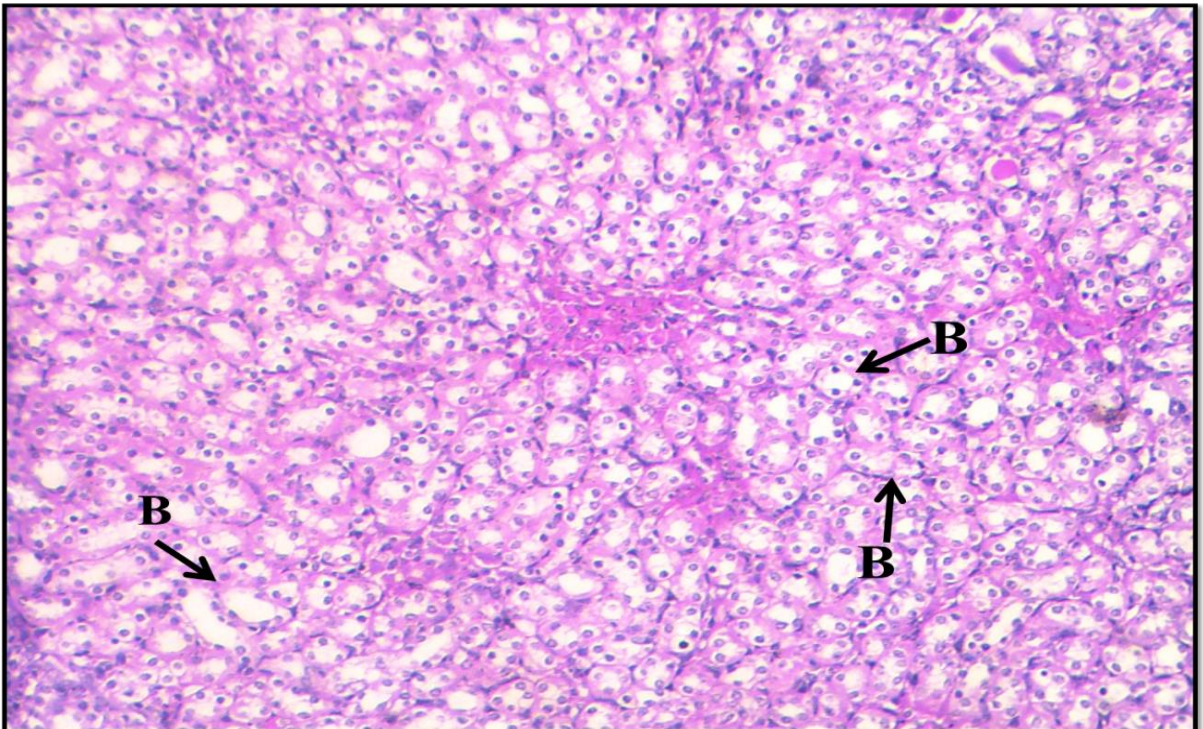


Fig 4-6. Transverse section of male kidney in control group which showed A- Descending convoluted tubule, B- Ascending convoluted tubule, C-Collecting duct. H&E stain 10X.

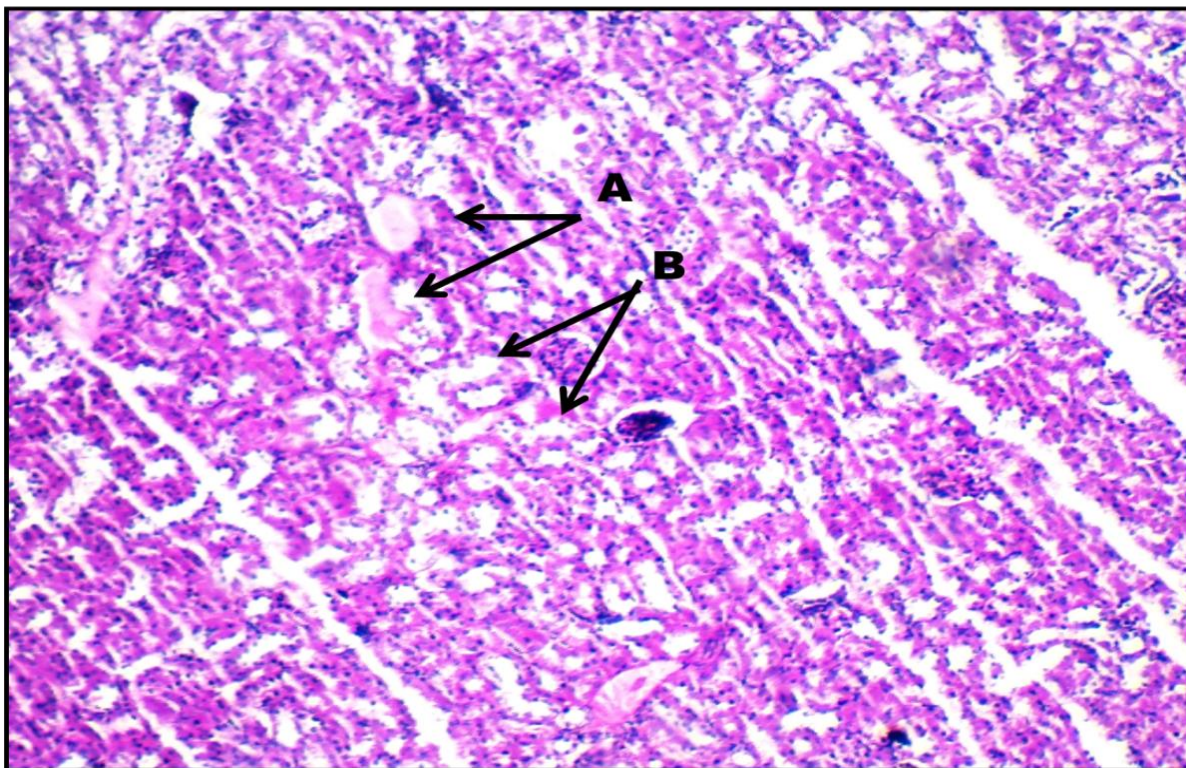


Fig 4-7. Transverse section of female kidney in treated group (formalin) which showed A- Vascular congestion, B- Degeneration. H&E stain 10X.

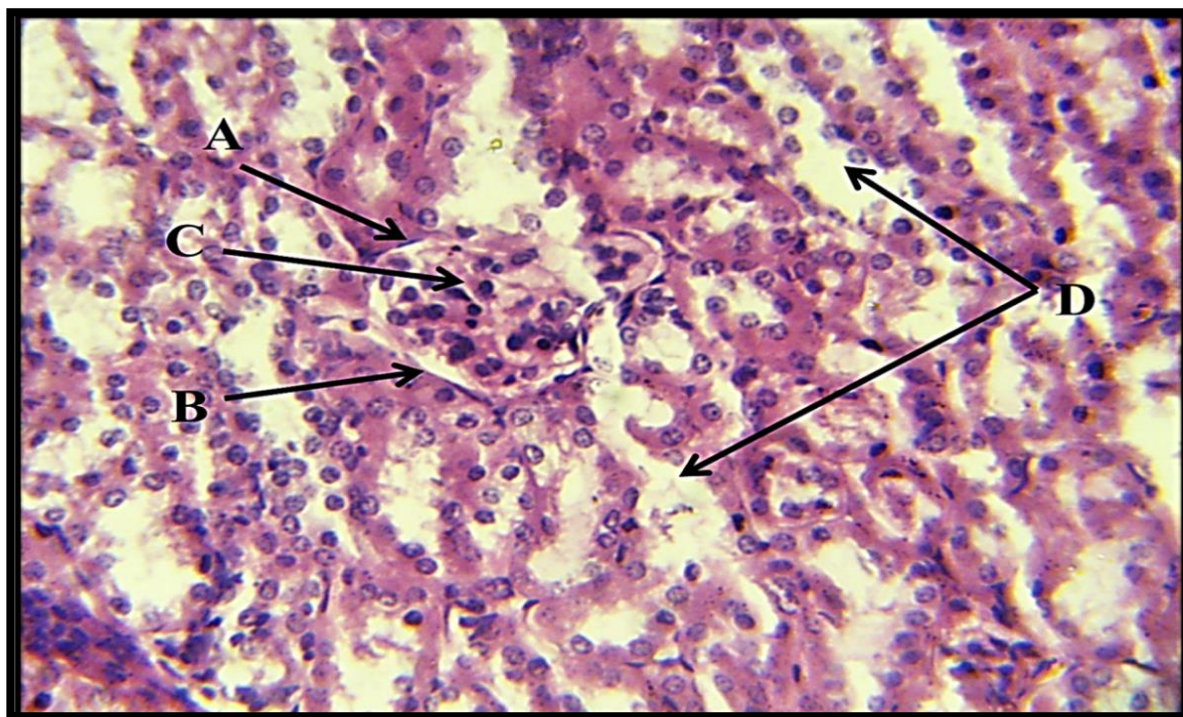


Fig 4-8. Transverse section of female kidney in treated group (formalin) which showed A- Parietal layer, B- Bowman's space, C- Mesangial cell, D- Degeneration area. H&E stain 40X.

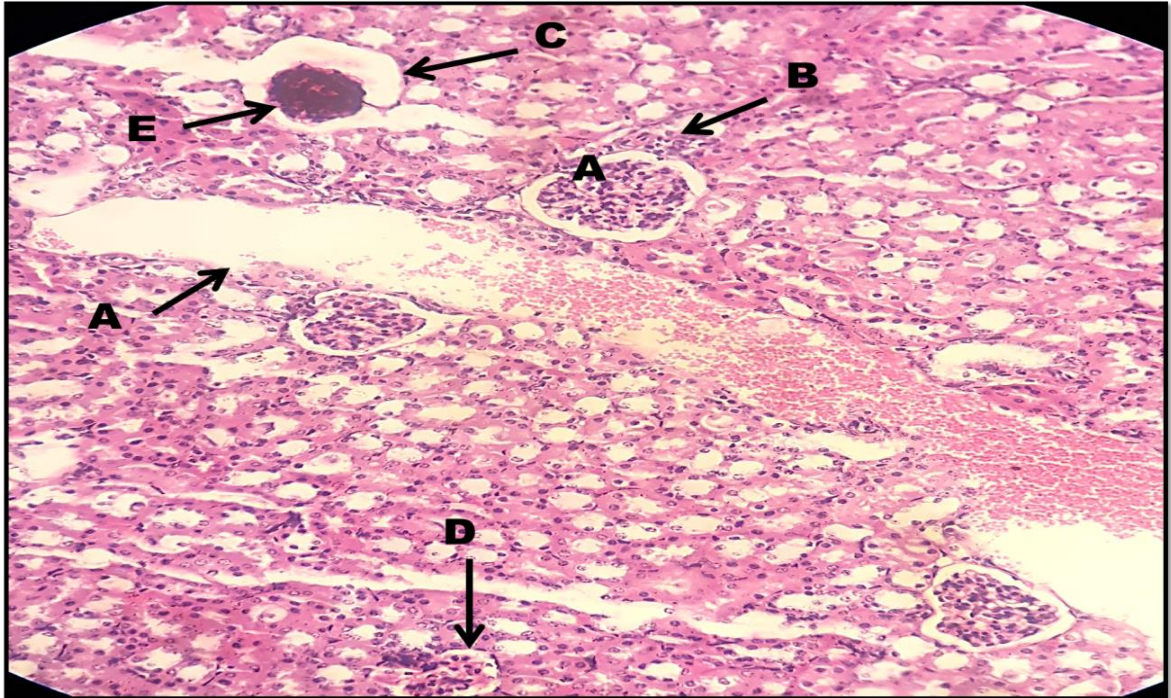


Fig 4-9. Transverse section of female kidney in treated group (formalin) which showed A-Cystic dilation, B-Aggregation inflammatory cells, C-Wide Bowmen space, D-Damage Bowmen Space E- progressive of glomerullus capilleries. H&E stain 20X.

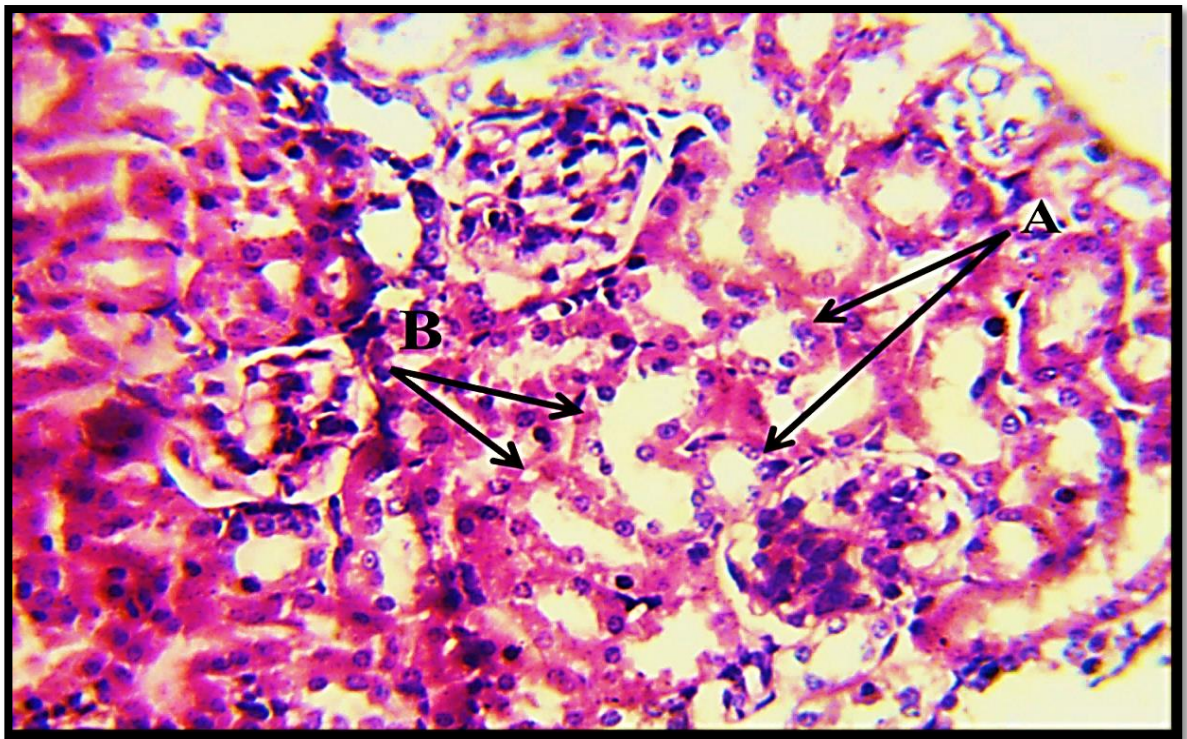


Fig 4-10. Transverse section of the female kidney in treated group (formalin) which showed exfoliated epithelia layer from: A-Proximal convoluted tubules, B-Distal convoluted tubules. H&E stain 40X.

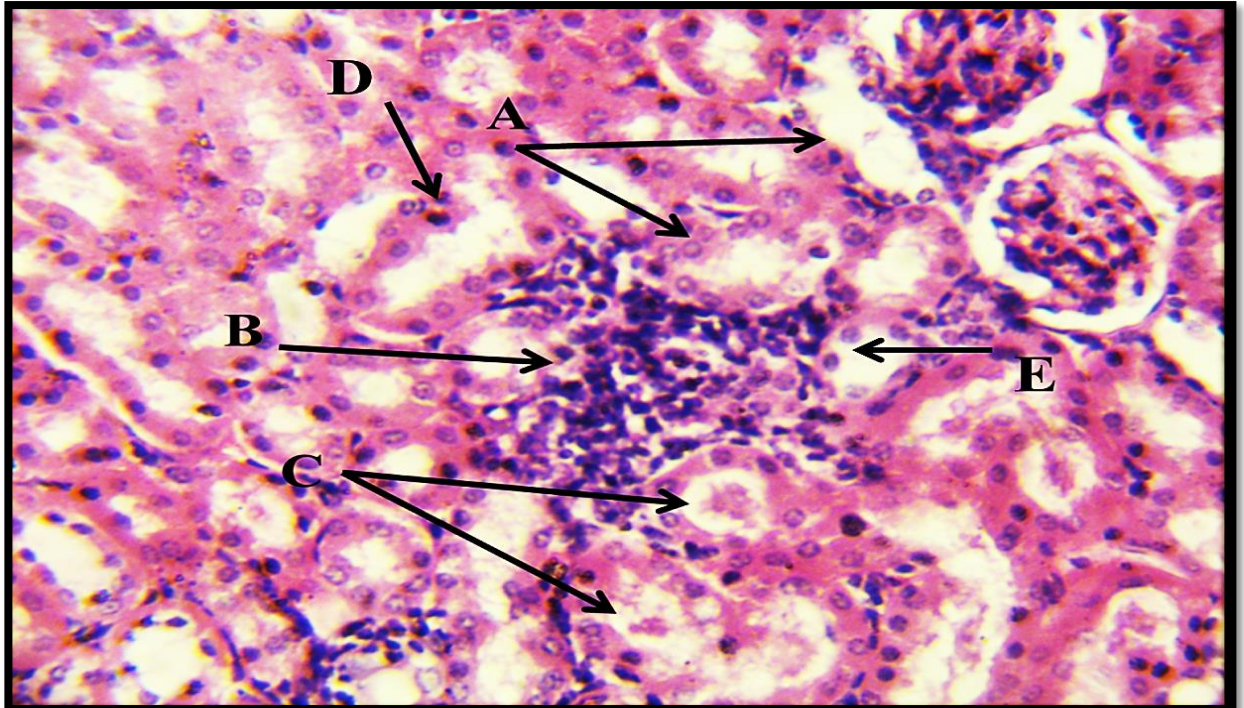


Fig 4-11. Transverse section of female kidney in treated group (formalin) which showed A- Degeneration in distal convoluted tubules, B- Aggregation of inflammatory cells, C- Infiltration in renal tubules D-Destruction epithelia layer E-Loss of brush border. H&E stain 40X.

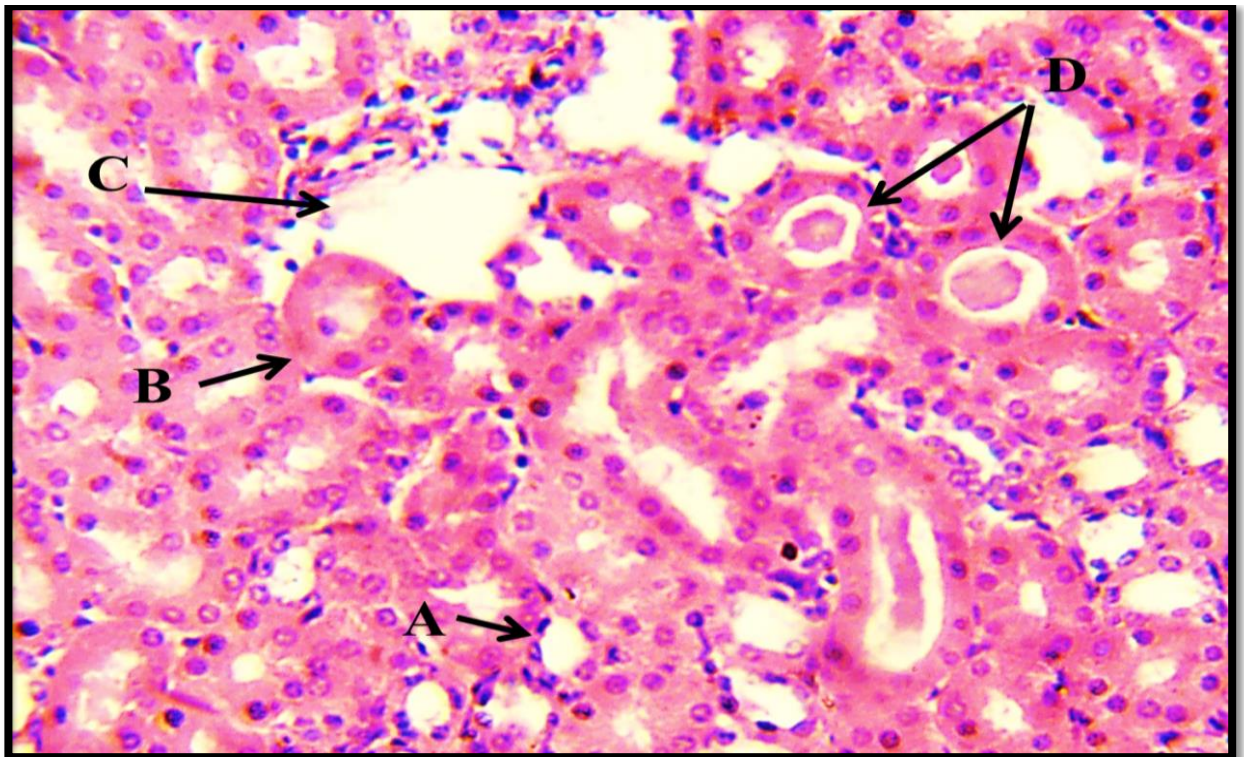


Fig 4-12. Transverse section of female kidney in treated group (formalin) which showed A- Descending branch of Henley loop, B- Ascending branch of Henley loop C- Cystic dilation, D- Thick spots of infiltration H&E stain 40X

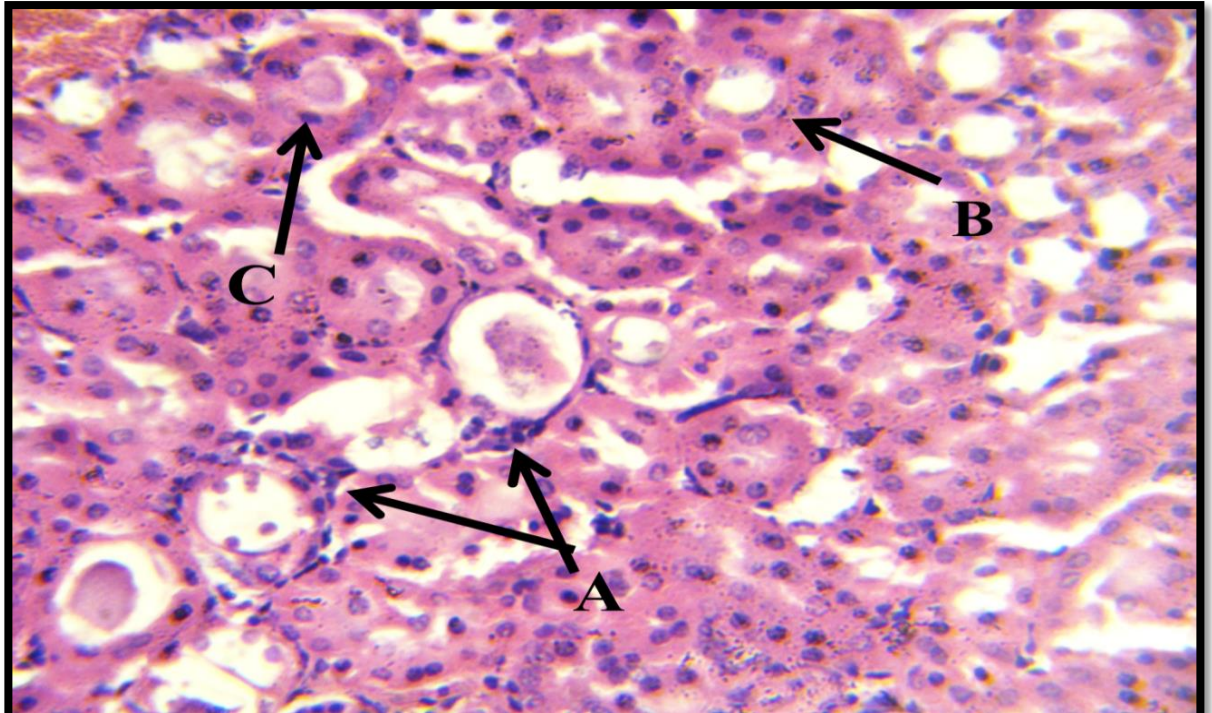


Fig 4-13. Transverse section of female kidney in treated group (formalin) which showed A-Aggregation of inflammatory cells, B-Descending branched C-Ascending branched filled with thick fluid H&E stain 40X.

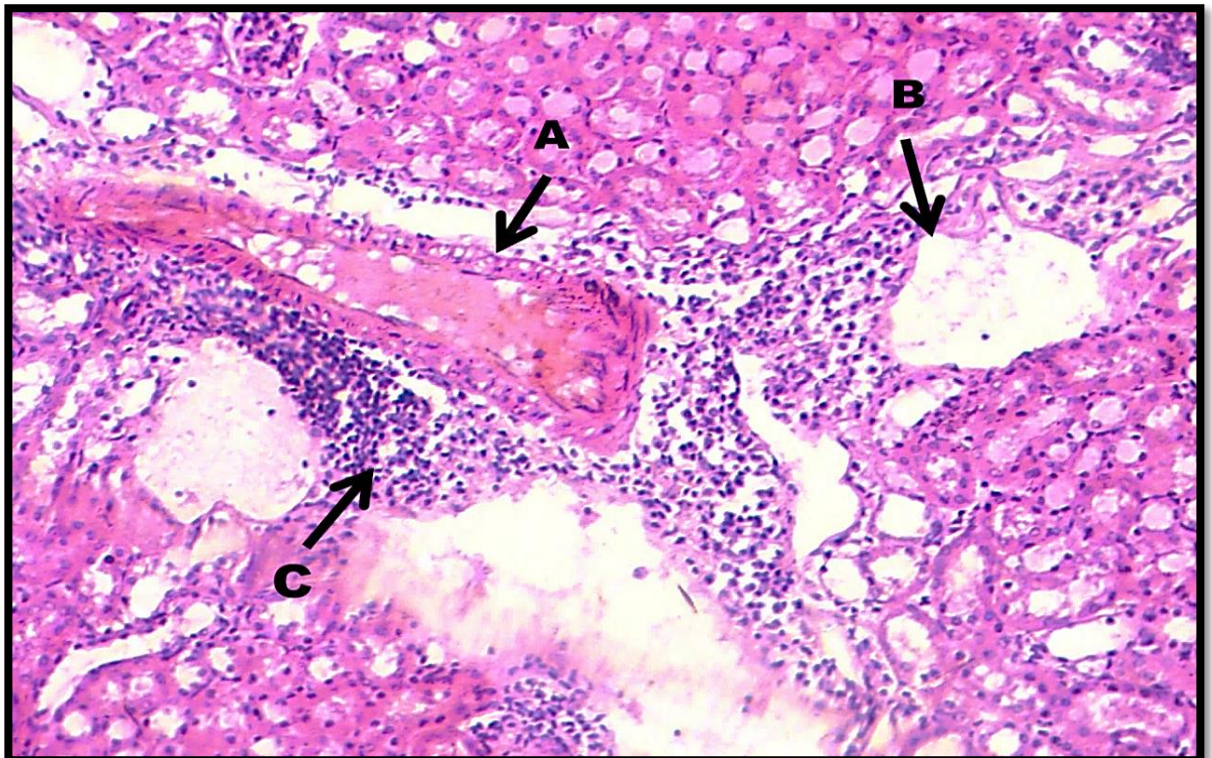


Fig 4-14. Transverse section of male kidney in treated group (formalin) which showed A- Vascular congestion, B- Degeneration area, C-Aggregations of inflammatory cells-.H&E stain 10X

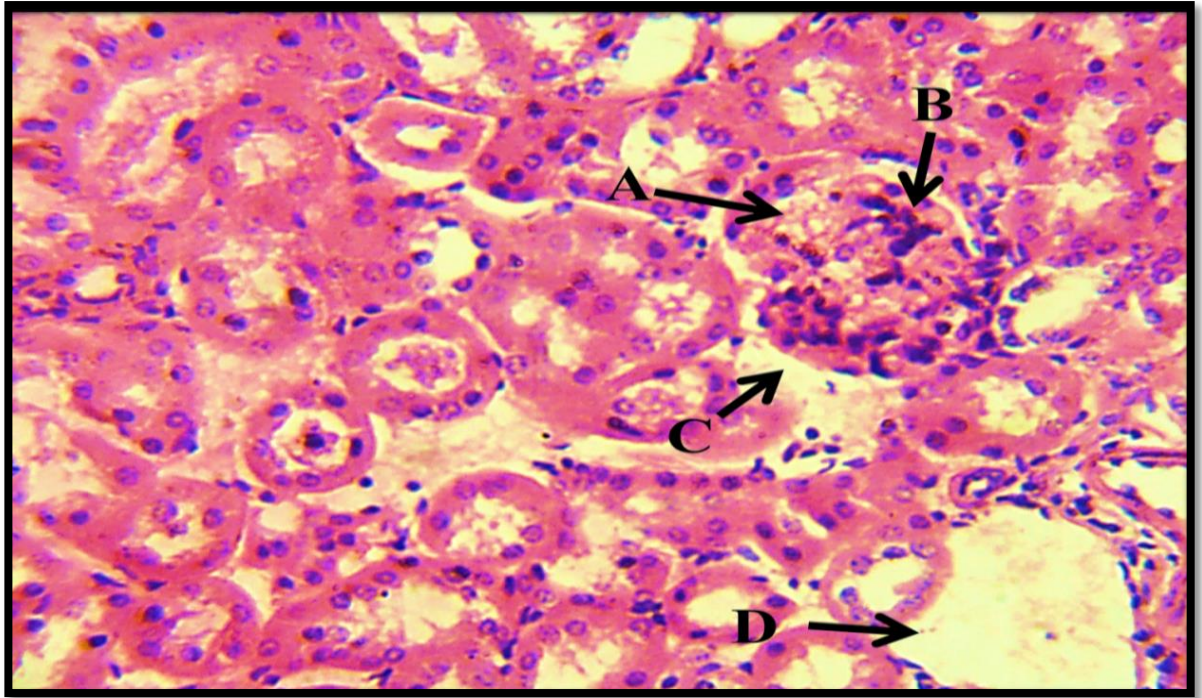


Fig 4-15. Transverse section of male kidney in treated group (formalin) which showed A-Hemorrhage glomerular capillaries, B-Dark nuclei of mesangial cell C-Damage in Bowman's space, D-Cystic dilation. H&E stain 40X.

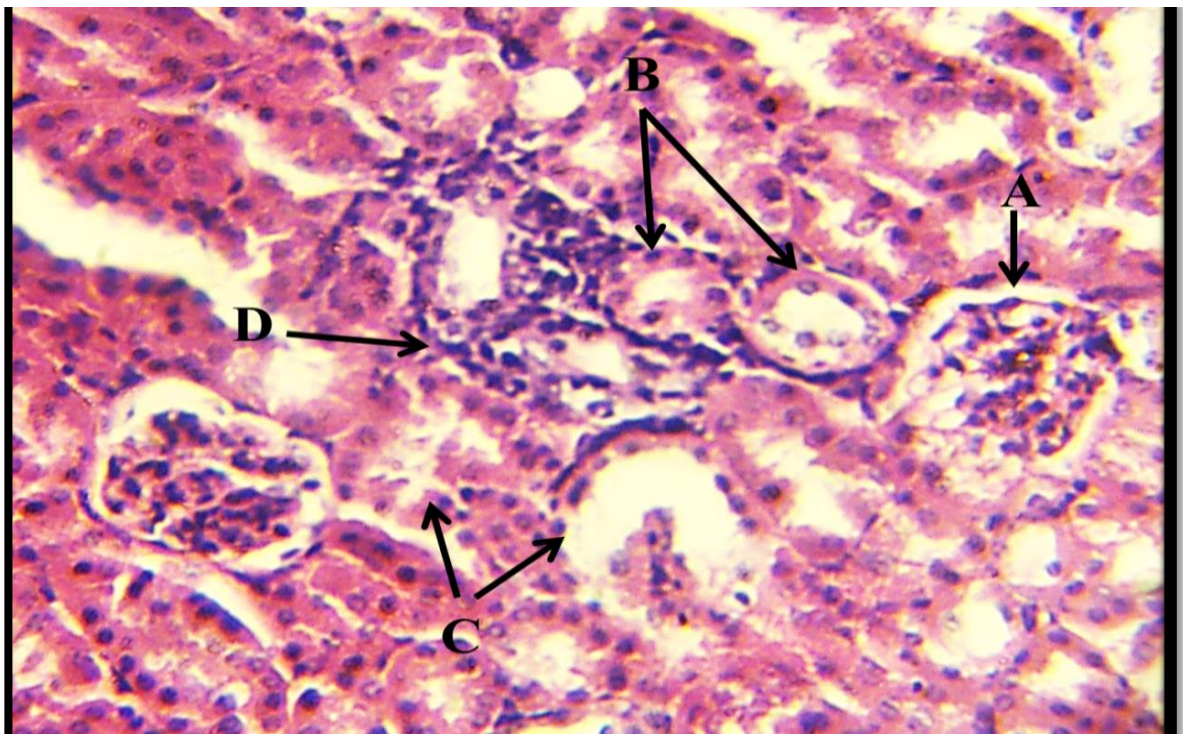


Fig 4-16. Transverse section of male kidney in treated group (formalin) which showed A-Renal corpuscle, B-Proximal convoluted tubules C-Distal convoluted tubules D-Aggregation of inflammatory cells. H&E stain 40X.

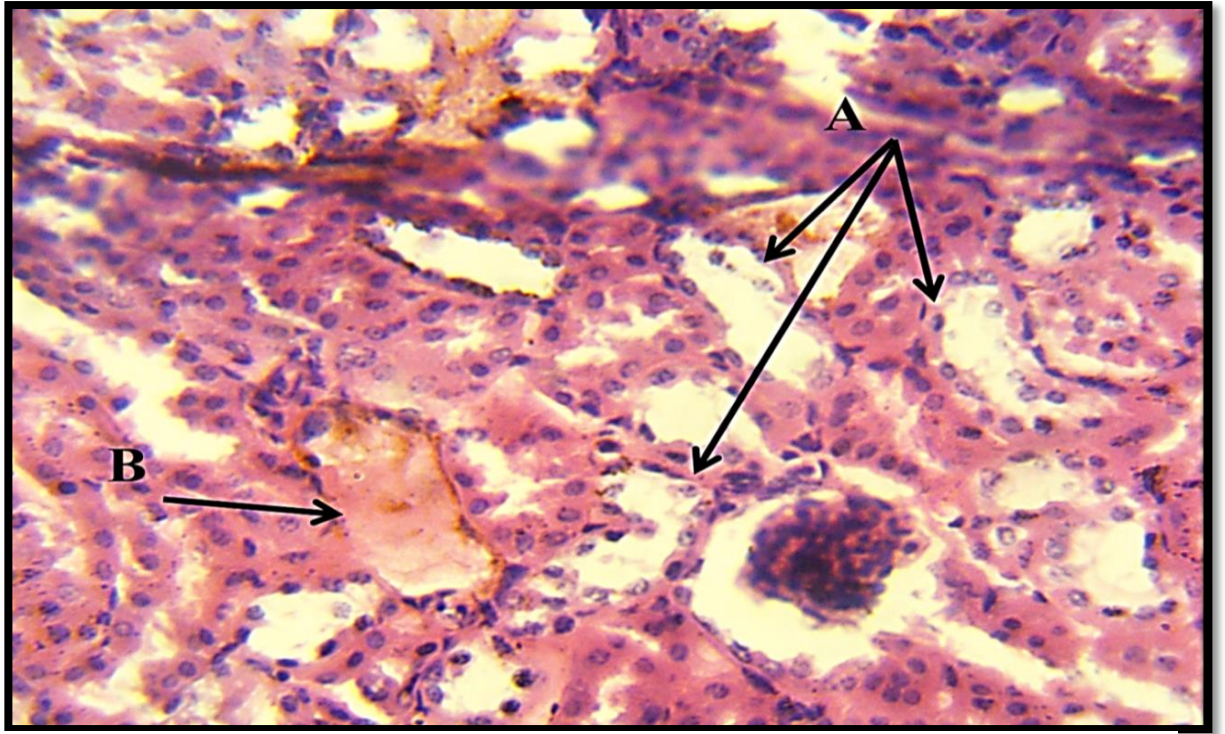


Fig 4-17. Transverse section of male kidney in treated group (formalin) which showed A- Damage in distal convoluted tubules B-Hemorrhage. H&E stain 40X.

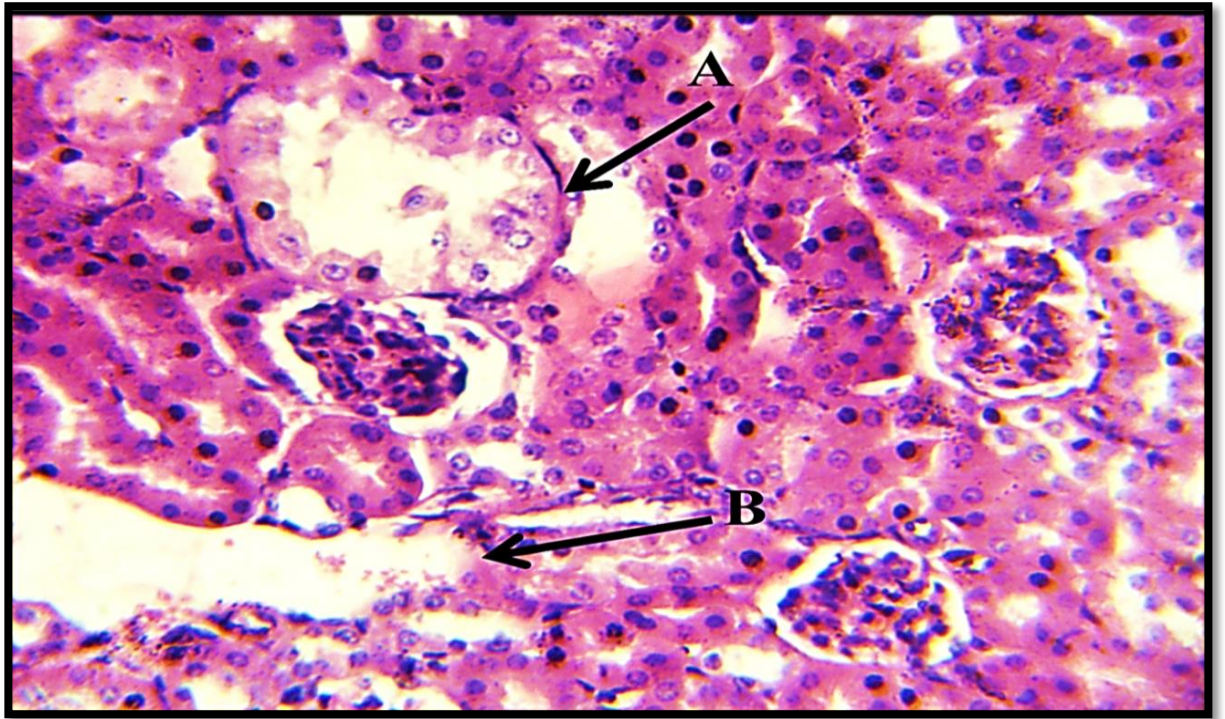


Fig 4-18. Transverse section of male kidney in treated group (formalin) which showed A- Enlargement in the distal convoluted tubules B-Degeneration tubules. H&E stain X 40.

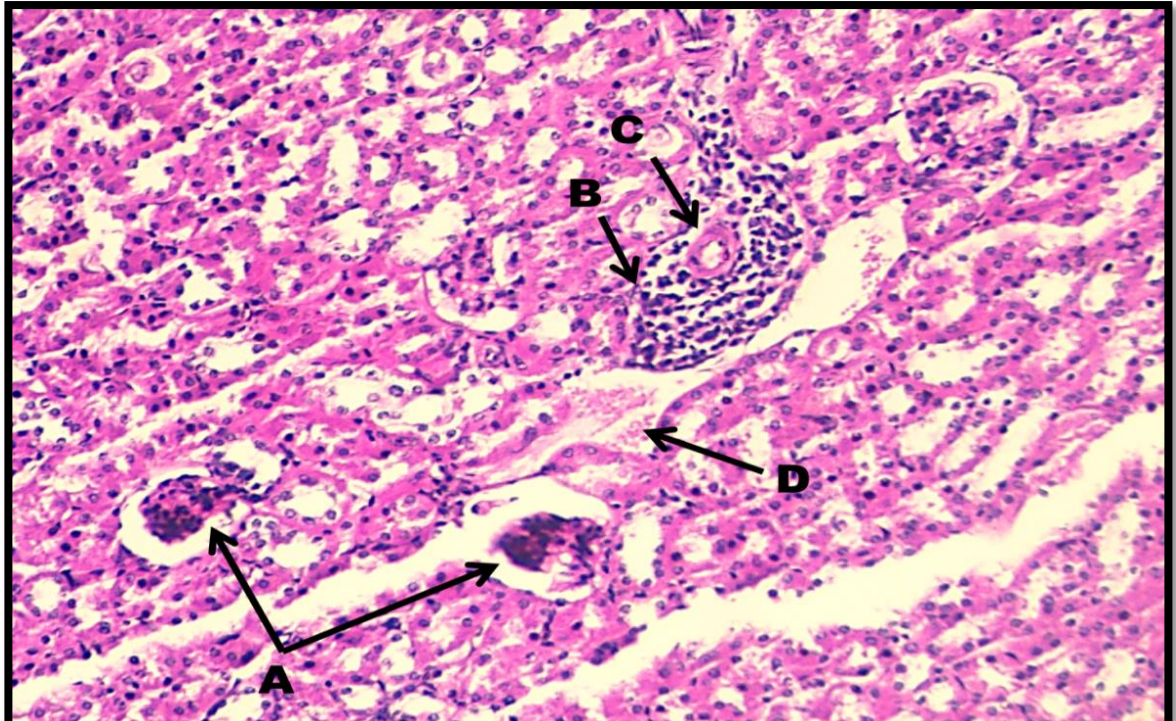


Fig 4-19. Transverse section of female kidney in treated group (citric acid) which showed A- Wide bowmans space, B-Aggregation of inflammatory cells, C-Vascular congestion, and D-Cystic dilation filled with blood. H&E stain 20X

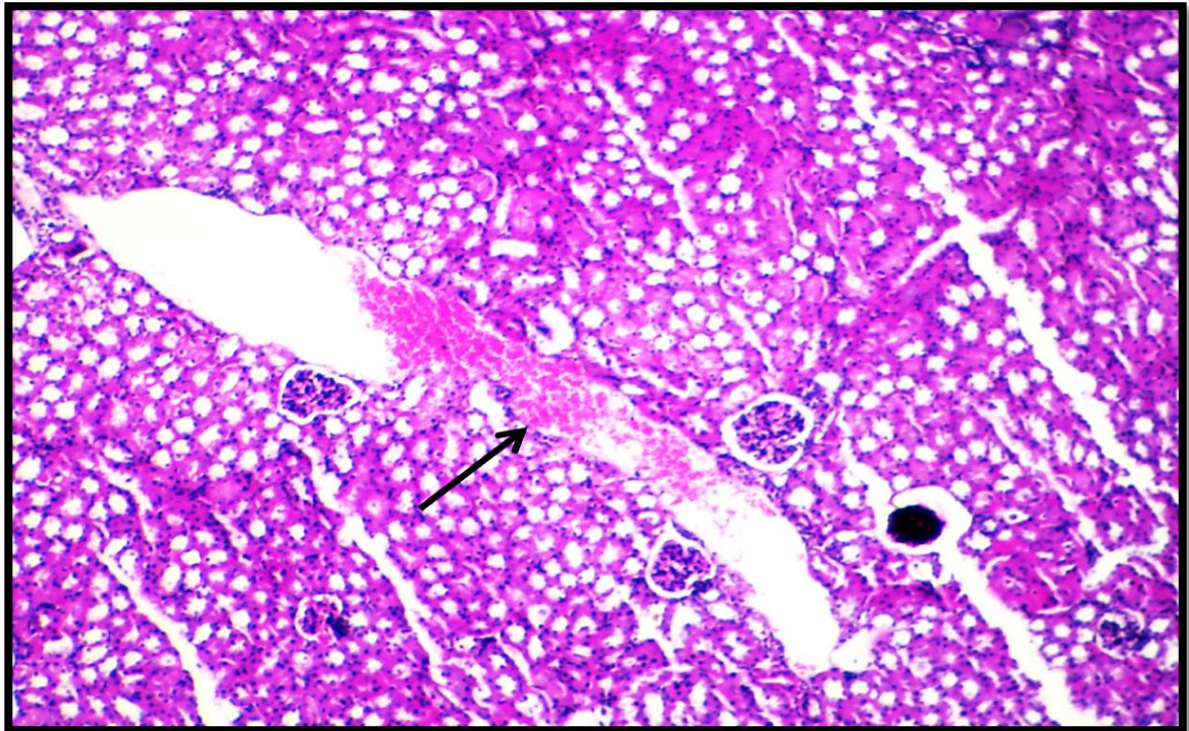


Fig 4-20. Transverse section of female kidney in treated group (citric acid) which showed long cystic dilation filled with blood (Black arrow). H&E stain 10X

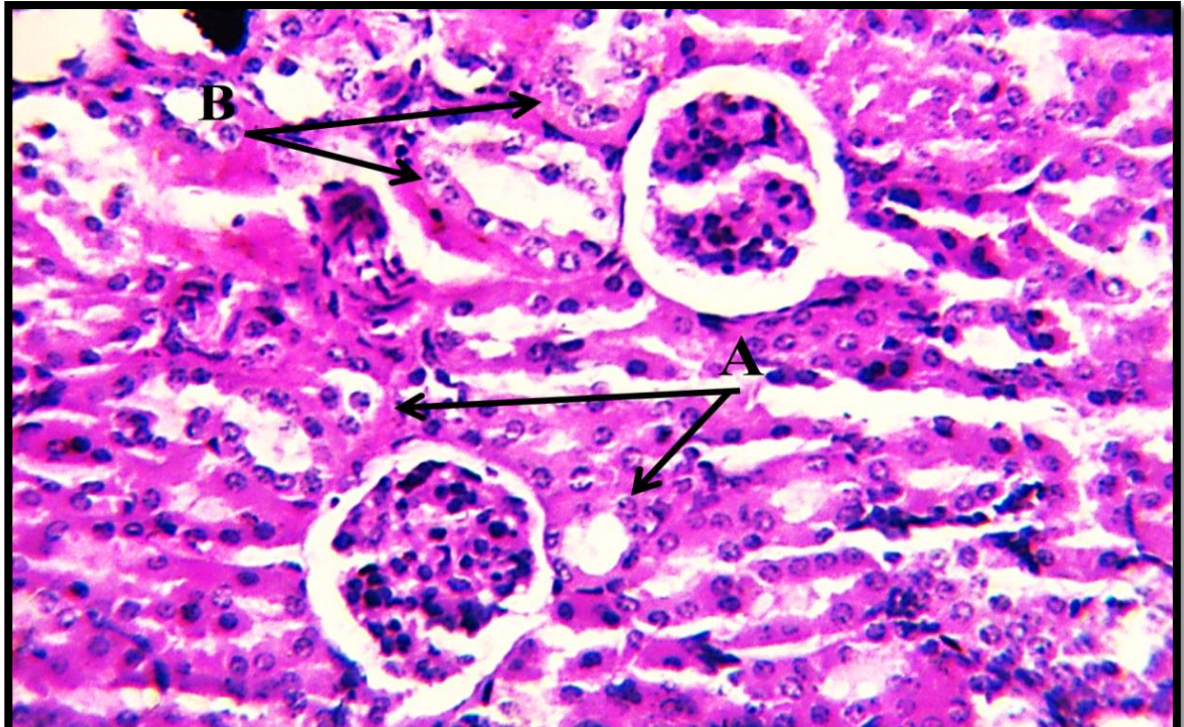


Fig 4-21. Transverse section of female kidney in treated group (citric acid) which showed A- Exfoliated epithelial layer of proximal convoluted tubules, B- Necrosis epithelial cells of distal convoluted tubules. H&E stain 40X.

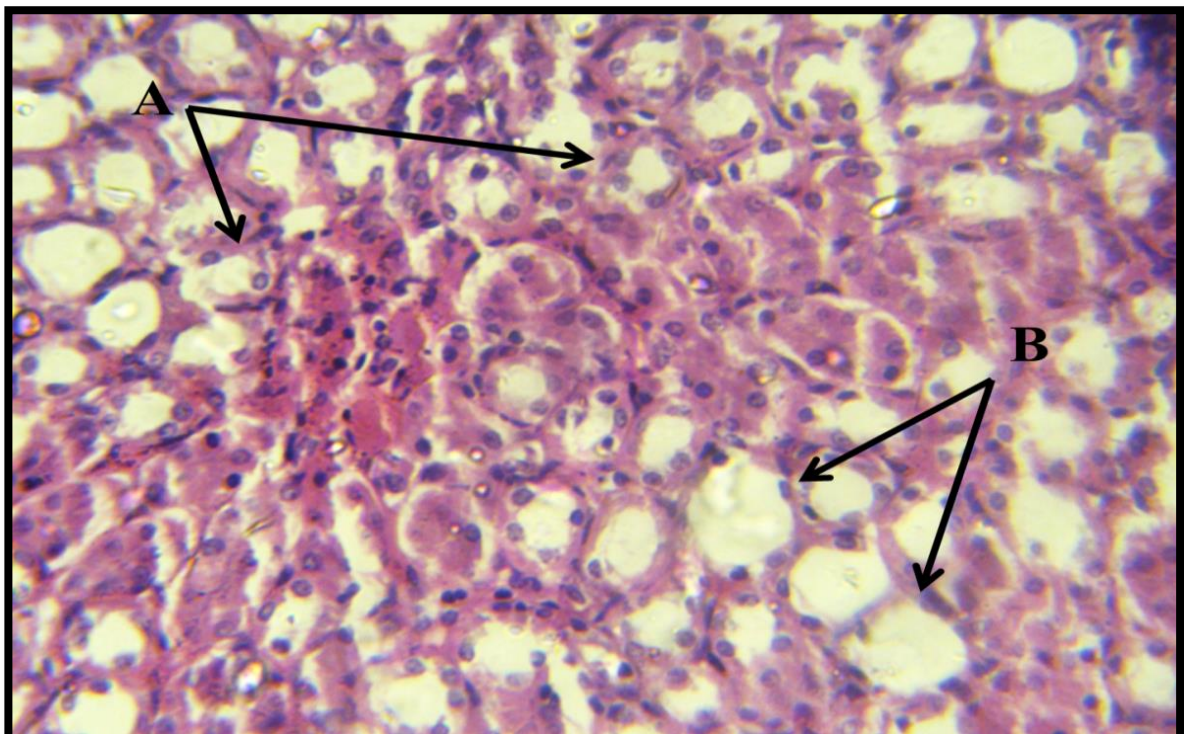


Fig 4-22. Transverse section of female kidney in treated group (citric acid) which showed Degeneration and Exfoliated epithelial layer of both A- Descending branched and B- Ascending branched. H&E stain 40X.

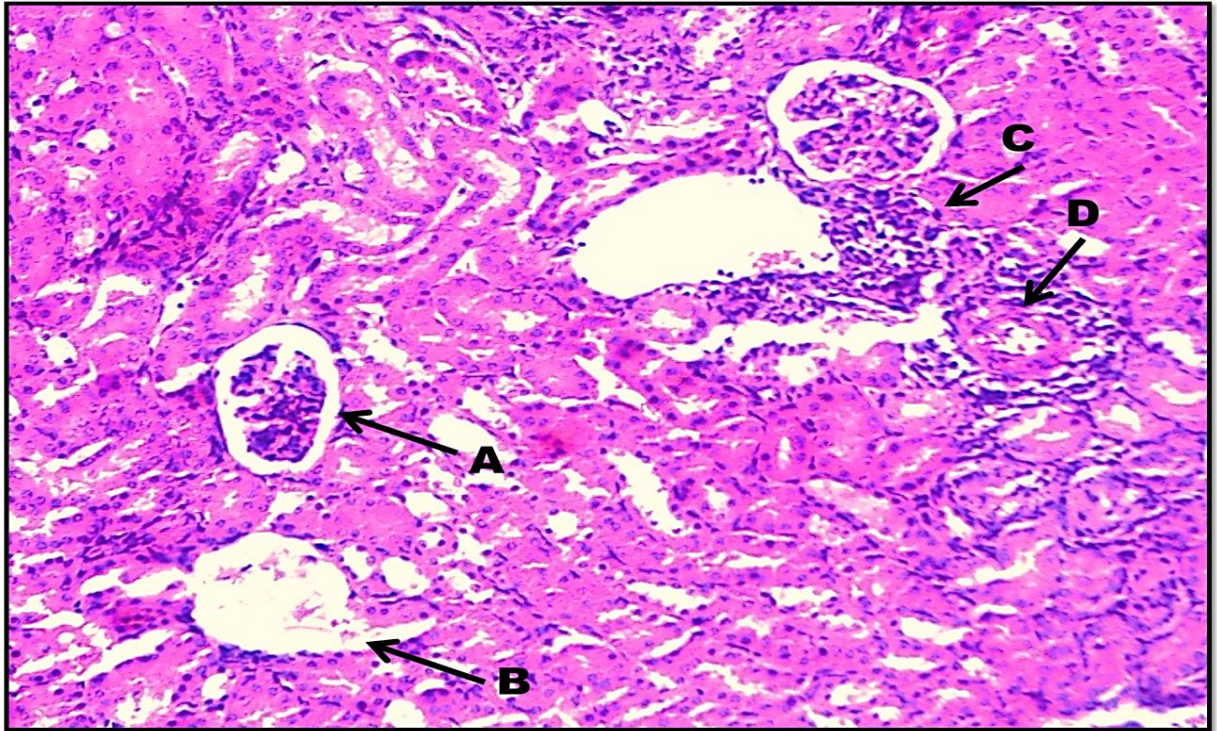


Fig 4-23. Transverse section of male kidney in treated group (citric acid) which showed A- Renal Corpuscle B-Necrosis C-Aggregation of inflammatory cells, D- Vascular congestion. H&E stain 20X.

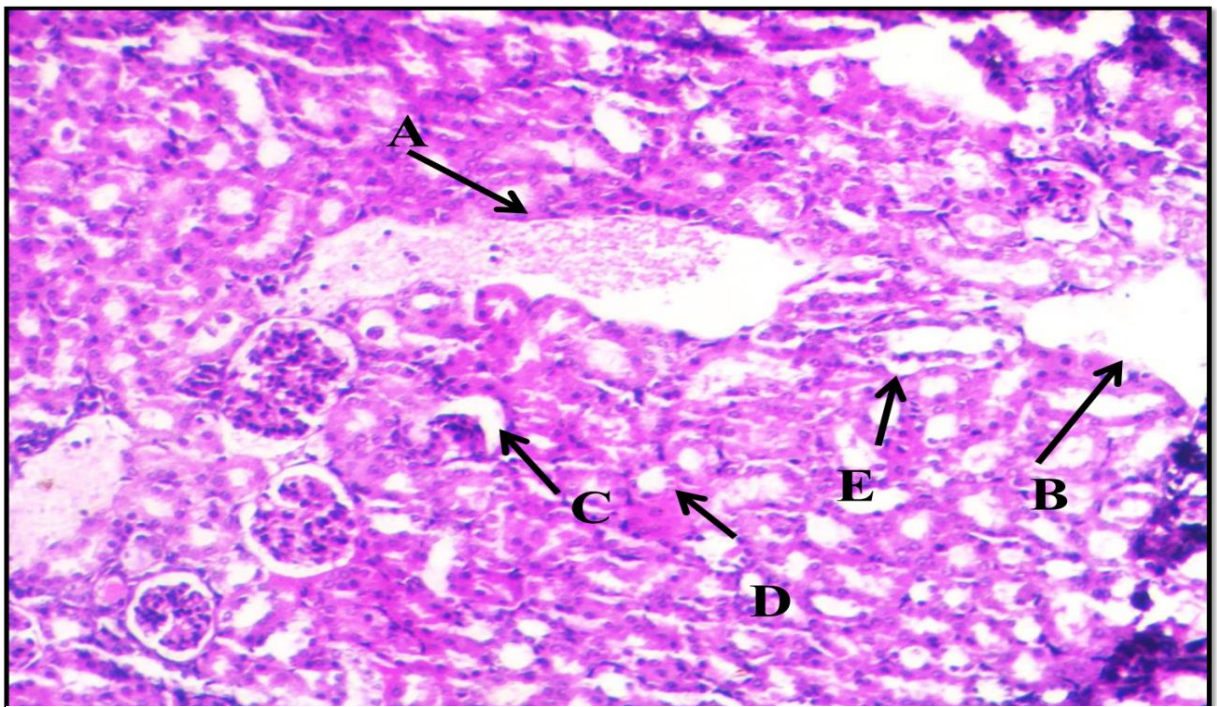


Fig 4-24. Transverse section of male kidney in treated group (citric acid) which showed A-Hemorrhage B-Cystic dilation, C-Bowman space D-Proximal convoluted tubule E- Distal convoluted tubule. H&E stain X 20.

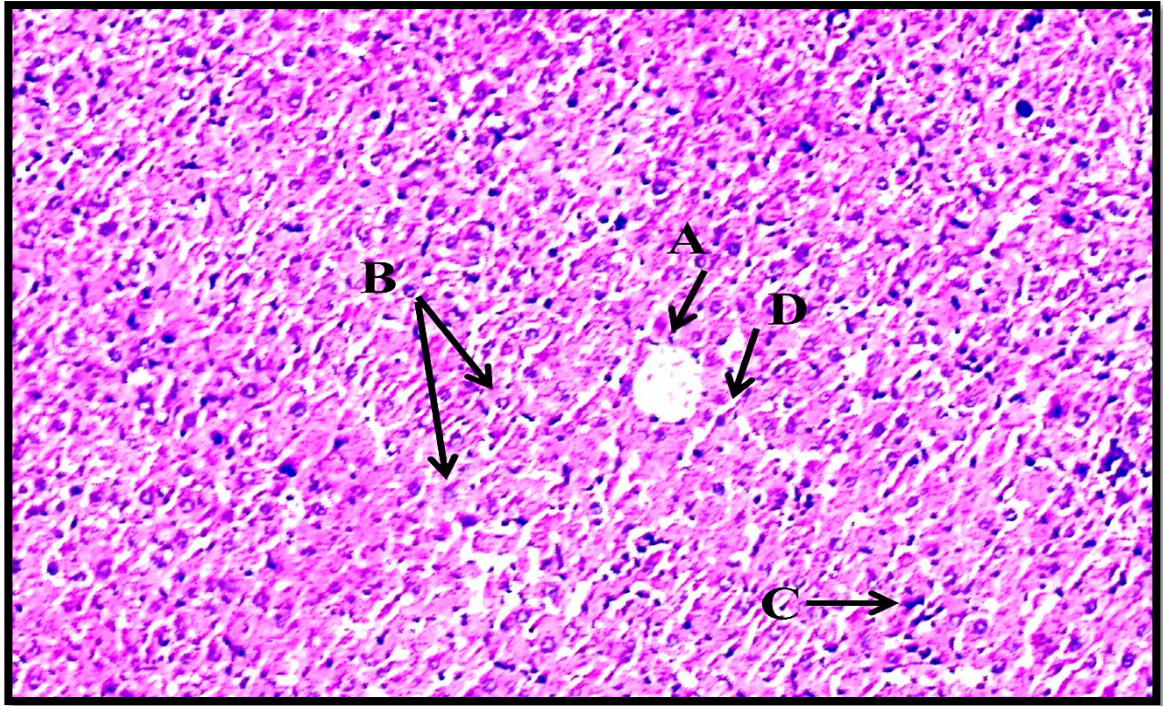


Fig 4-25. Transverse section of female liver in control group which showed A-Central vein, B-Hepatocytes C- Kupper cells D-Sinusoids. H&E stain 10X.

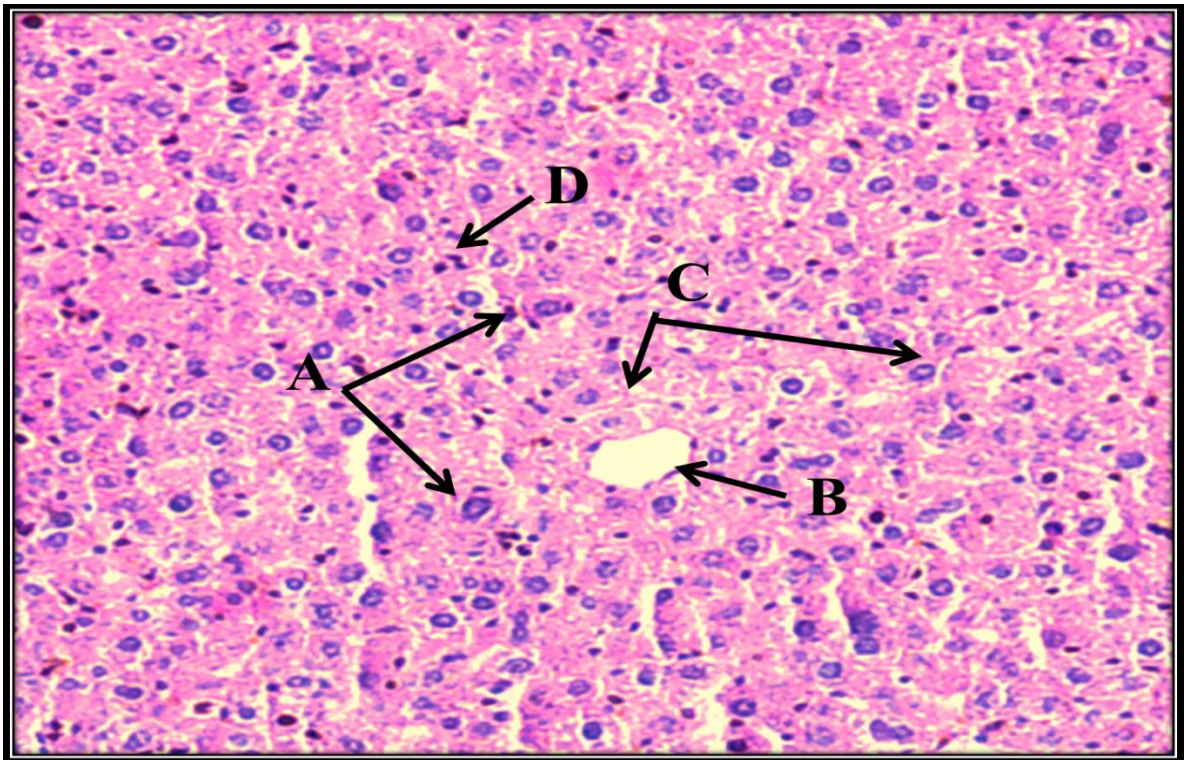


Fig 4-26. Transverse section of section of male liver in control group which showed A- Hepatocyte, B-Central vein, C-Sinusoids, D-Kupper cells H&E stain 20X.

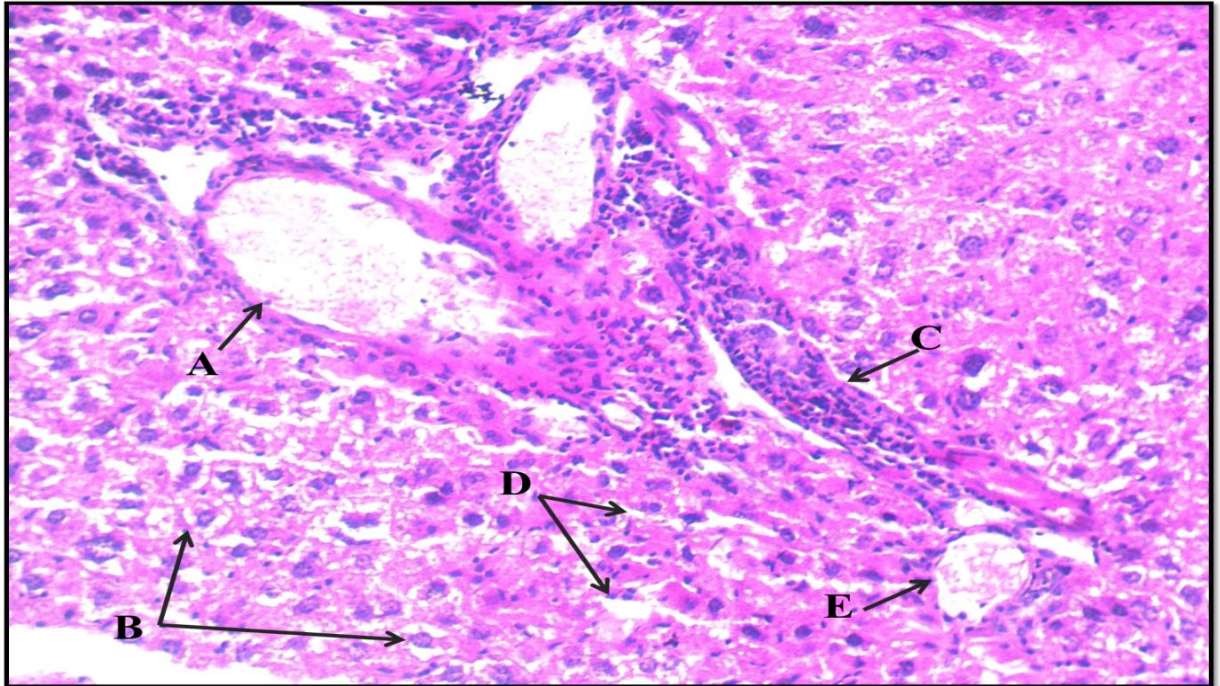


Fig 4-27 Transverse section of female liver in treated group (Formalin) which showed A- Necrosis area, B-Enlargement of hepatocytes, C-Aggregation of inflammatory cells and D- Damage in hepatic cord E-Hemorrhage. H&E stain 20X.

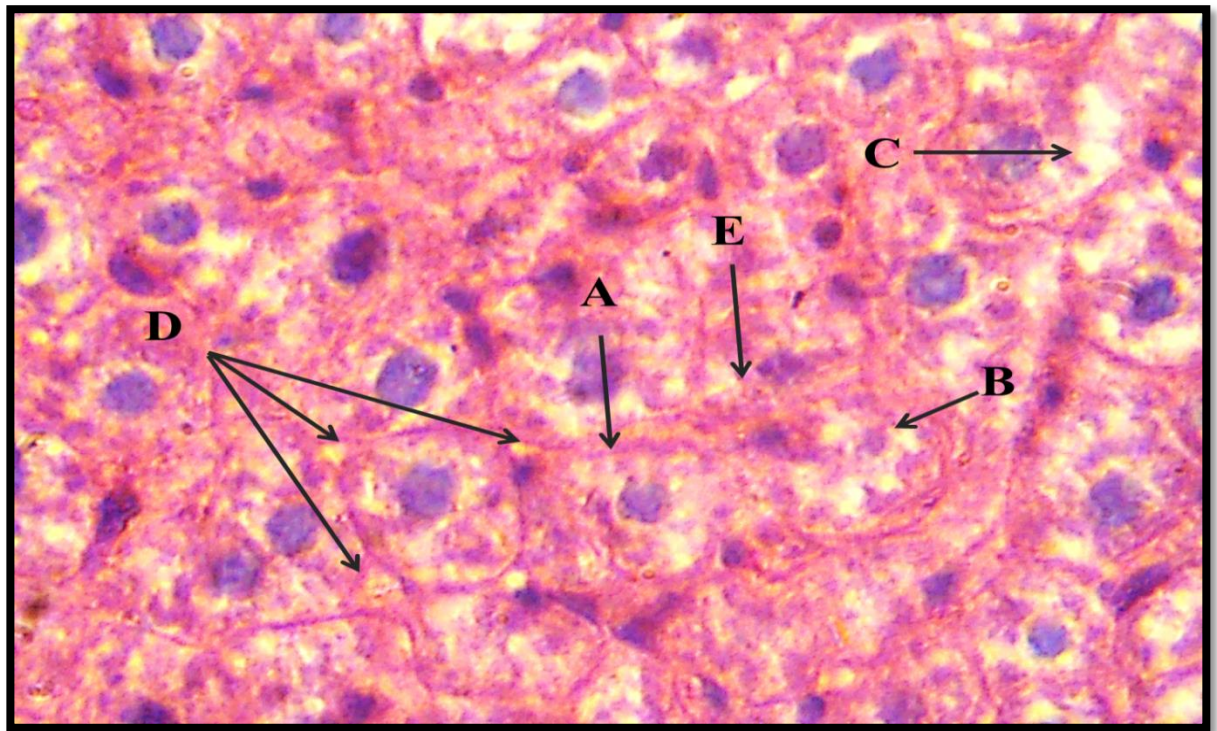


Fig 4-28. Transverse section of female liver in treated group (Formalin) which showed A- Hypertrophy in hepatocyte, B-Destruction nuclei C-Vacuole in cytoplasm of hepatocyte, D-Fatty aggregation, E-Interlobular duct filled with secretion. H&E stain 100X.

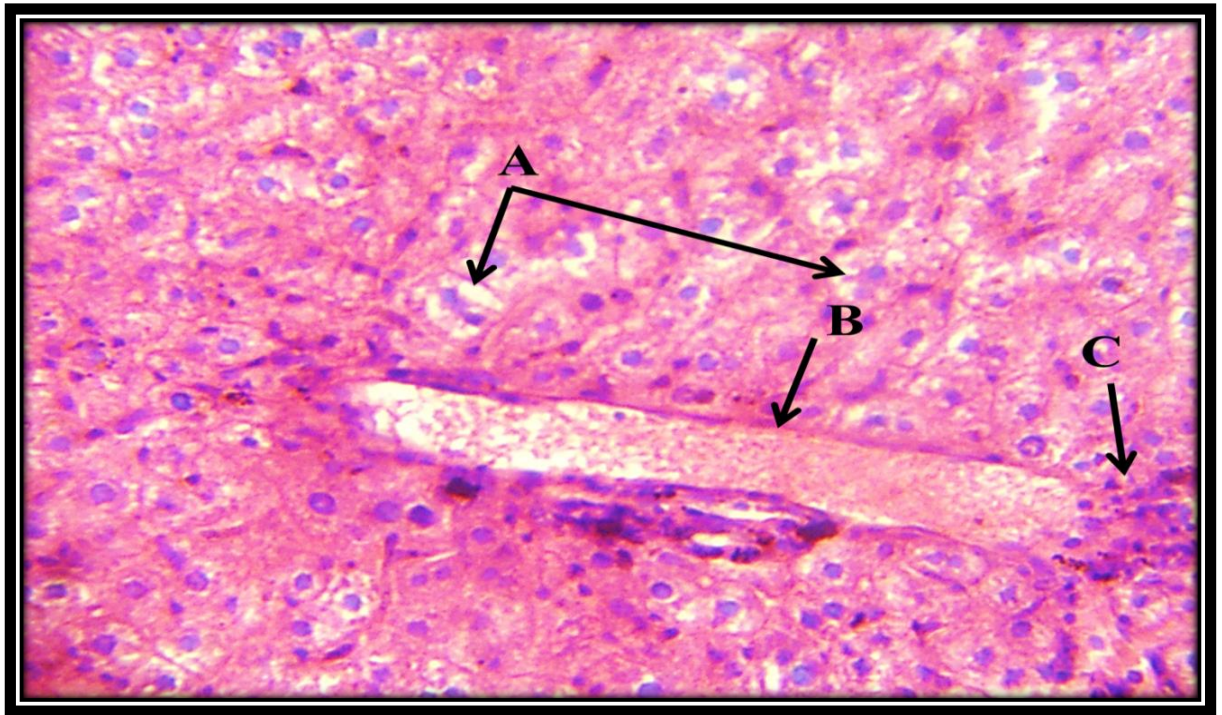


Fig 4-29. Transverse section of female liver in treated group (Formalin) which showed A-Mitotic in nuclei of hepatocyte, B-Hemorrhage C-Aggregation of inflammatory cells. H&E stain 40X.

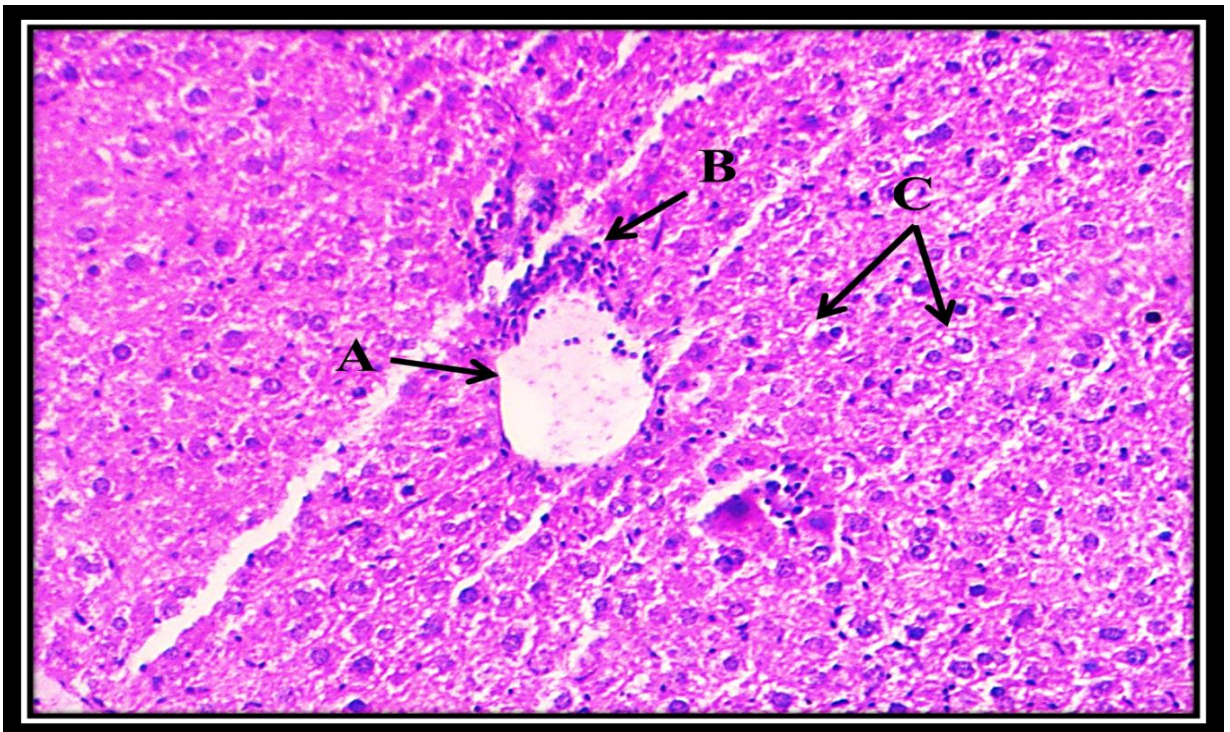


Fig 4-30. Transverse section of male liver in treated group (Formalin) which showed A- Cystic dilation filled with blood, B-Aggregation of inflammatory cells, C- Vacuole in cytoplasm of hepatocyte. H&E stain 20X.

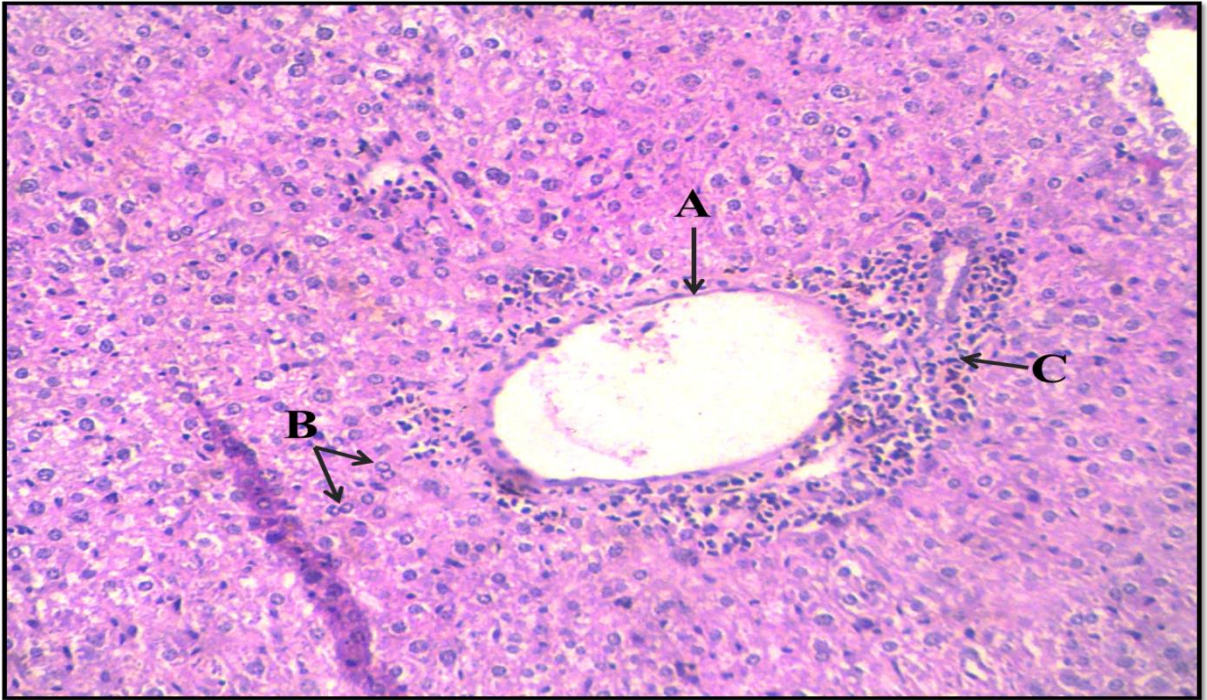


Fig 4-31. Transverse section of male liver in treated group (Formalin) which showed A-Cystic dilation filled with blood, B- Mitotic division in nuclei of hepatocyte, C-Aggregation of Kupper cells,. H&E stain 20X.

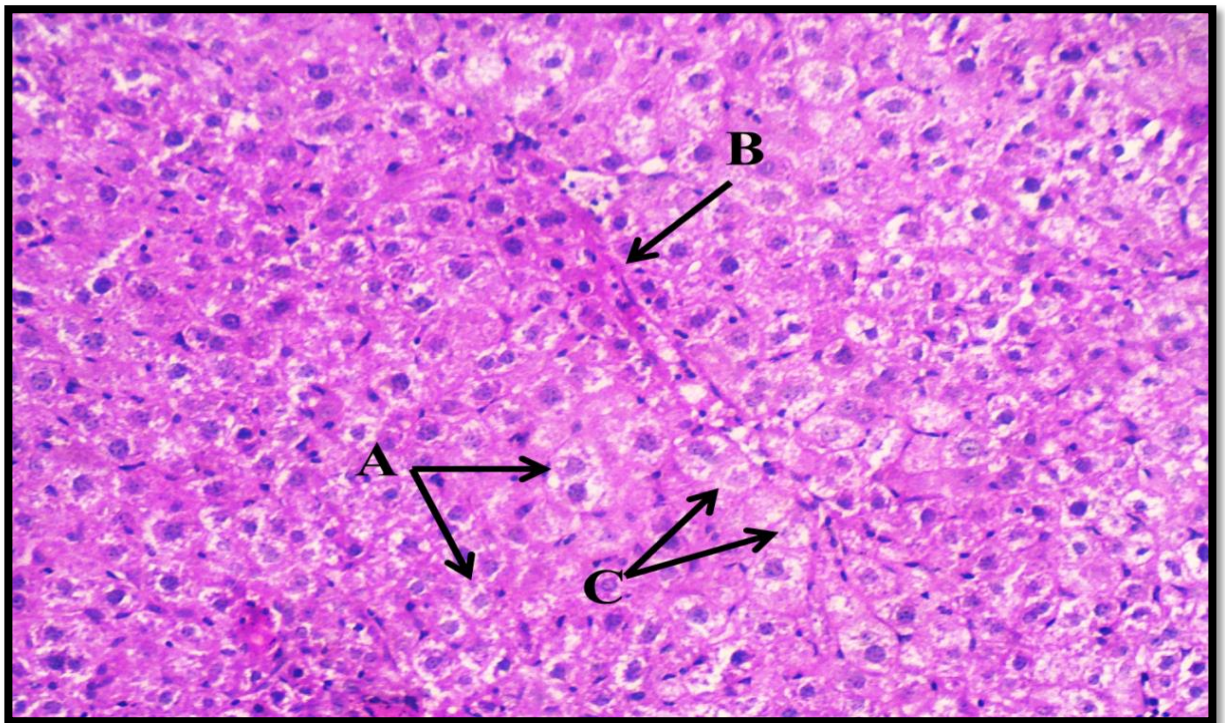


Fig 4-32. Transverse section of male liver in treated group (Formalin) which showed A- Vacuole in cytoplasm of hepatocyte, B-Hemorrhage between sinusoids, C- Destruction nuclei. H&E stain 20X.

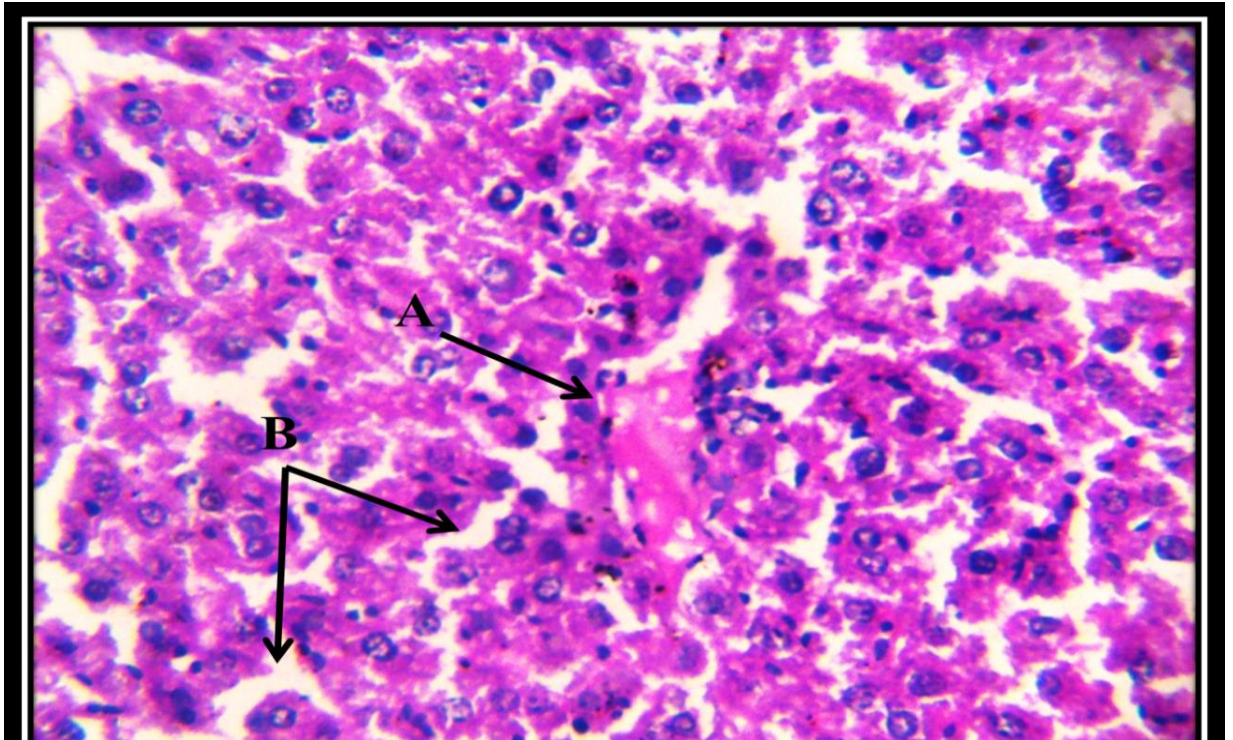


Fig 4-33. Transverse section of female liver in treated group (Citric acid) which showed A- Damage in hepatic cord, B- Dilation in the sinuses with hemorrhage between sinusoid. H&E stain 40X.

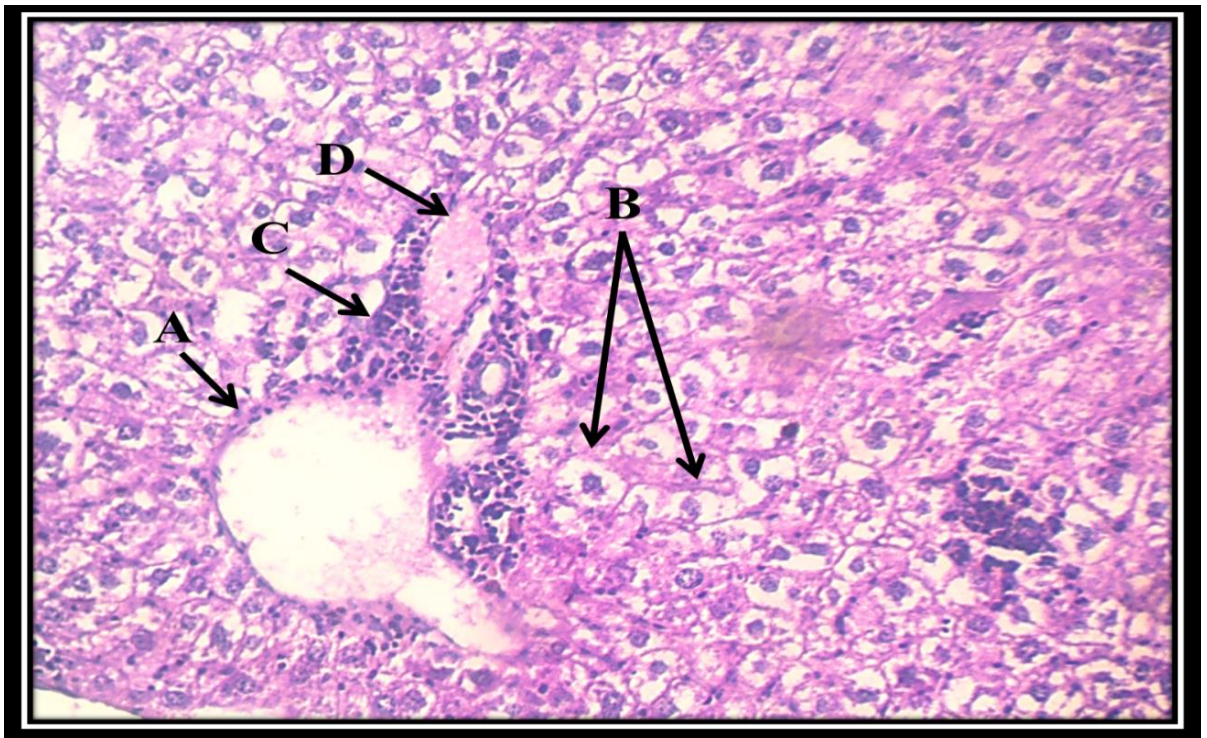


Fig 4-34. Transverse section of female liver in treated group (Citric acid) which showed A- Central vein, B-Enlargement of hepatocytes C-Aggregation of inflammatory cells D- Hemorrhage. H&E stain 40X.

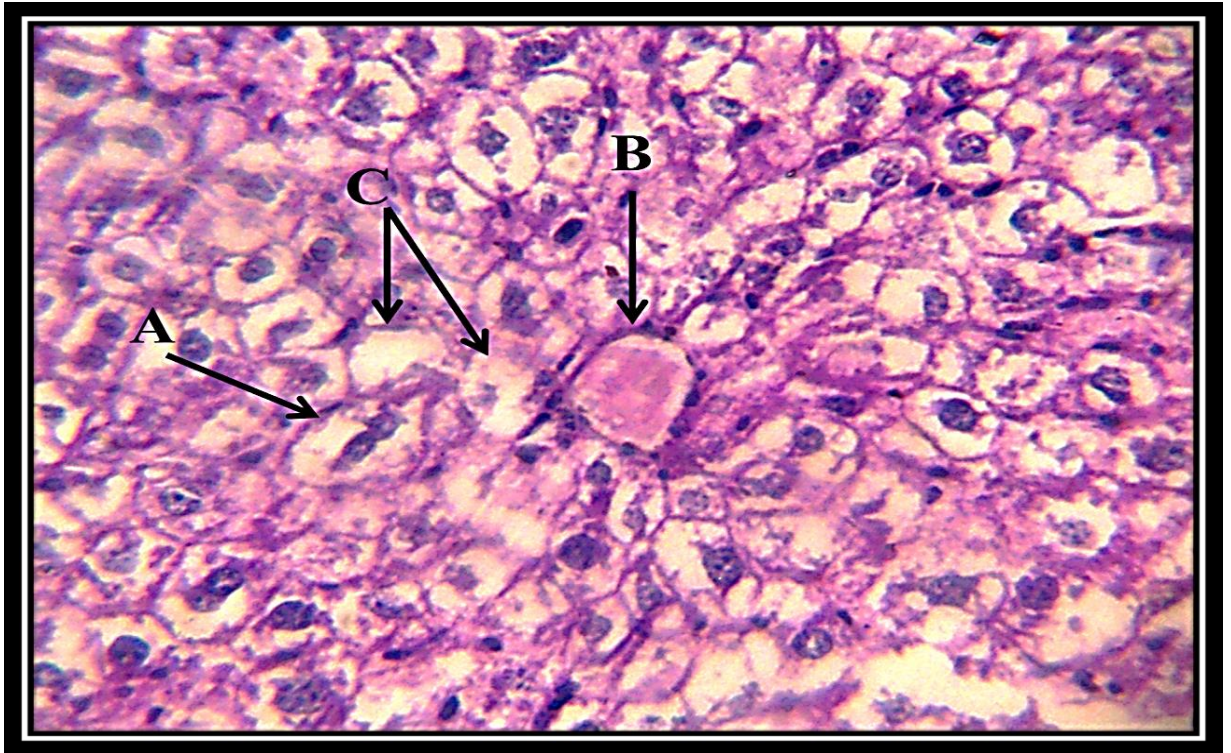


Fig 4-35. Transverse section of female liver in treated group (Citric acid) which showed A- Enlargement, and mitotic division in nuclei of hepatocytes, B- Hemorrhage in central vein, C- Hepatocytes lost nuclei. H&E stain 100X.

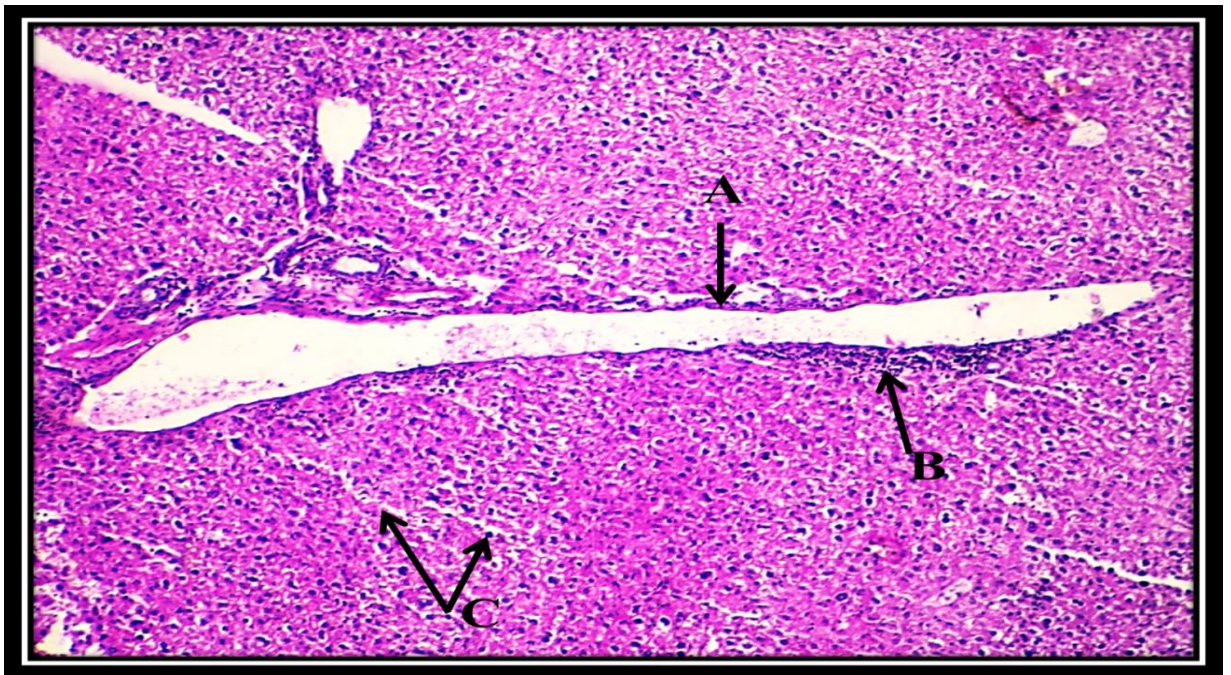


Fig 4-36. Transverse section of male liver in treated group (Citric acid) which showed A- Cystic dilation filled with blood, B-Aggregation of inflammatory cells, C-Damage in hepatic cord. H&E stain 10X.

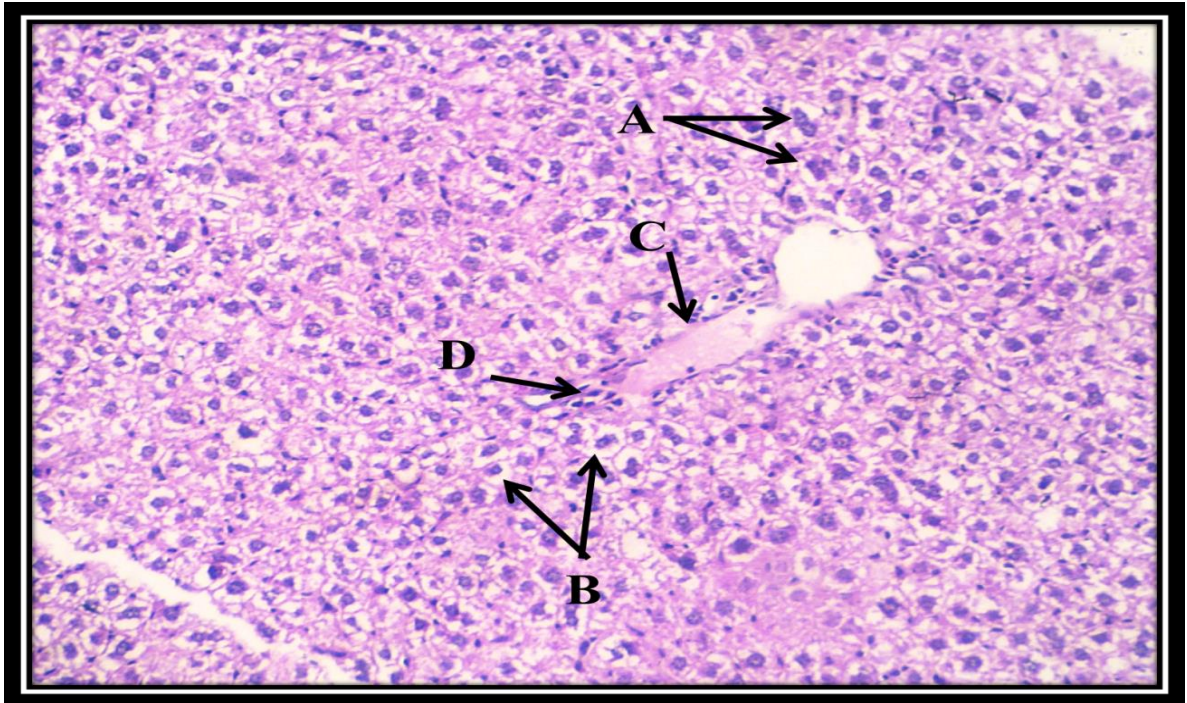


Fig 4-37. Transverse section of male liver in treated group (Citric acid) that noted A-Enlargement hepatocytes, B-Vacuous in the cytoplasm of hepatocytes, C- Dilated central vein with hemorrhage D-Aggregation of Kopper cells. H&E stain 20X.

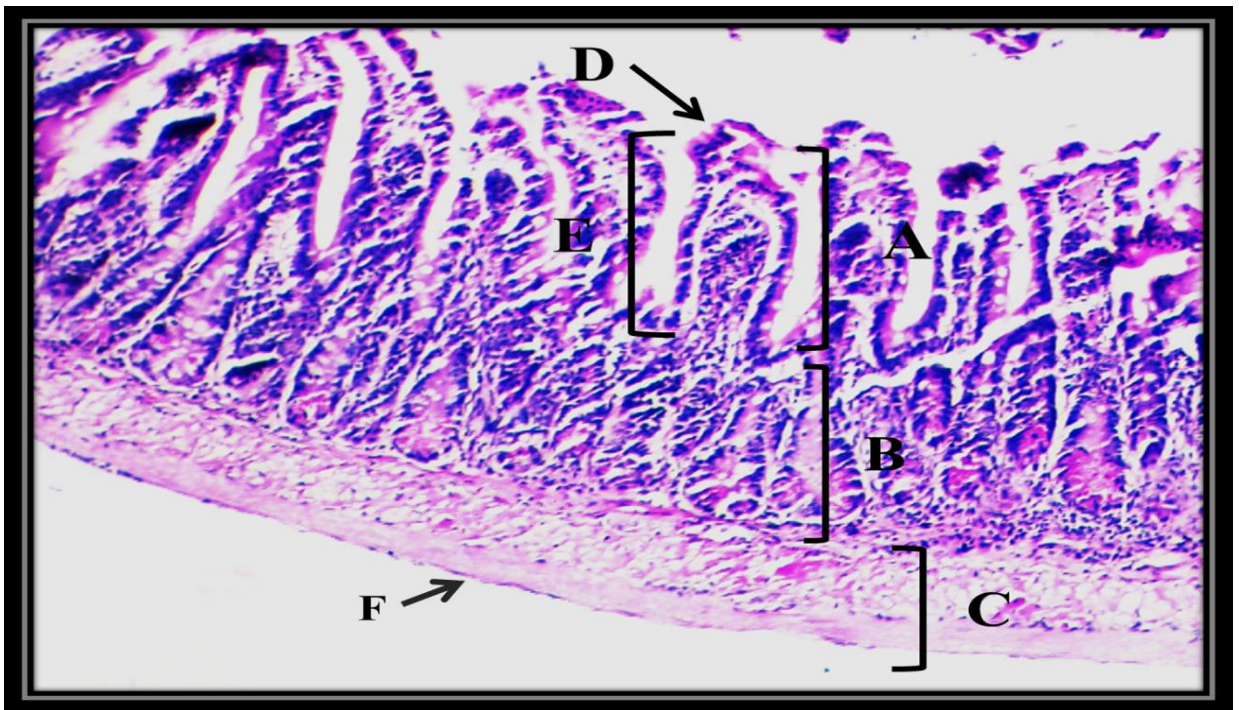


Fig 4- 38. Transverse section of female duodenum in control group which showed A-layer B-Submucosa layer C-Muscular layer, D-Epithelia layer, E- Villi and F- Serosa layer. H&E Mucosa stain 20X.

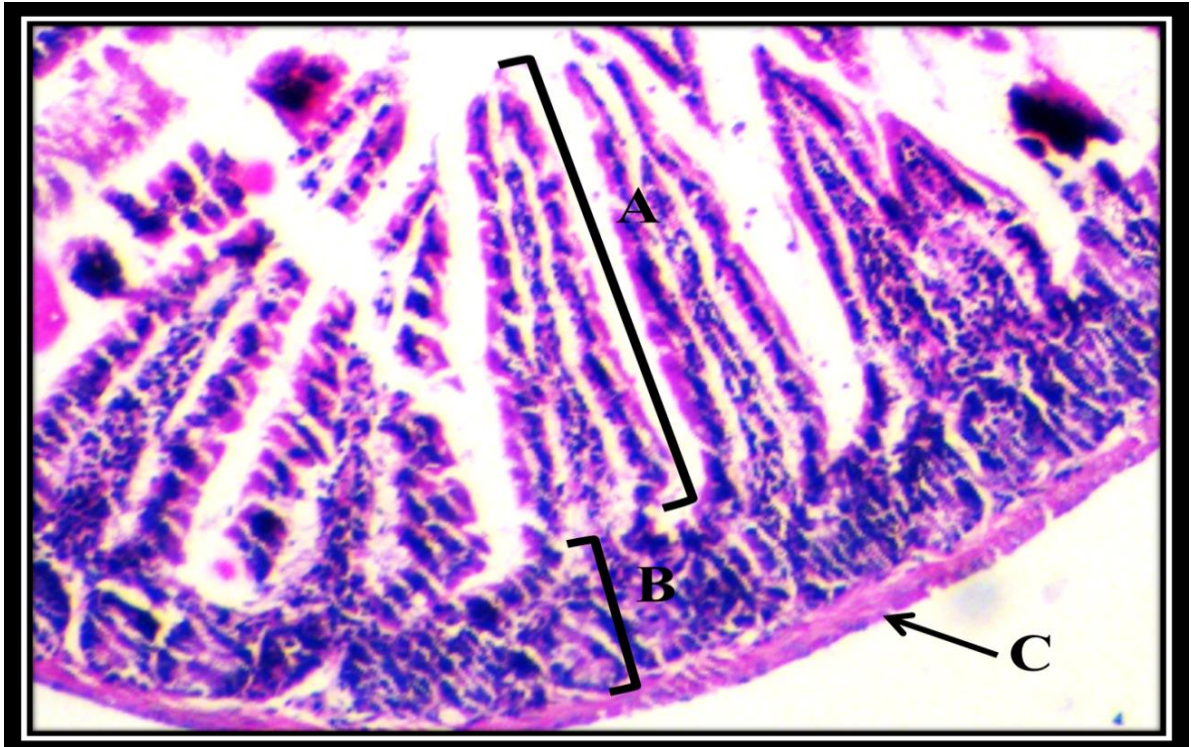


Fig 4-39. Transverse section of male duodenum in control group which showed A- Mucosa layer, B-Submucosa layer, C-Muscular layer. H&E stain 20X.

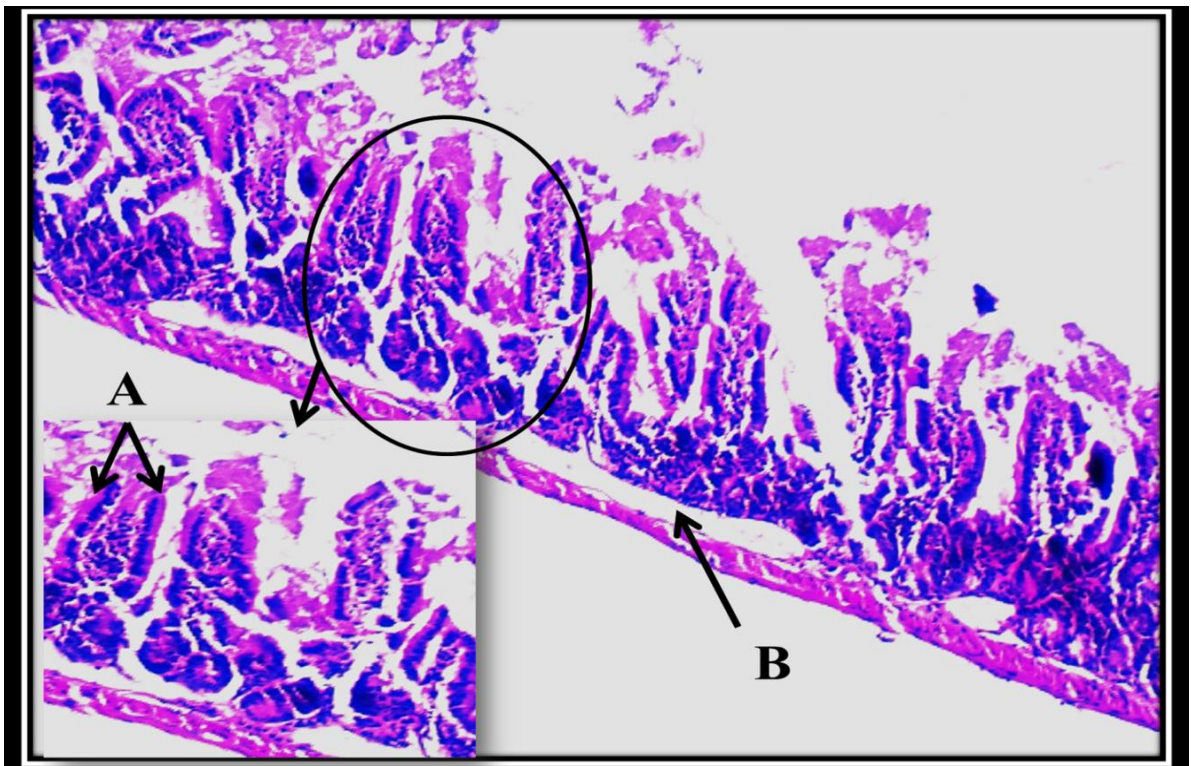


Fig 4-40. Transverse section of female duodenum in treated group (formalin) which showed A- Epithelia layer, B-Cystic dilation between mucosal and submucosal layers. H&E stain 20X.

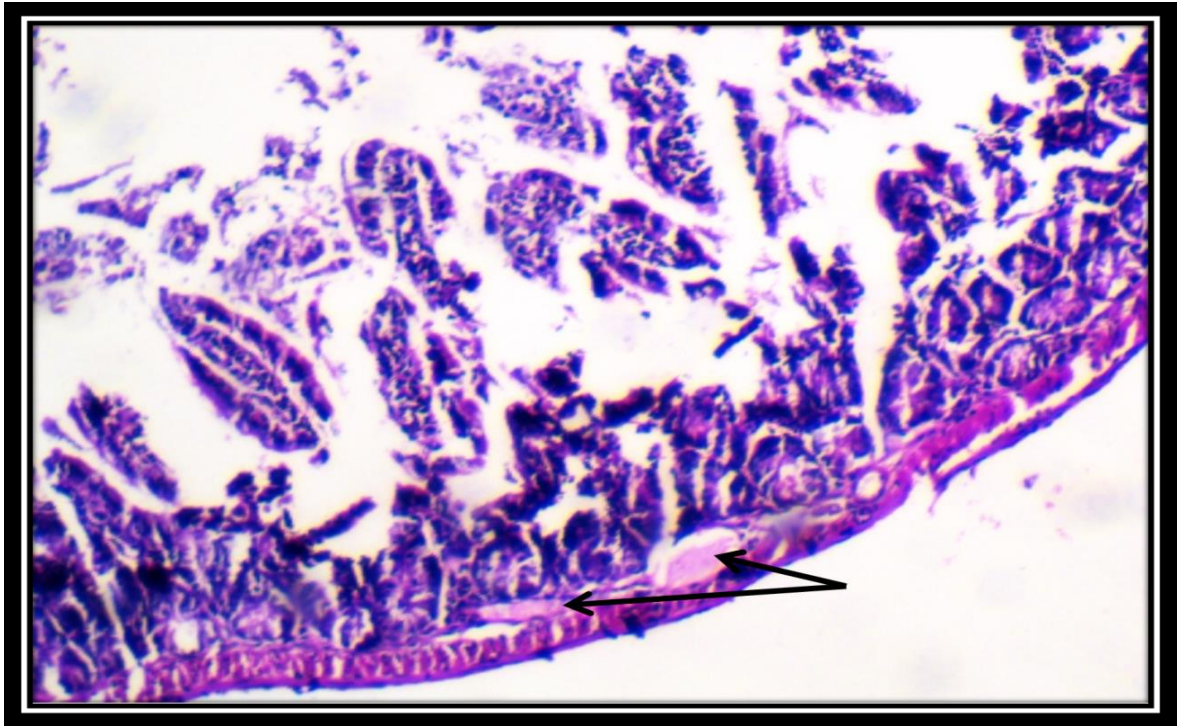


Fig 4-41. Transvers section of female duodenum in treated group (formalin) which noted vascular congestions in the submucosal and muscular layer of duodenum (Black arrow). H&E stain 20X.



Fig 4-42. Transvers section of section of male duodenum in treated group (formalin) which showed A-Mucosa layer, B-Submucosa layer C-Degeneration in submucosa, D-Damage in muscular layer. H&E stain 10X.

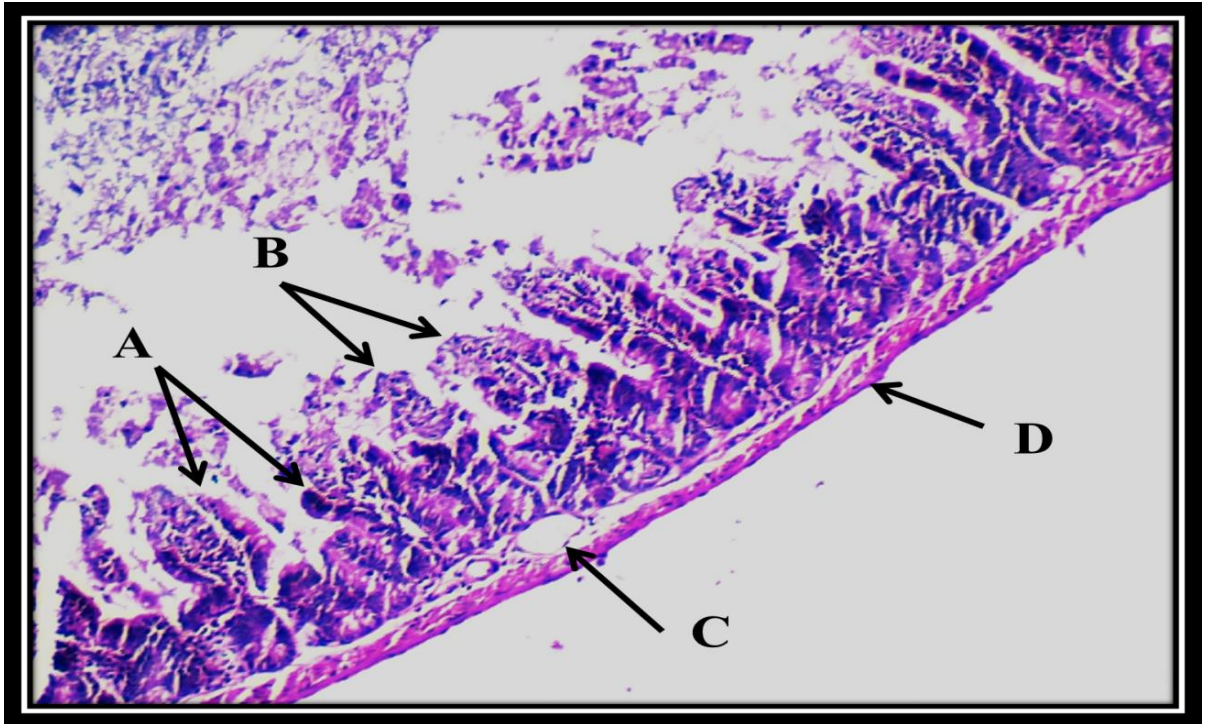


Fig 4-43. Transverse section of female duodenum in treated group (citric acid) which showed A-Exfoliated the epithelial layer, B-Exodus lamina propira, C-Cystic dilation and D-Degeneration in the muscular layer. H&E stain 10X.

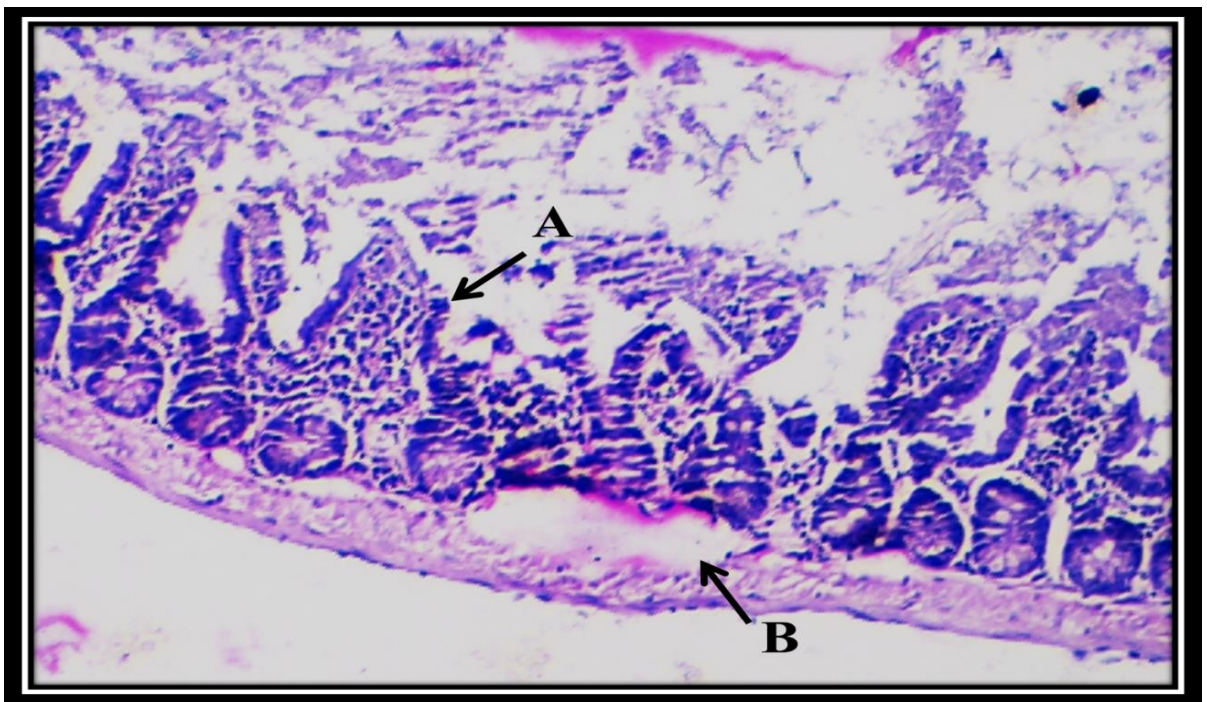


Fig 4-44. Transvers section in the duodenum in male treated group (citric acid) which showed A- Exfoliated epithelia layer, B -Cystic dilation. H&E stain 20X.

4.2 The Biochemical results

4.2.1 The Biochemical Results of Creatinine

A- Control Groups

Table (4-4) showed the normal value of creatinine in the serum of female control group was (0.36190 ± 0.00728 mg/dl), while in the serum of male control group was ($0.36188 \pm 0.00718 \mu\text{m}$). Creatinine consider a waste product from breakdown of creatine in skeletal muscle which eliminated by kidney, when increased the concentration of serum creatinine may be due to glomerular dysfunction of the nephron to filtrated it or damage in proximal convoluted tubules lead to increased reabsorption the creatinine concentration. This change in concentration of creatinine may be due to kidney dysfunction (tubules).

B-Formalin Groups

In (Table 4-4) showed the level of creatinine in the serum of female treated group with formalin was (0.4557 ± 0.0303 mg/dl) while in male was (0.4259 ± 0.0570 mg/dl). The current result showed the treated groups with formalin have significantly increased in the level of creatinine compared with the control group (Appendix-49, 50). Also the result showed slight differences between female and male group, (Appendix-51). These biochemical change of creatinine in the treated group with formalin were agreement with (Oliveira Ramos *et al.*, 2017) which showed an increase in the level of creatinine and urea in the isolated serum from rates after treated with formaldehyde may be due to toxicity of it lead to damage in kidney.

C- Citric Acid Groups

The results in (Table 4-4) showed the level of creatinine in the female serum treated group with citric acid was (0.4860 ± 0.0729 mg/dl), while in the serum of the male was (0.4271 ± 0.0621 mg/dl). The result noted the level of serum creatinine in treated group

with citric acid has significantly increased compared with the control group (Appendix-52, 53). Also the result showed there are significant differences in the level of creatinine between male and female (Appendix-54). These biochemical changes of creatinine after treated with citric acid were deal with (Abd-ALGadir *et al.*, 2010) which showed an increase in the serum creatinine level in the rats with increasing the dose of citric acid.

4.2.2 The Biochemical Results of Urea

A-Control Groups

The biochemical result in (Table 4-4) noted the level of serum urea in the female control group was (22.000 ± 0.795 mg/dl), while in the male control group was (22.20 ± 1.20 mg/dl). The present result noted non-significant differences in the level of urea between male and female control groups. Urea is resulted from a breakdown of protein, consider a waste product of metabolism and eliminated or exclusively by kidney in the urine. Serum urea concentration reflects the balance between urea production in the liver and urea elimination by kidney in the urine. High level of urea in the blood may be due to failed kidney (damage in the kidney) to excreted urea.

B-Formalin Groups

The current result (Table 4-4) noted the level of urea in female treated group with formalin was (37.40 ± 4.88 mg/dl), while in the male treated group with formalin was ($35.85 \pm 5.15 \mu\text{m}$). These result noted treated group with formalin have significantly increased compared with the control group (Appendix-55, 56). Also, the result showed no significant increase in the level of urea between the male and female treated group, (Appendix-57).

The results noted the formalin has effects more than citric acid on the level of urea in the blood of female and male treated group. High level of urea in blood may be due to failed kidney (damage in kidney) to excreted urea that results from metabolism of formalin that converted to FA then to ammonia in the liver which converted to urea lead

to increased level urea in blood that referred to failure the kidney to excrete it. These biochemical change of urea after treated with formalin in treated groups were an agreement with (Dorairajan, 2010) which showed the blood urea have significantly increased in rats exposed to formaldehyde.

C-Citric Acid Groups

The (Table 4-4) showed the value of serum urea in female treated group with citric acid were ($34.10 \pm 4.32 \text{ mg/dl}$), while in the male treated group was ($34.00 \pm 4.32 \mu\text{m}$), which have significantly increased compared with control group diagram (Appendix-58, 59). Also, the result noted the experimental groups that treated with citric acid didn't have significant differences in the level of urea between male and female which noted in (Appendix-60). These results of high concentration of urea in the blood may be due to the gastrointestinal hemorrhage which is associated with increased protein intake (blood in the gut is effectively a high protein meat) and thereby increased urea production and consequently increased urea level.

The main findings of the present study noted after treated the formalin have prominent effects on the experimental animal which lead to significantly increase the level of serum urea in the treated group with formalin more than the treated group with citric acid. These physiological change of urea after treated with citric acid were constant with (Boj *et al.*, 2003) which showed there was a statistically significant increase in urea levels after administered formaldehyde in rats.

4.2.3 The Biochemical Results of AST Enzyme

A-Control Groups

(Table 4-4) showed the level of AST enzyme in the serum of female control group was ($273.90 \pm 1.94 \text{ U/L}$), while the level of AST in serum male control group was ($273.75 \pm 1.80 \text{ U/L}$). The value of AST in female and male control groups didn't have significant differences in both control groups.

B-Formalin Groups

Table (4-4) showed the level of AST in the female treated group with formalin was ($438.5 \pm 61.1 \mu\text{m}$) which have significantly increased compared with control group, while in the male group was ($457.5 \pm 63.7 \text{ U/L}$) which have significant increased compared with the control group that noted in (Appendix-61, 62). Also, the result noted no significant differences in the level of AST between male and female groups that treated with formalin (Appendix-63).

The biochemical test of the level of AST in serum of treated group, the results referred to increase in the level of AST, this change may be due to the acute destruction in the liver tissue. The destruction hepatocytes lead to release the AST and ALT from their cytoplasm that leads to an increase the level of AST in the serum of treated groups. These biochemical change of AST enzyme after treated with formalin were not coincide with (Olooto, 2010) which showed there was no significant increase in the level of ALT and AST after exposure to formaldehyde.

C-Citric Acid Groups

The result showed the level of AST in the (Table 4-4) of female treated group with citric acid was ($383.8 \pm 67.2 \text{ U/L}$), while in male treated group with citric acid was ($344.1 \pm 38.0 \text{ U/L}$) which have significantly increased compared with control group (Appendix-64, 65). Also, the (Table 4-4) noted in the groups treated with citric acid showed there are no high significant differences in the level of AST between female and male (Appendix-66). These results may be to a toxic effect of citric acid on liver tissue.

The present result showed the level of AST in female treated group with formalin have significant increased compared with the female treated group with citric acid, this result referred to the formalin have effects on the liver tissue more than citric acid, formalin may be causes acute degeneration of hepatocytes leads to an increase in the level of AST in current female treated group with formalin. These physiological change of AST enzyme after treated with citric acid were disagreement with (Aktaş *et al.*, 2003)

which showed ALT and AST there were no high significant increase in serum of mice after treated with citric acid.

4.2.4 The Biochemical Results of ALT Enzyme

A-Control Groups

The present result noted the level of serum ALT that showed in (Table 4-4), the female control group was (363.60 ± 4.65 U/L) while in male control group was (360.25 ± 6.58 U/L), both male and female control group which have the same level of ALT that noted in the statistical analysis which recorded to non-significant difference in the level of ALT between them.

B-Formalin Groups

The result showed the level of ALT (Table 4-4) in serum female treated group with formalin was (595 ± 123 U/L) while in male treated group with formalin was ($585 \pm 117 \mu\text{m}$), (Appendix-67, 68). These results appeared the treated groups with formalin have significant increased compared with the control group. Also the result showed no significantly differences in the level of ALT between the male and the female in the groups that treated with formalin (Appendix-69). This increased in the level of ALT may be due to damage in hepatocyte which increased this enzyme that transports to blood. These biochemical changes of ALT enzyme after treated with formalin were agreement with (Abushofa *et al.*, 2019) which showed the rats that treatment with preservatives (sodium nitrite) caused a significant increase in serum (ALT) and (AST).

C-Citric acid Groups

In (Table 4-4) the result noted the level of ALT in serum female treated group that treated with citric acid was (478.2 ± 60.9 U/L), while in the serum of male treated group with citric acid was (441.0 ± 63.3 U/L) which have significantly increased compared with

the control group (Appendix-70, 71). Also, the result noted in the groups treated with citric acid showed there are non-significant differences in the level of ALT between the female and the male (Appendix-72).

These changes in the levels of ALT in serum treated group may be referred to as the high effect of formalin on the tissue structure of the liver more than citric acid. These biochemical changes of ALT enzyme after treated with citric acid were constant with (Ortiz *et al.*, 2006) which showed the cytotoxic effect of monosodium glutamate (flavor enhancer) induced liver damage and enzyme release increasing their serum levels of ALT and AST.

Table 4-4. Level of Creatinine, Urea, AST, and ALT in the serum of treated group

Descriptive Statistics (N=20)				
	SEX	Treatment	Mean±SE	Std. D
Creatinine	Female	Control	0.36190±0.0016	0.00728
		Formalin	0.4557±0.0068	0.0303
		Citric acid	0.4860±0.016	0.0729
	Male	Control	0.36188±0.0016	0.00718
		Formalin	0.4259±0.013	0.0570
		Citric acid	0.4271±0.014	0.0621
Urea	Female	Control	22.000±0.18	0.795
		Formalin	37.40±1.1	4.88
		Citric acid	34.10±0.96	4.32
	Male	Control	22.20±0.27	1.20
		Formalin	35.85±1.2	5.15
		Citric acid	34.00±0.97	4.32
AST	Female	Control	273.90±0.43	1.94
		Formalin	438.5±14	61.1
		Citric acid	383.8±15	67.2
	Male	Control	273.75±0.40	1.80
		Formalin	457.5±14	63.7
		Citric acid	344.1±8.5	38.0
ALT	Female	Control	363.60±1.0	4.65
		Formalin	595±27	123
		Citric acid	478.2±14	60.9
	Male	Control	360.25±1.5	6.58
		Formalin	585±26	117
		Citric acid	441.0±14	63.3

Chapter Five

Conclusions and Recommendations

5.1. Conclusions

- 1) The formalin effect on the kidney, which lead to hypertrophy nephron, necrosis in in parenchyma of kidney, vascular congestion between glomeruli capillaries and renal tubules, and shrinkages the renal corpuscle while the formalin effect on the liver caused acute degradation, blood hemorrhage, hepatocytes enlargement and cystic dilation. The formalin effect on the duodenum noted a significantly increasing in the thickness of the epithelial layer, several damages in mucosa layer and vascular congestion in the wall.
- 2) The 3% concentration of citric acid effect on the kidney, which lead to hemorrhage between the renal corpuscle, aggregation of inflammatory cells, cystic dilation filled with blood and exfoliated of the epithelial layer of tubules while the effect of it on the liver caused damage of hepatic cords, significant increase in the diameter of hepatocyte nuclei, aggregation of fatty material in the different area of liver, enlargement hepatocyte and dilation in the sinusoids with hemorrhage. The citric acid effect on the duodenum showed a significant increase in the thickness of the epithelial layer, exfoliated the epithelial layer that covering the intestinal villi and separated between layers.
- 3) The effect of formalin and citric on the biochemical results for both males and females showed a significant increase in the levels of ALT, AST, also noted a significant increase in the level of creatinine and urea compared with control groups which referred the food preservatives which lead to excess of AST, ALT, creatinine and urea level in treated group
- 4) There is no difference between the male and female regarding the effect of formalin and citric acid on histological sections.

5.2. Recommendations

- 1) To study the effects of food preservatives on liver and kidney after treating with these foods for long period for detecting about the cancer antigen.
- 2) Immunohistochemically studies of liver and kidney after treated with food preservatives to detect about specific protein such as alanine, bilirubin and glutathione.
- 3) Ultra-structural studies of the urinary and gastrointestinal system after treating with food preservatives
- 4) Histochemical studies of the endocrine gland in the body after treating with food preservatives.
- 5) Conducting studies aimed at test the effect of food preservatives on the embryos.

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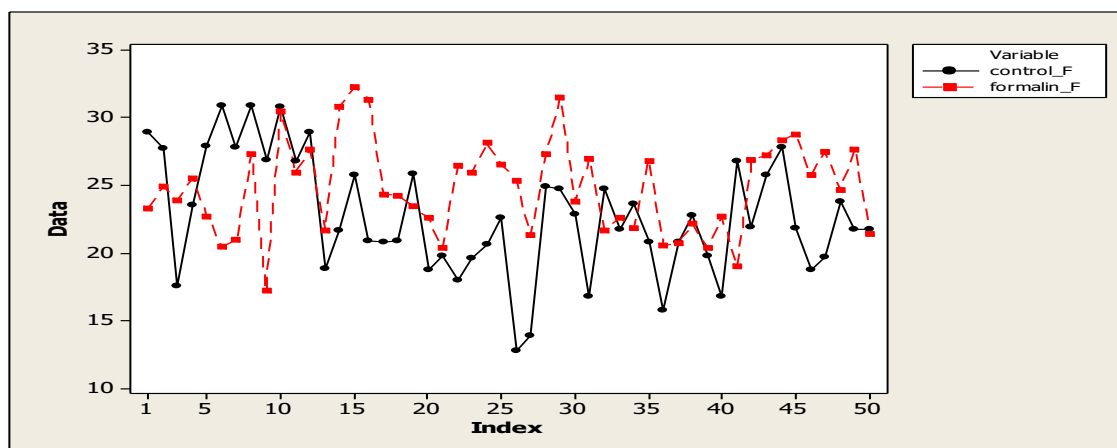
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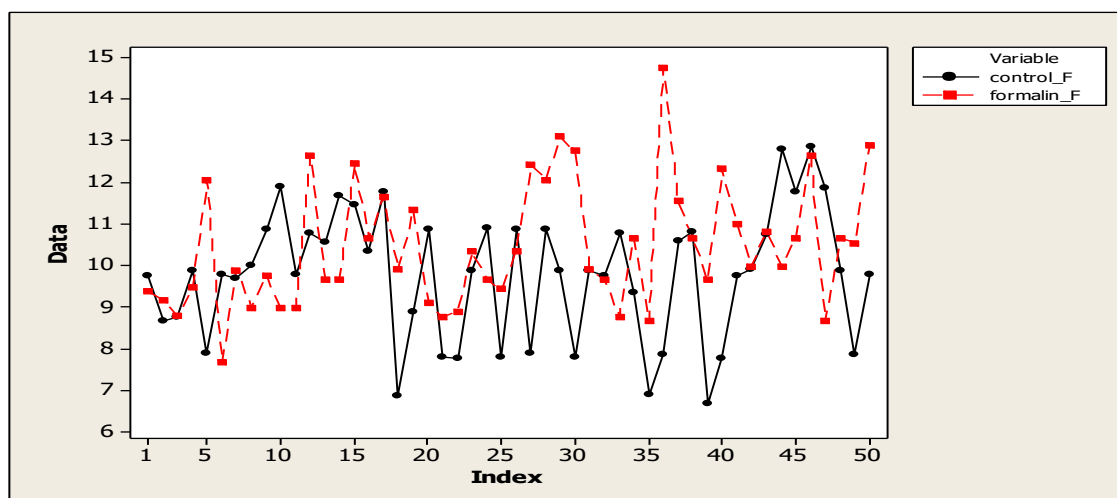
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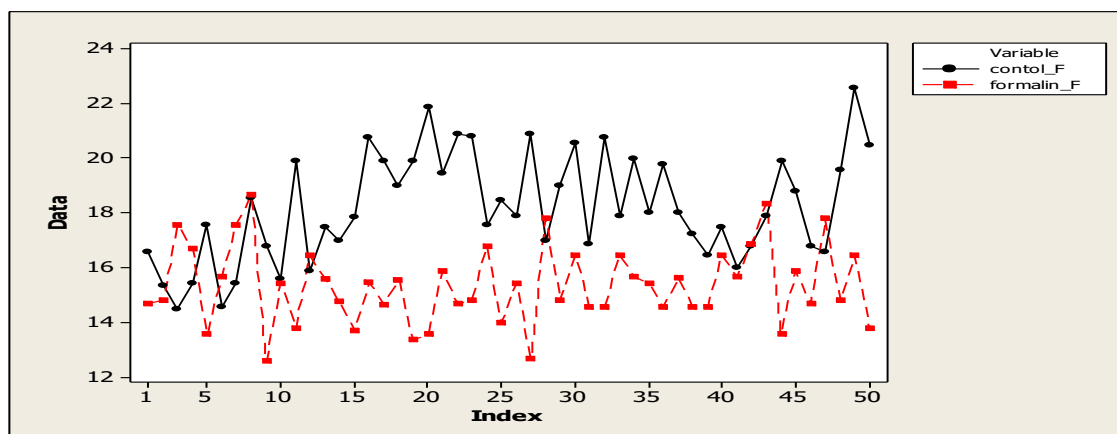
Appendix



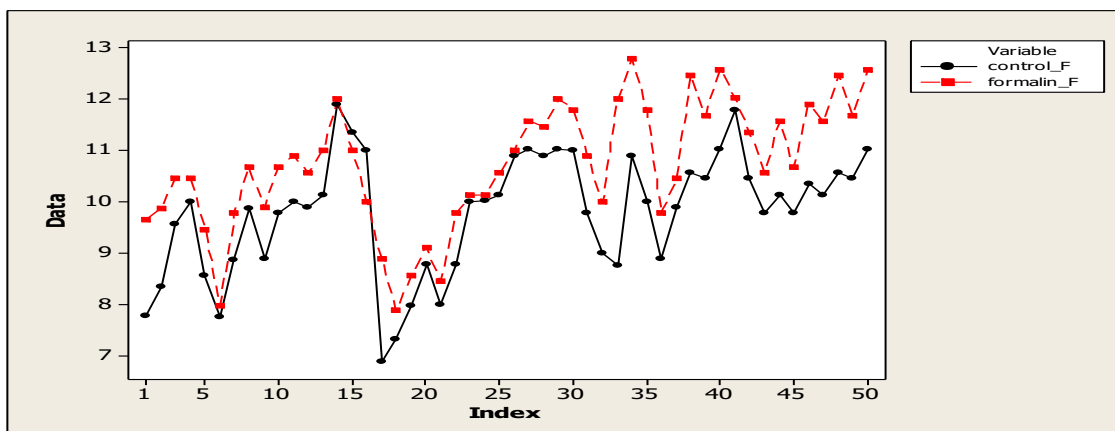
(Appendix-1) Diameter of renal corpuscle (the female treated group with formalin)



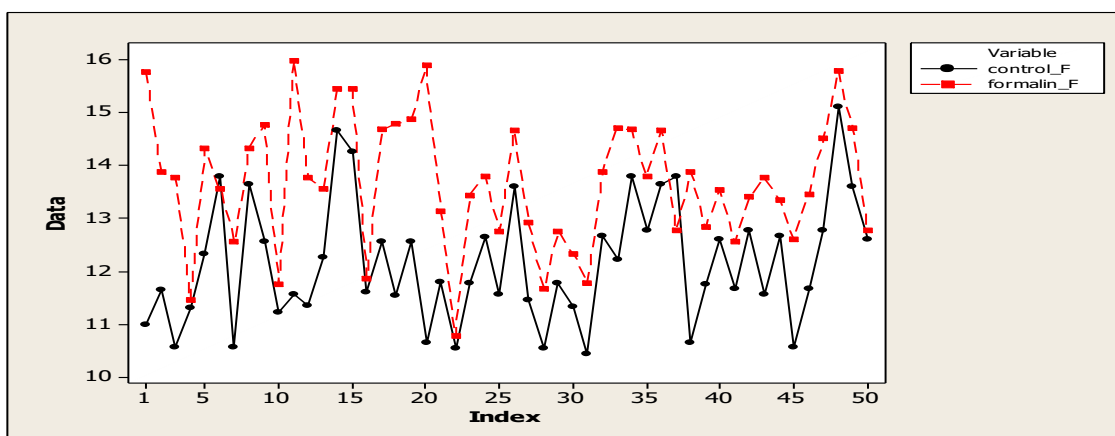
(Appendix-2) Diameter of proximal convoluted tubules (the female treated group with formalin)



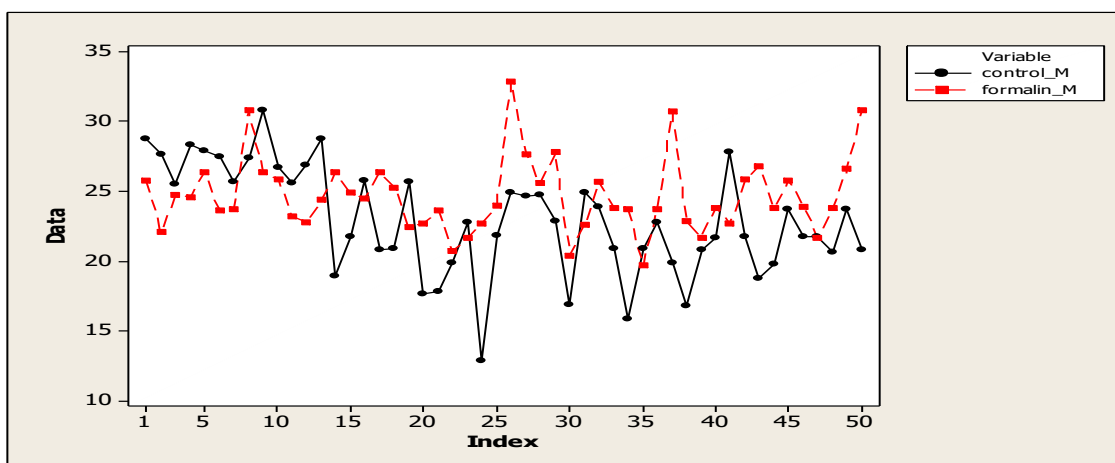
(Appendix-3) Diameter of distal convoluted tubules (the female treated group with formalin)



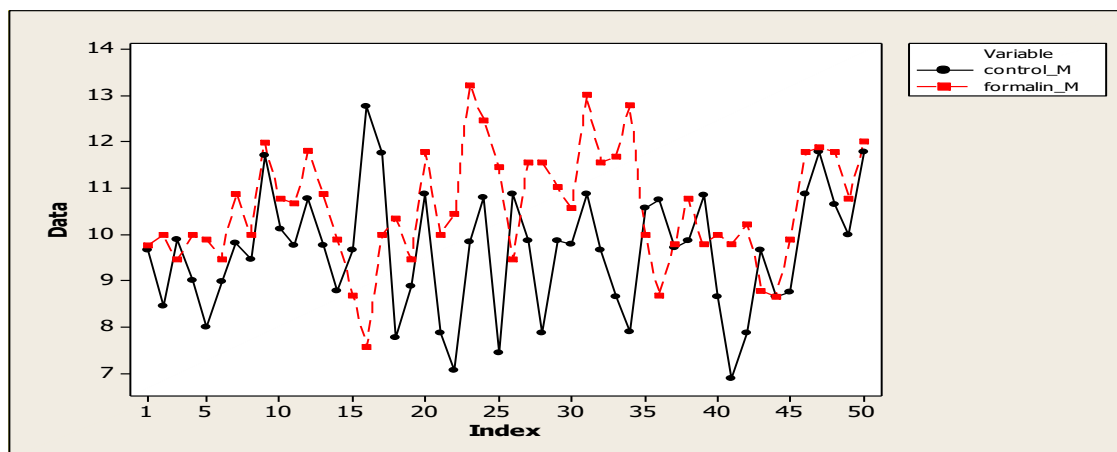
(Appendix-4) Diameter of descending limb of Henley loop (the female treated group with formalin).



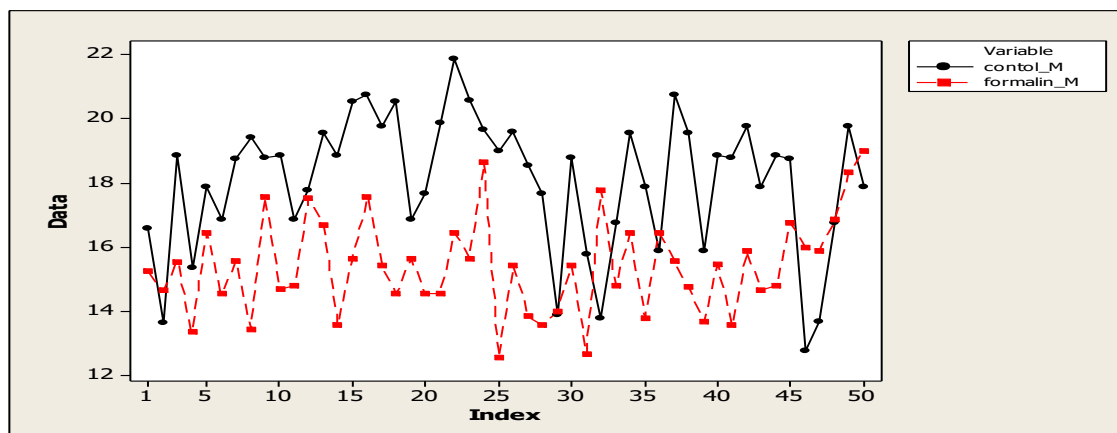
(Appendix-5) Diameter of ascending limb of Henley loop (the female treated group with formalin)



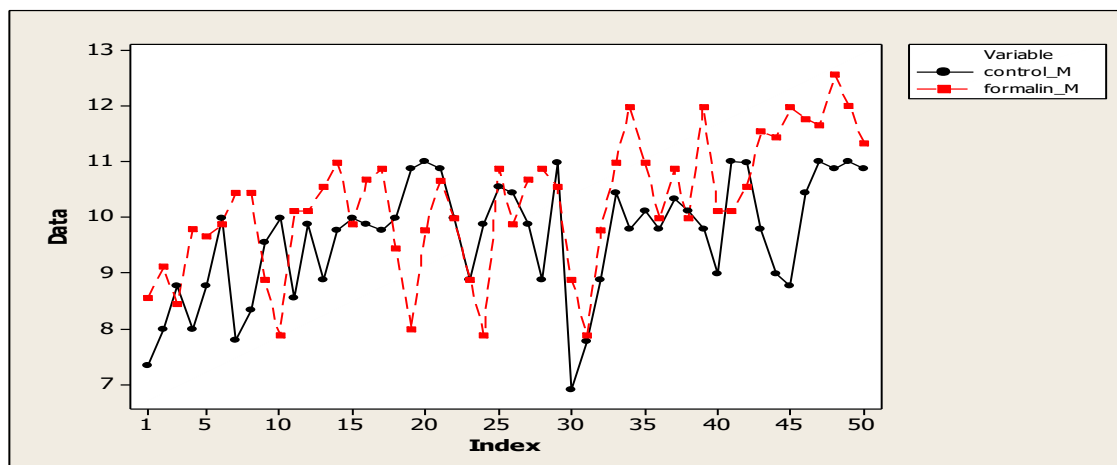
(Appendix-6) Diameter of renal corpuscle (the male treated group with formalin)



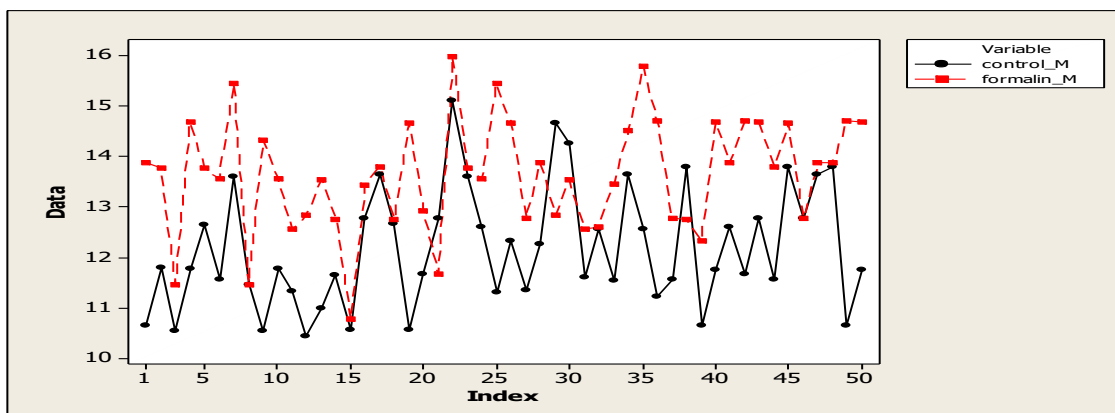
(Appendix-7) Diameter of proximal convoluted tubules (the male treated group with formalin)



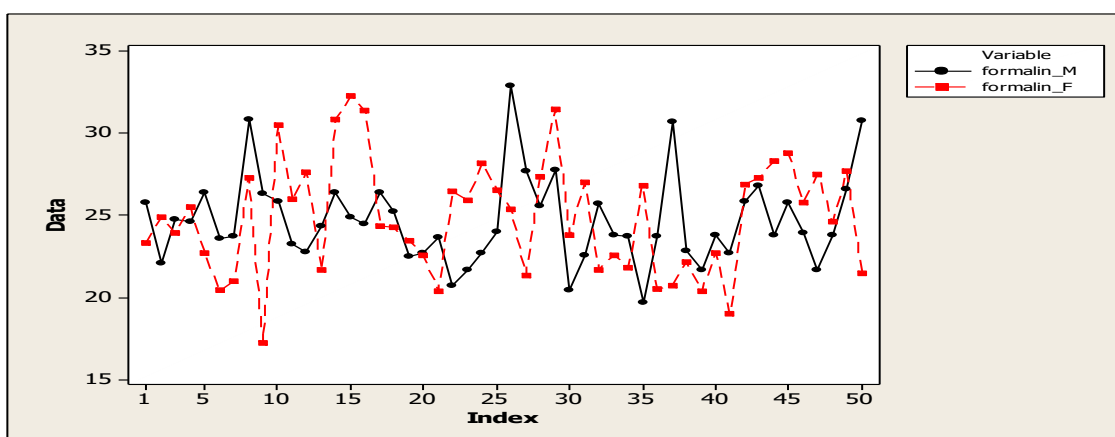
(Appendix-8) Diameter of distal convoluted tubules of kidney (the male treated group with formalin)



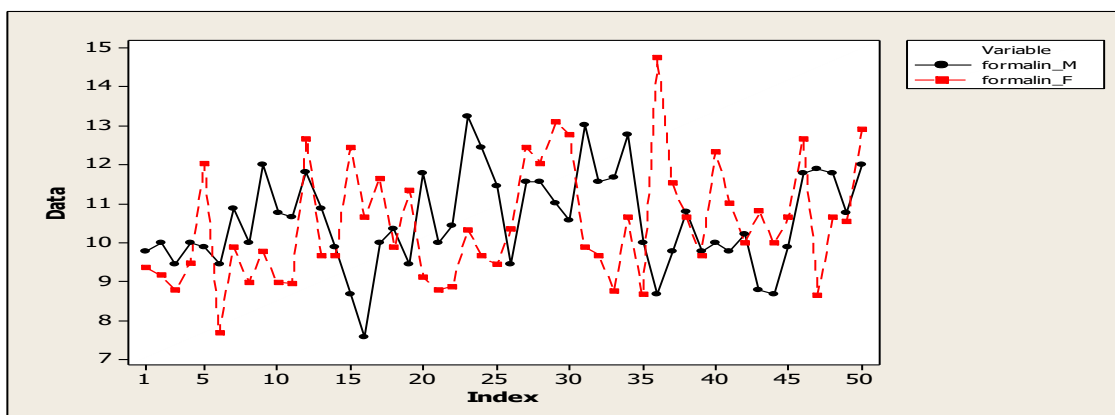
(Appendix-9) Diameter of descending limb of Henley loop (the male treated group with formalin).



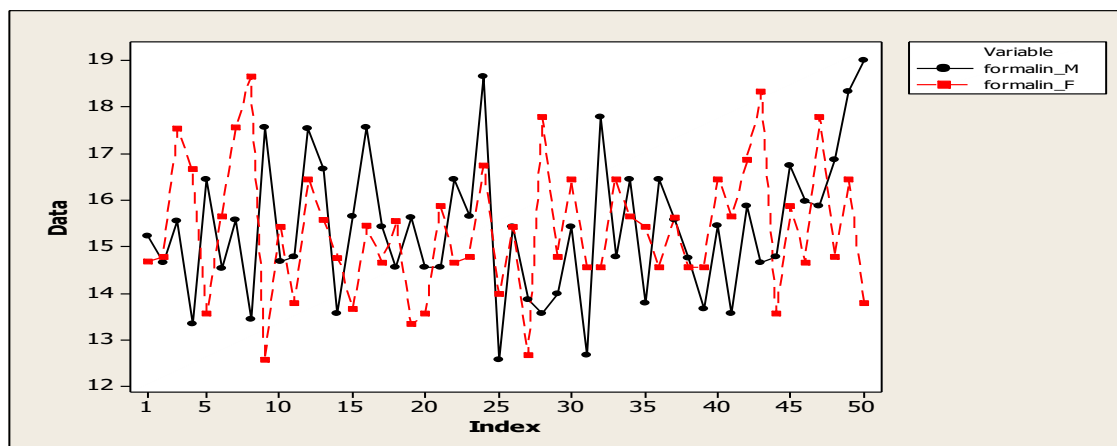
(Appendix-10) Diameter of ascending limp of Henley loop (the male treated group with formalin)



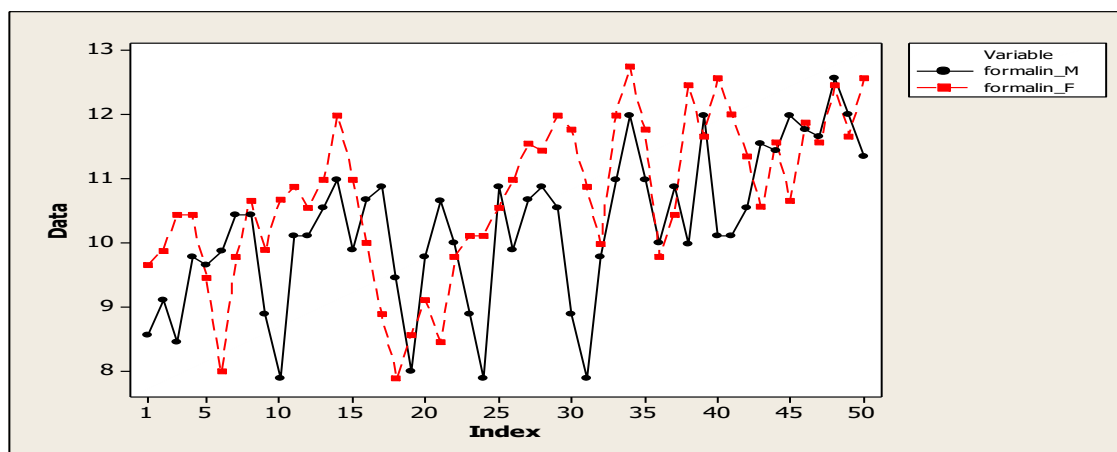
(Appendix-11) Diameter of renal corpuscle (between the male and female treated group with formalin).



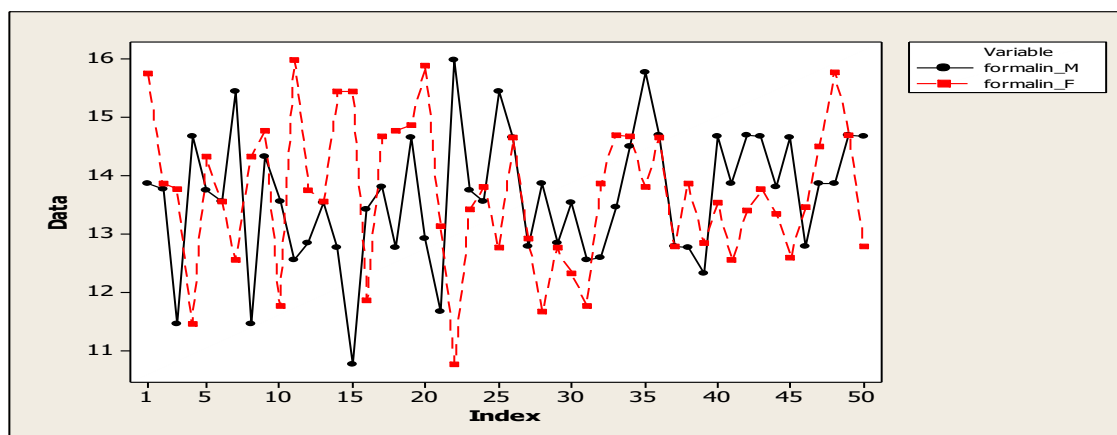
(Appendix-12) Diameter of proximal convoluted tubules (between the male and female treated group with formalin)



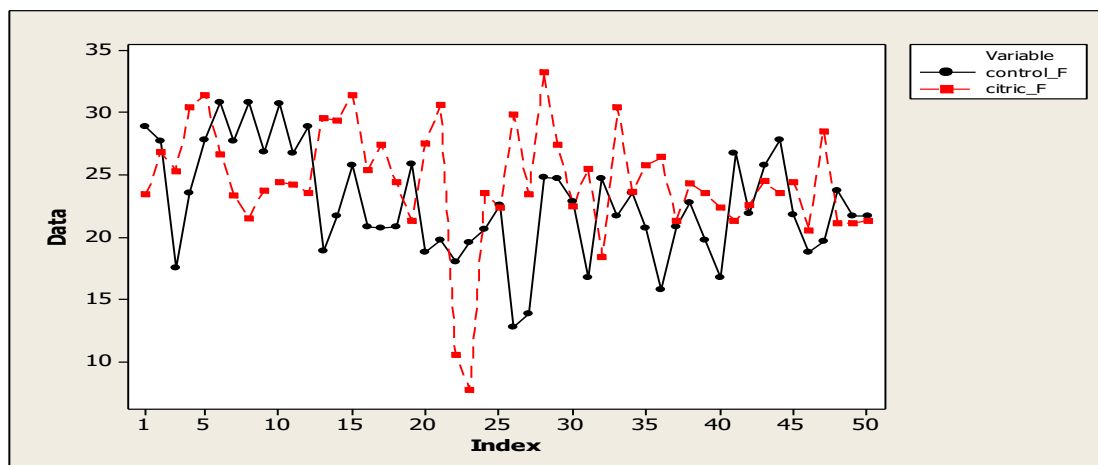
(Appendix-13) Diameter of distal convoluted tubules (between the male and female treated group with formalin)



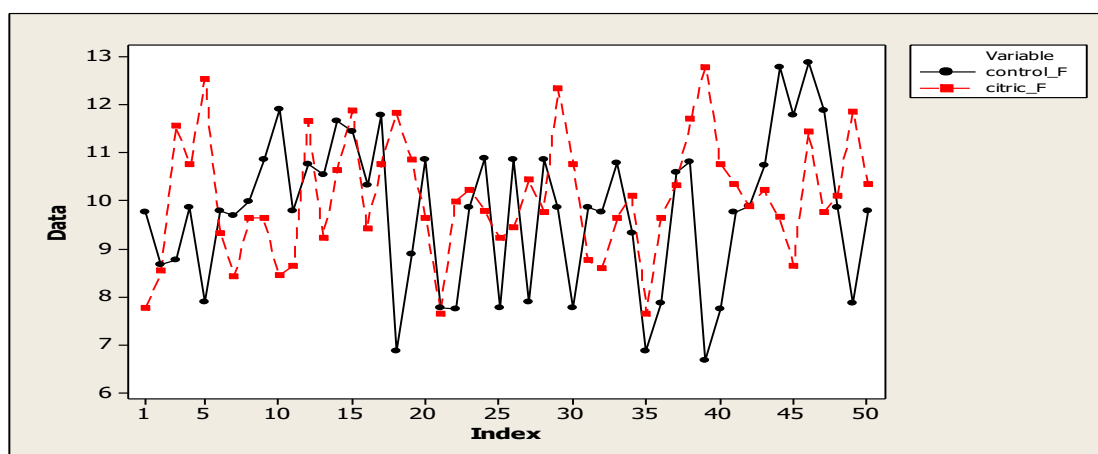
(Appendix-14) Diameter of descending limb of Henley loop (between the male and female treated group with formalin).



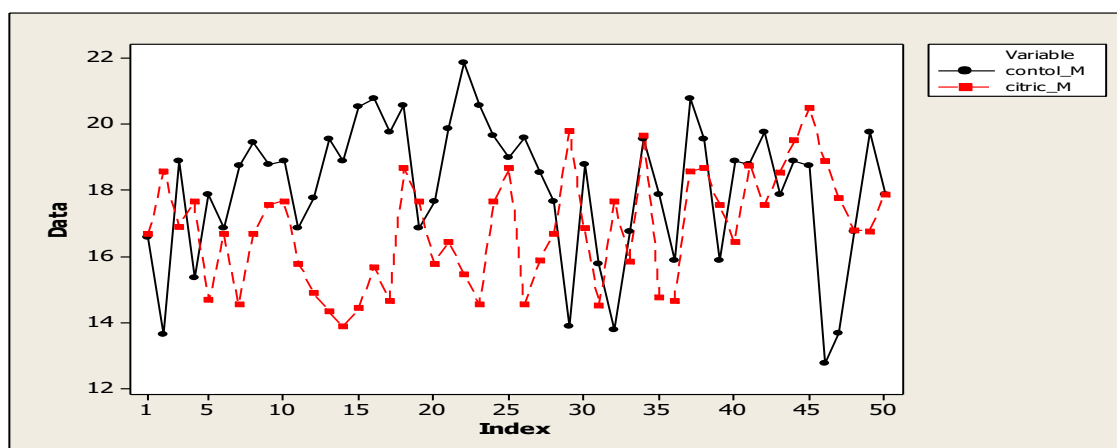
(Appendix-15) Diameter of ascending limb of Henley loop (between the male and female treated group with formalin)



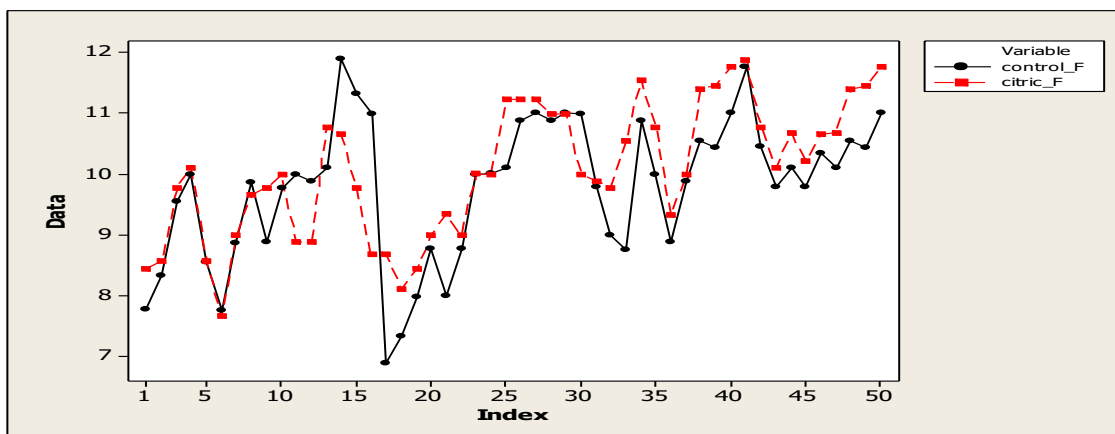
(Appendix-16) Diameter of renal corpuscle (the female treated group with citric acid).



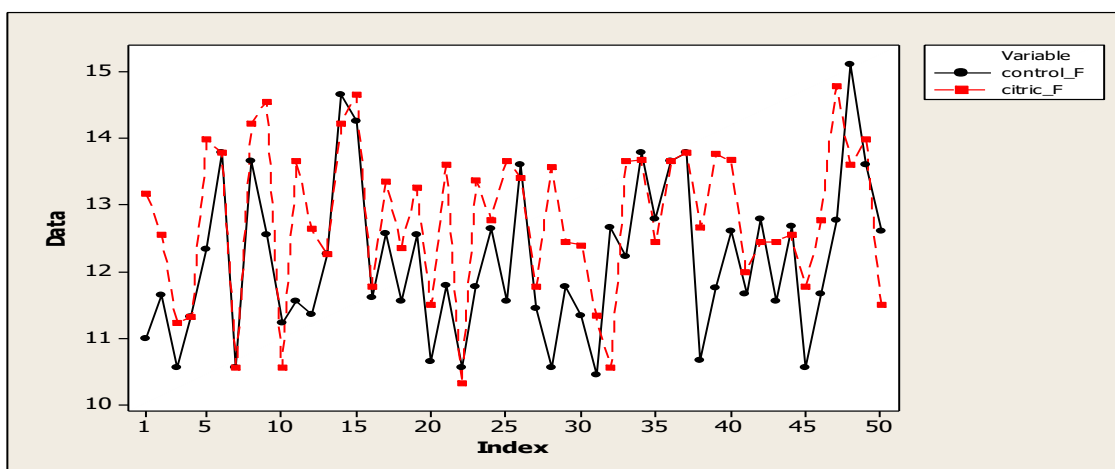
(Appendix-17) Diameter of proximal convoluted tubules (the female treated group with citric acid).



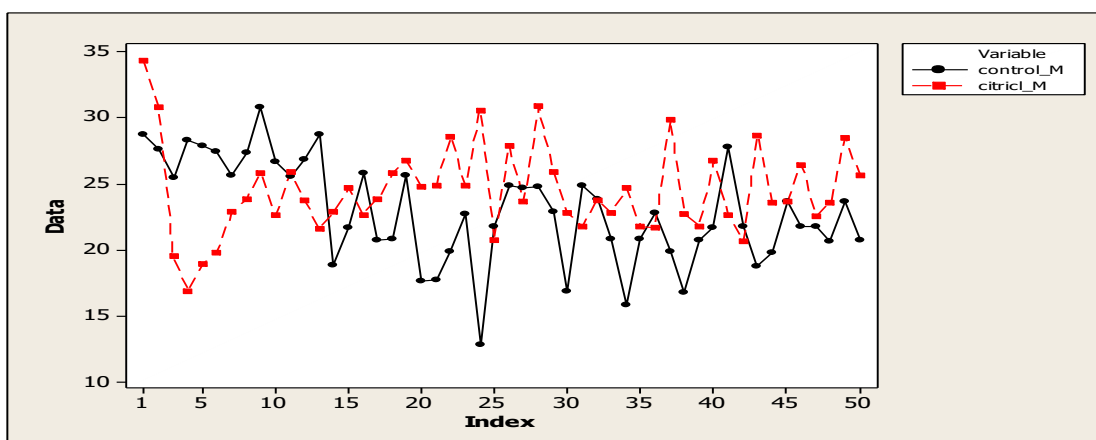
(Appendix-18) Diameter of distal convoluted tubules (the female treated group with citric acid).



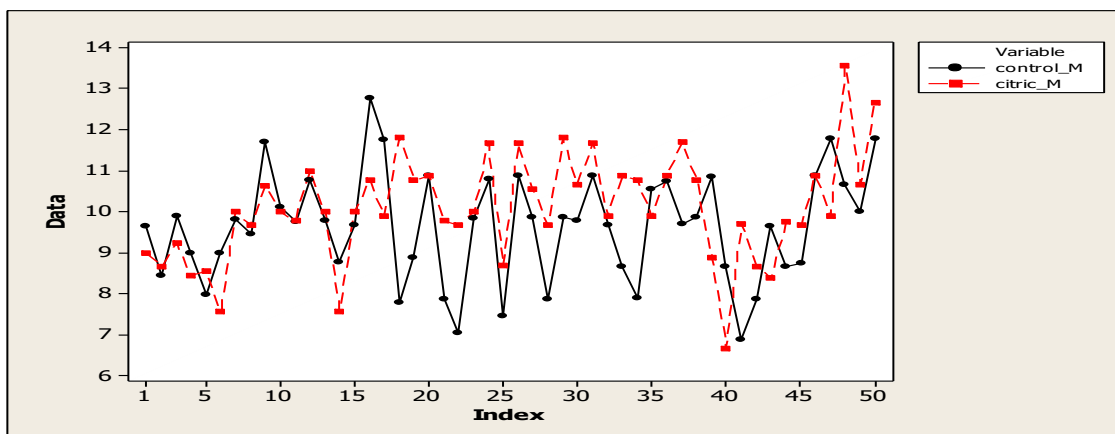
(Appendix-19) Diameter of descending limb of Henley loop (the female treated group with citric acid).



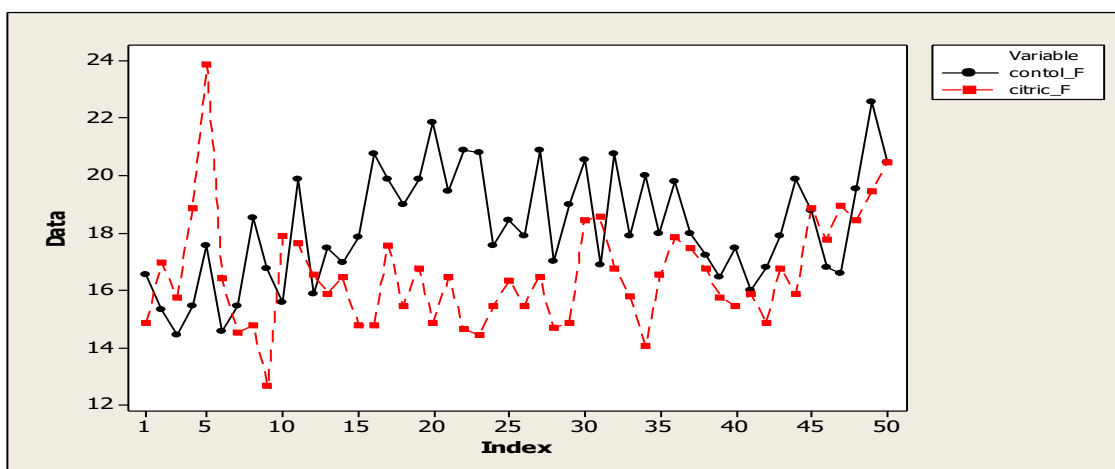
(Appendix-20) Diameter of ascending limb of Henley loop (the female treated group with citric acid).



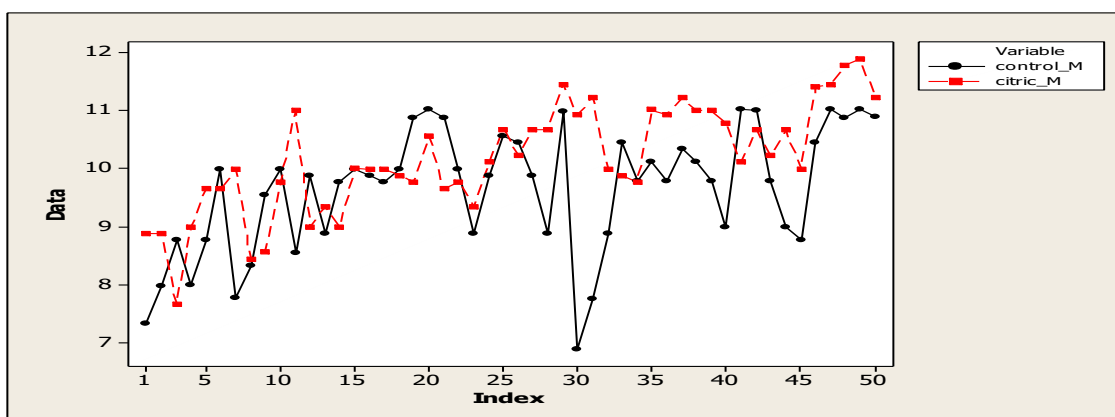
(Appendix-21) Diameter of renal corpuscle (the male treated group with citric acid).



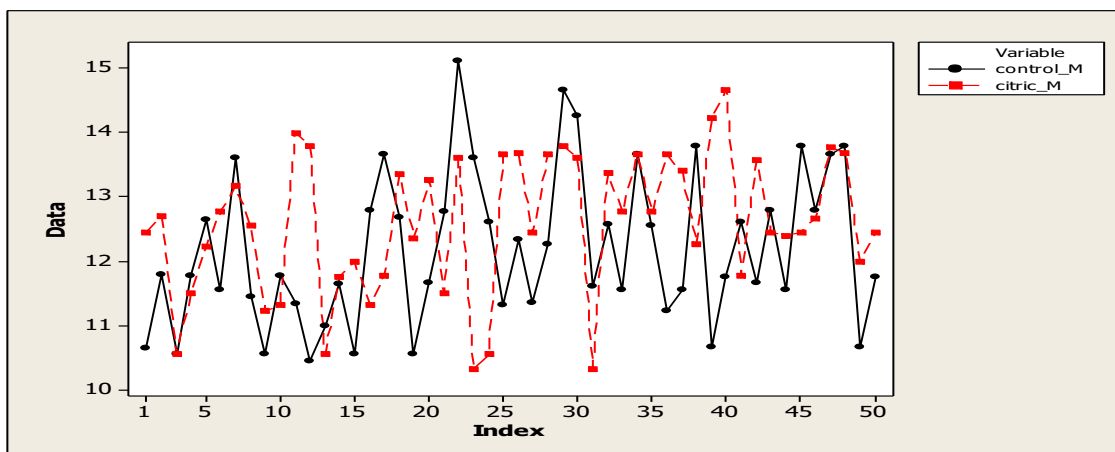
(Appendix-22) Diameter of proximal convoluted tubules (the male treated group with citric acid).



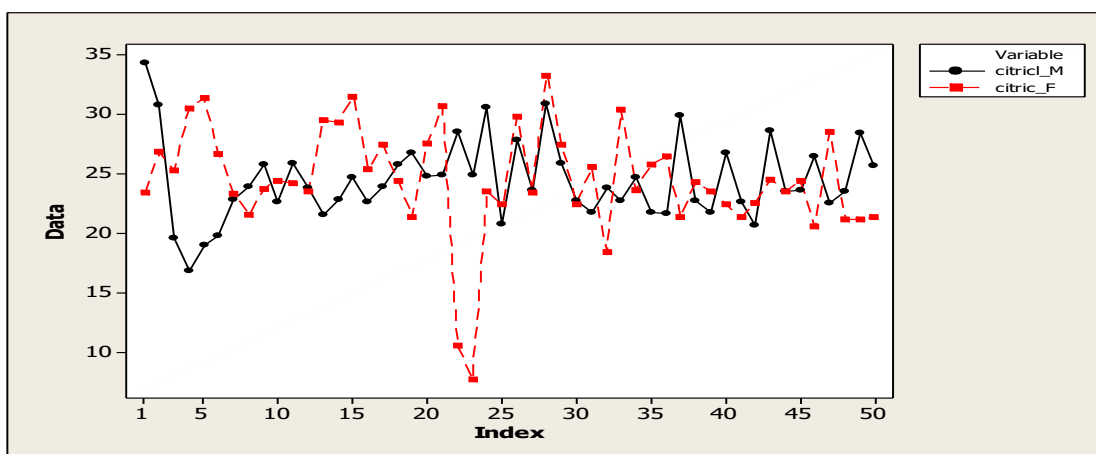
(Appendix-23) Diameter of distal convoluted tubules (the male treated group with citric acid).



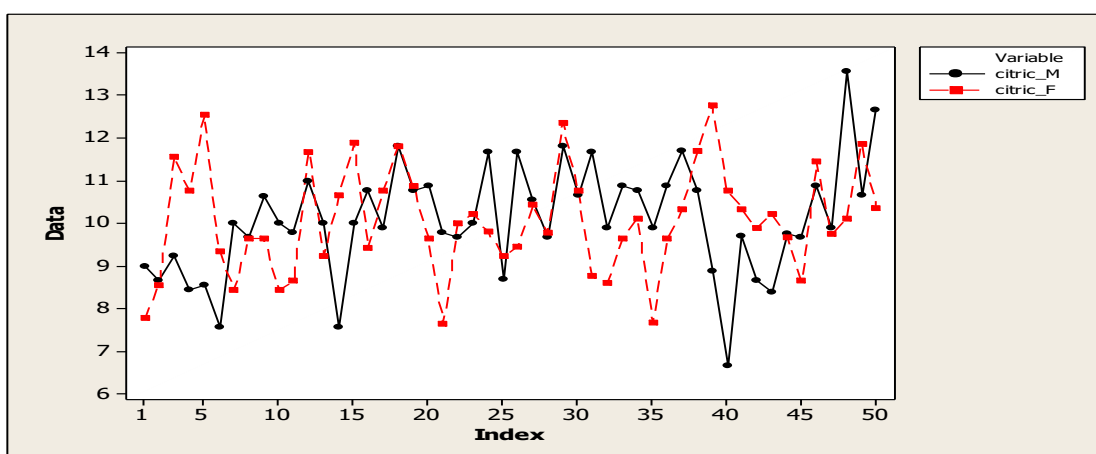
(Appendix-24) Diameter of descending limb of Henley loop (the male treated group with citric acid).



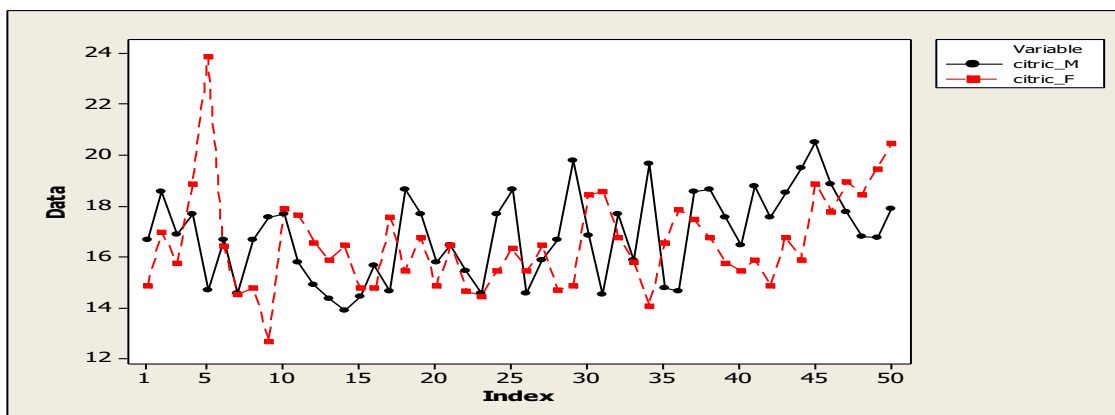
(Appendix-25) Diameter of ascending limb of Henley loop (the male treated group with citric acid).



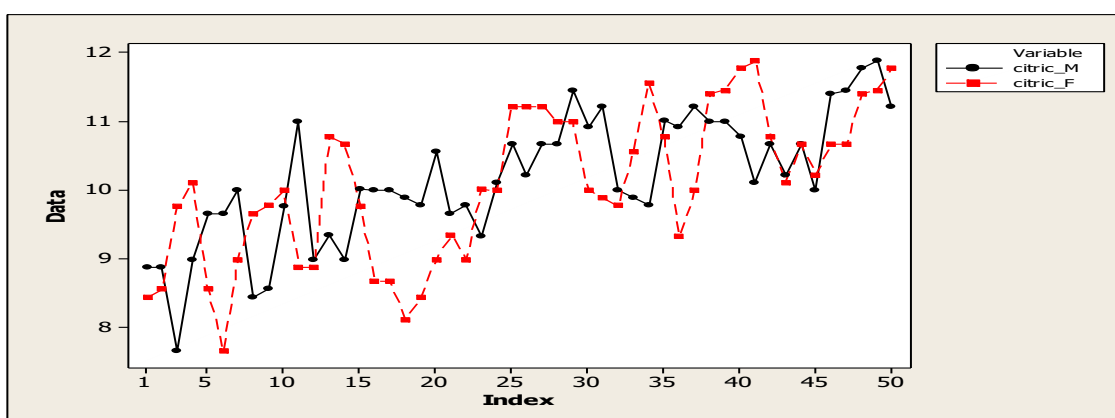
(Appendix-26) Diameter of renal corpuscle (between the male and female treated group with citric acid).



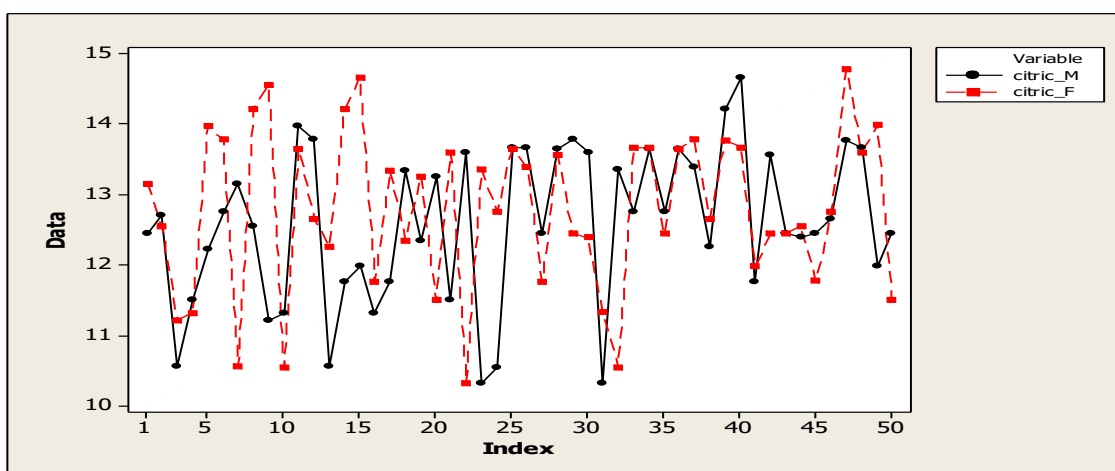
(Appendix-27) Diameter of proximal convoluted tubules (between the male and female treated group with citric acid).



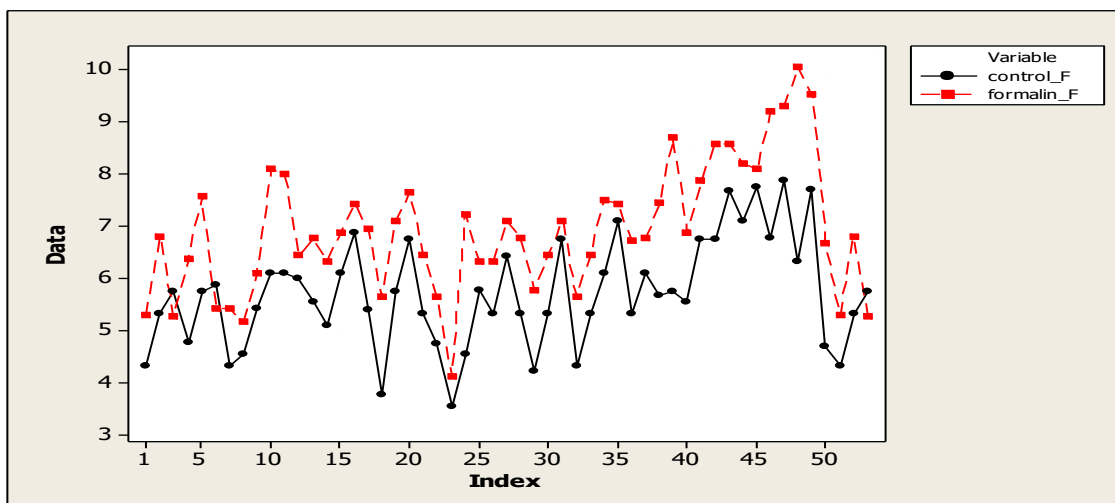
(Appendix-28) Diameter of distal convoluted tubules (between the male and female treated group with citric acid).



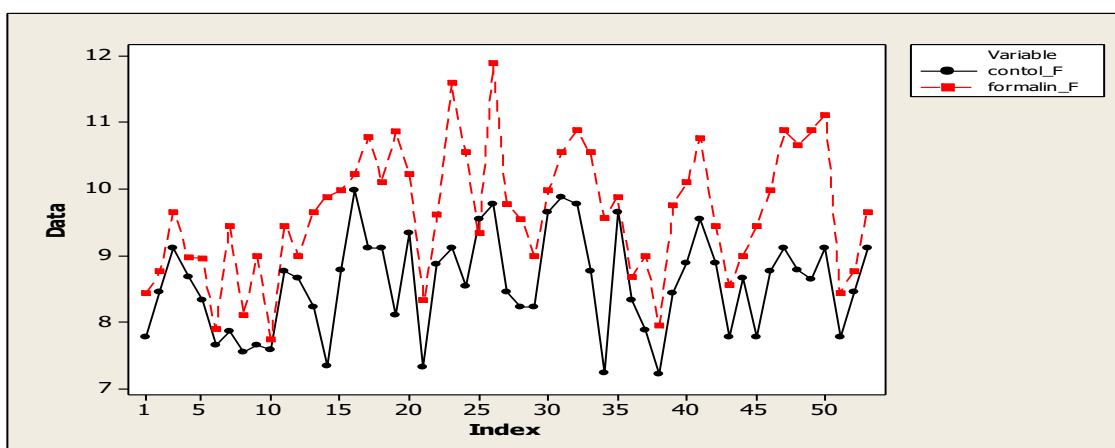
(Appendix-29) Diameter of descending limb of Henley loop (between the male and female treated group with citric acid).



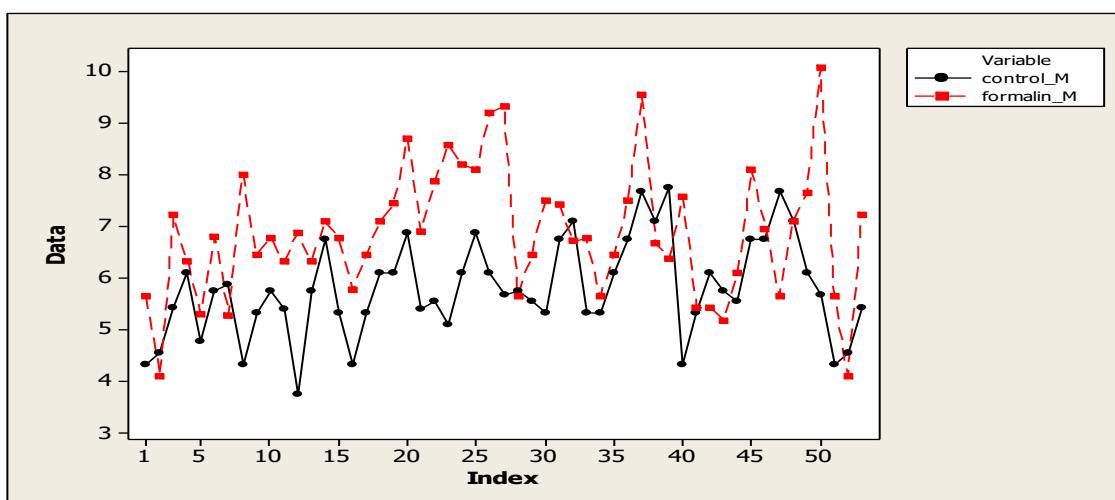
(Appendix-30) Diameter of ascending limb of Henley loop (between the male and female treated group with citric acid).



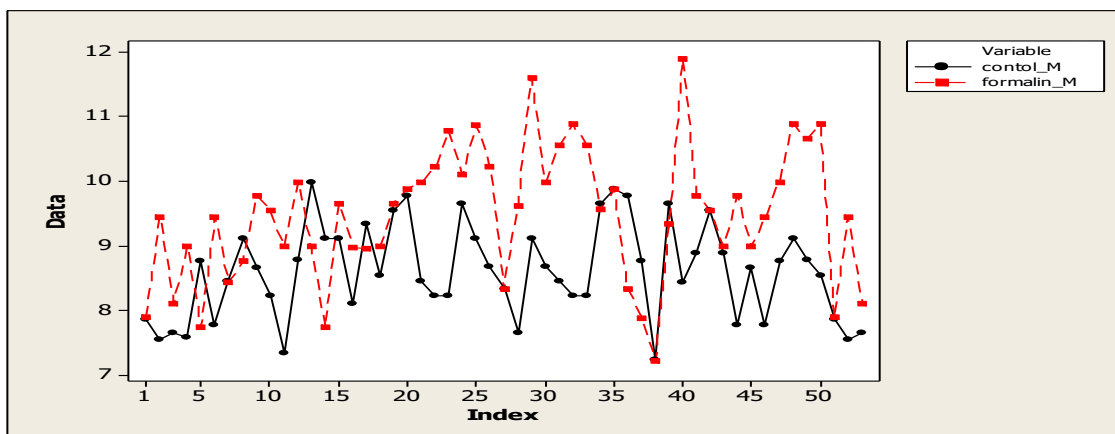
(Appendix-31) Thickness of liver capsule (the female treated group with formalin).



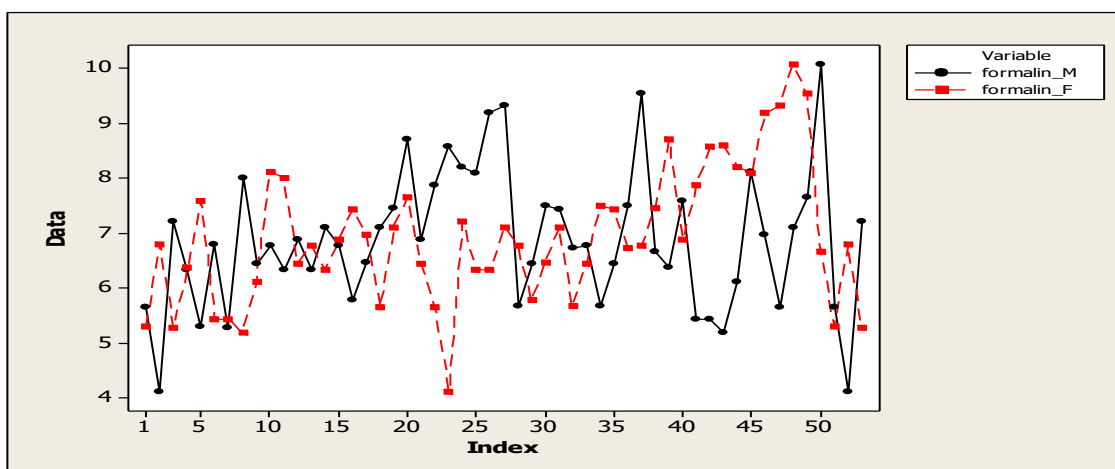
(Appendix-32) Diameter of hepatocyte nuclei in liver (the female treated group with formalin).



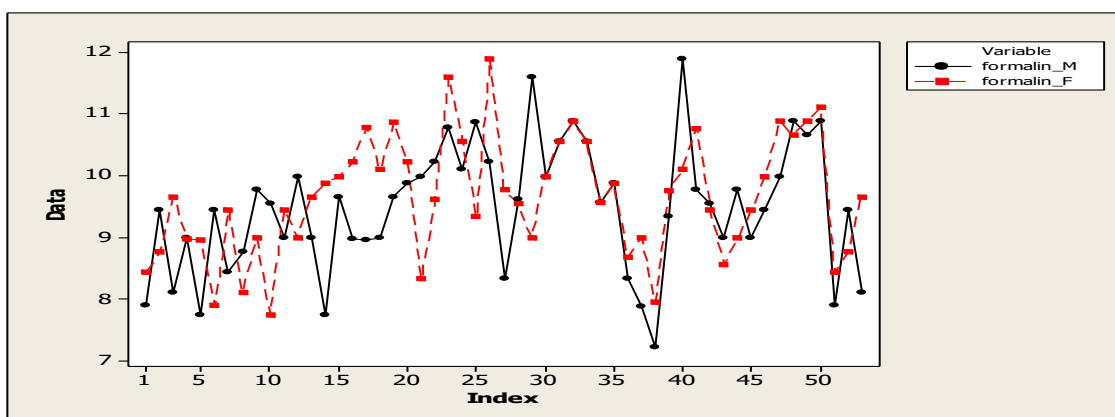
(Appendix-33) Thickness of liver capsule (the male treated group with formalin).



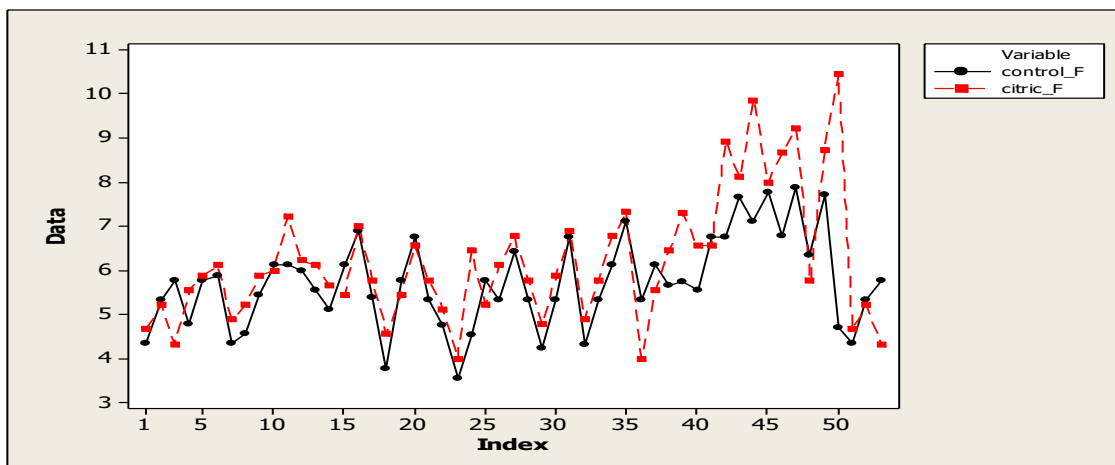
(Appendix-34) Diameter of hepatocyte nuclei in liver (the male treated group with formalin).



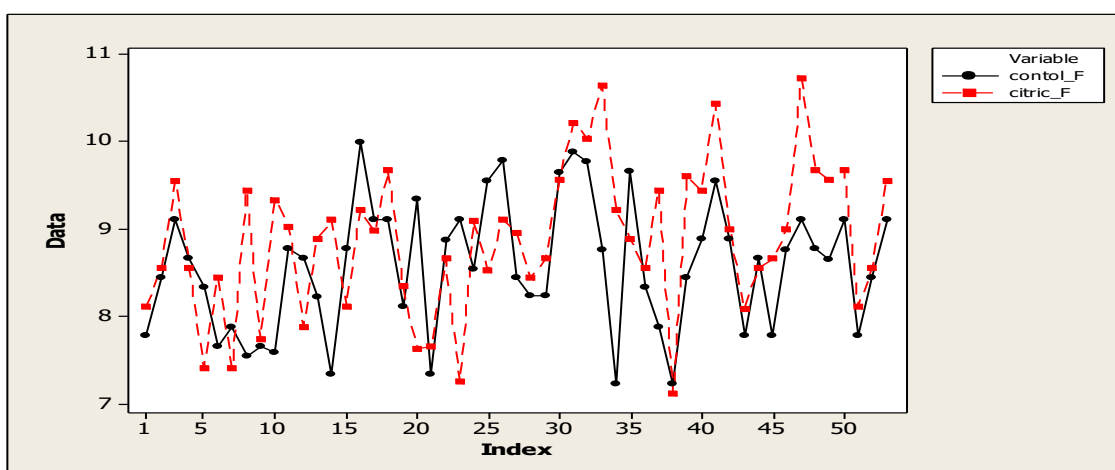
(Appendix-35) Thickness of liver capsule (between the male and female treated group with formalin).



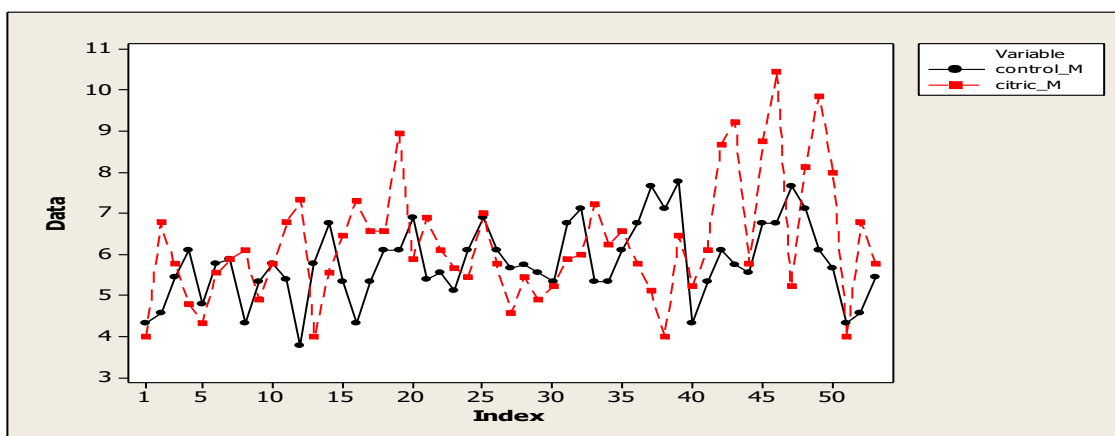
(Appendix-36) Diameter of hepatocyte nuclei in liver (between the male and female treated group with formalin).



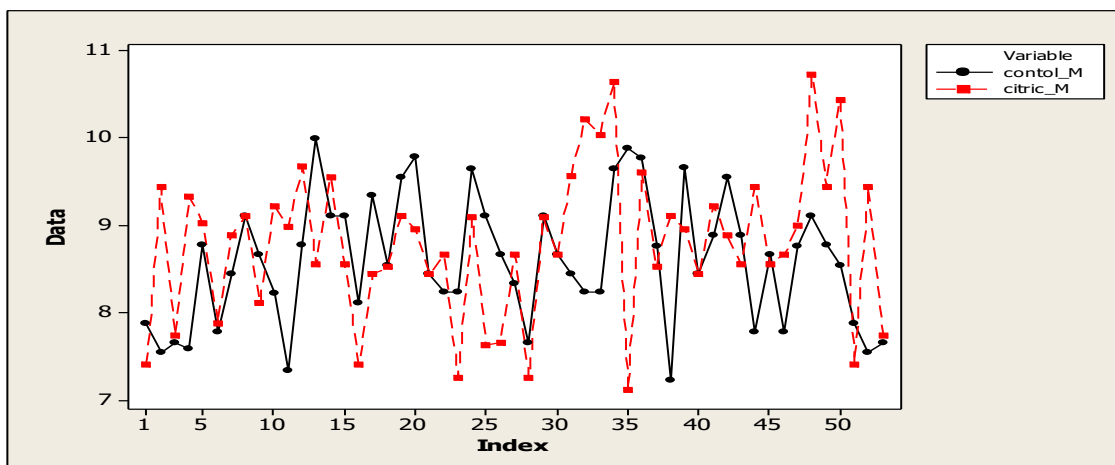
(Appendix-37) Thickness of liver capsule (the female treated group with citric acid).



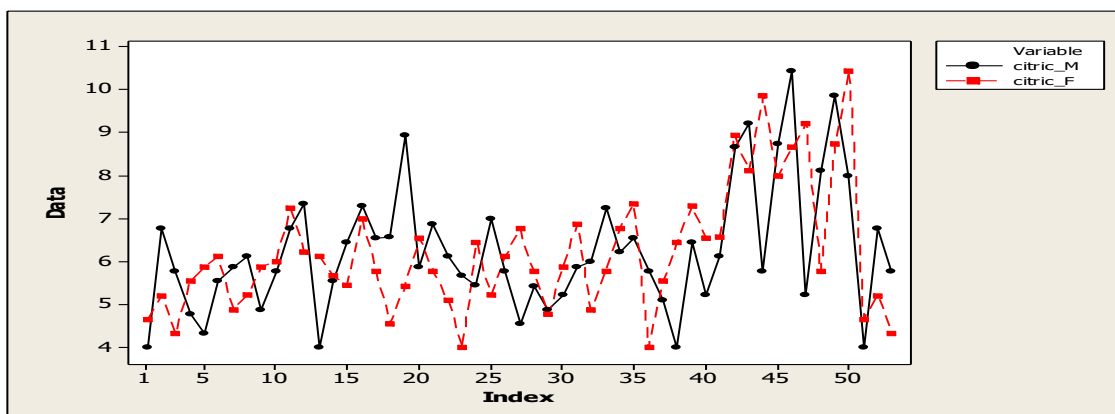
(Appendix-38) Diameter of hepatocyte nuclei in liver (the female treated group with citric acid).



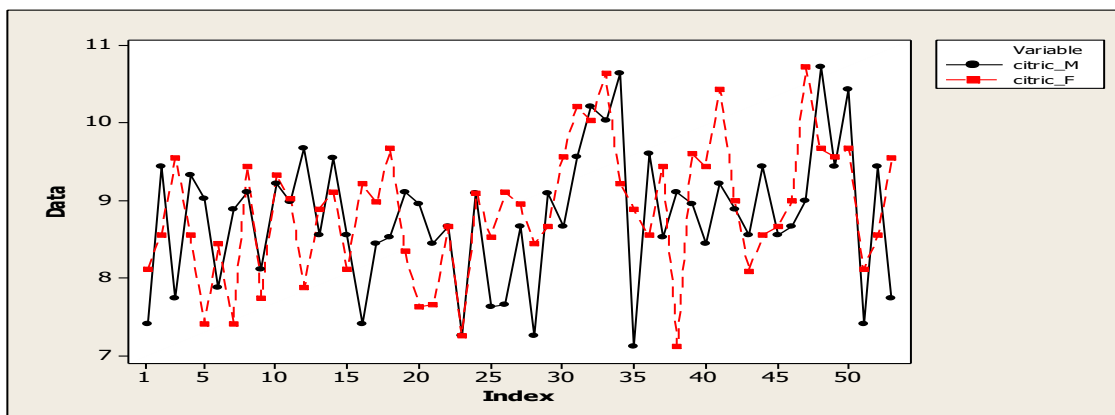
(Appendix-39) Thickness of liver capsule (the male treated group with citric acid).



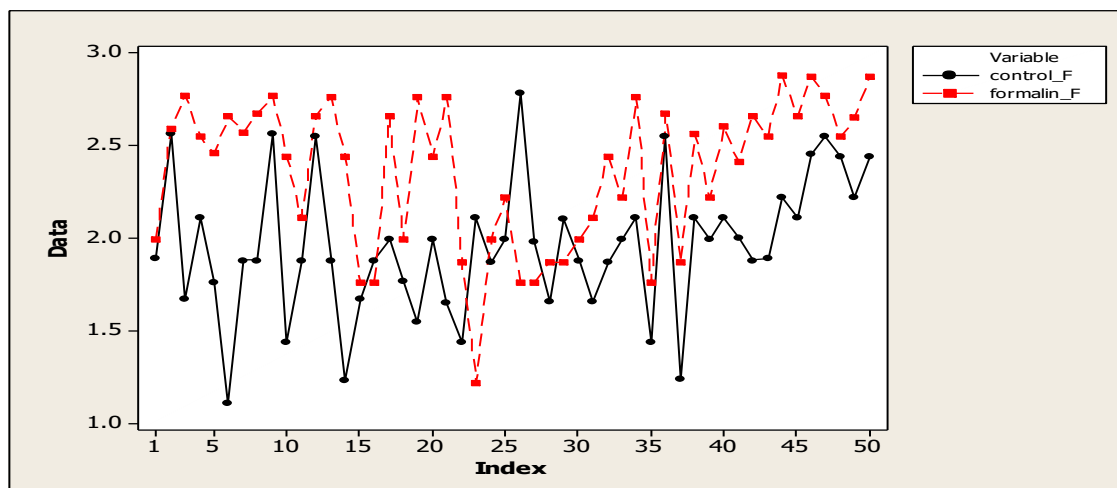
(Appendix-40) Diameter of hepatocyte nuclei in liver (the male treated group with citric acid).



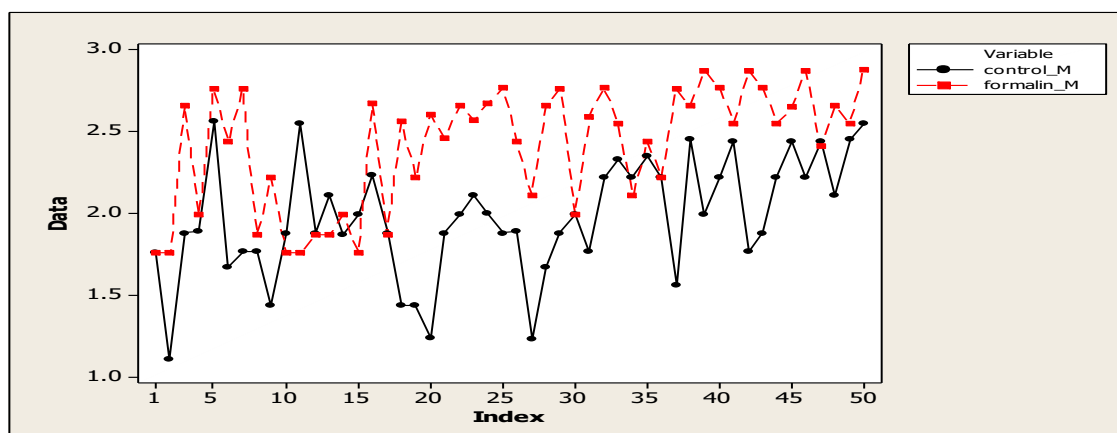
(Appendix-41) Thickness of liver capsule (between the male and female treated group with citric acid).



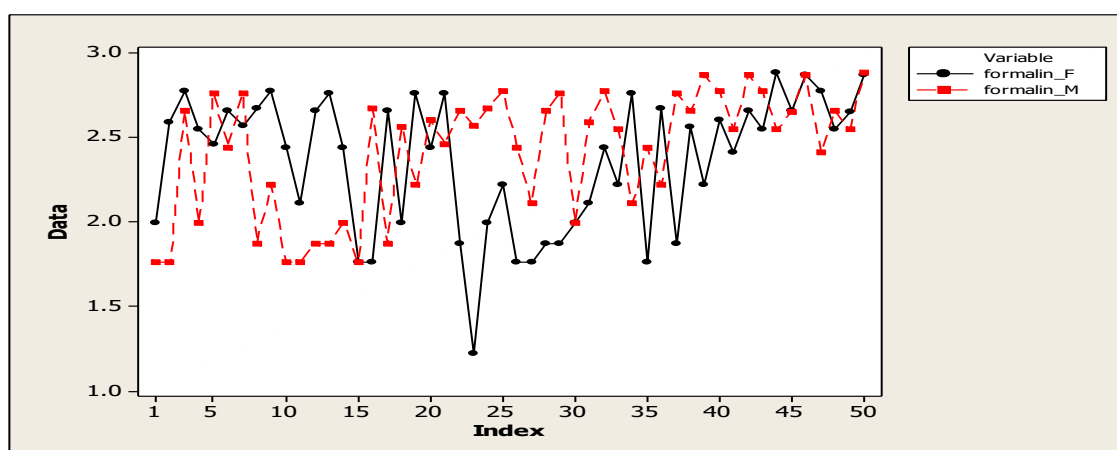
(Appendix-42) Diameter of hepatocyte nuclei in liver (between the male and female treated group with citric acid).



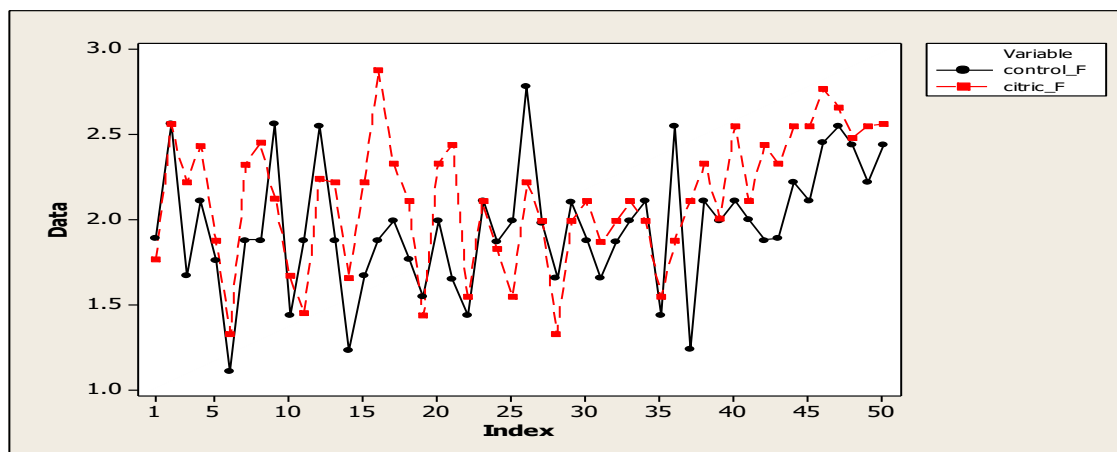
(Appendix -43) Thickness of the epithelial layer in treated groups with formalin (Female).



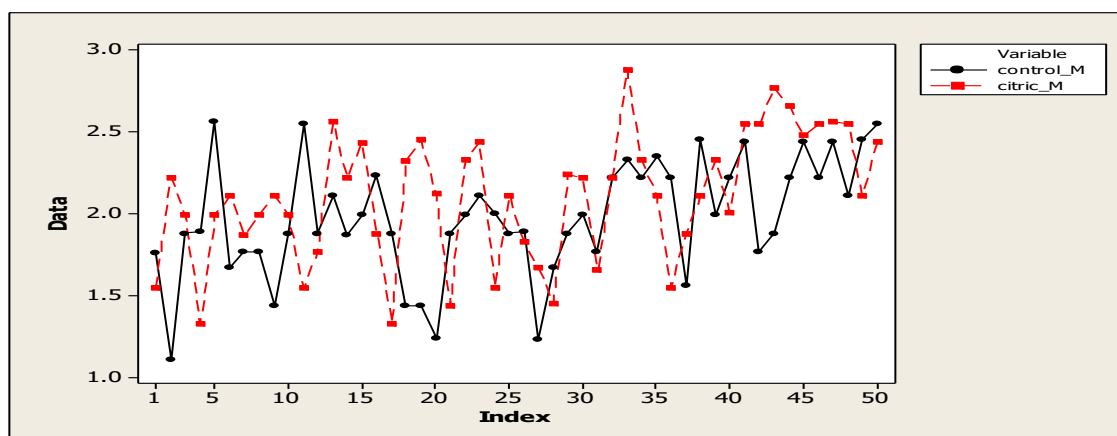
(Appendix -44) Thickness of the epithelial layer in treated groups with formalin (Male).



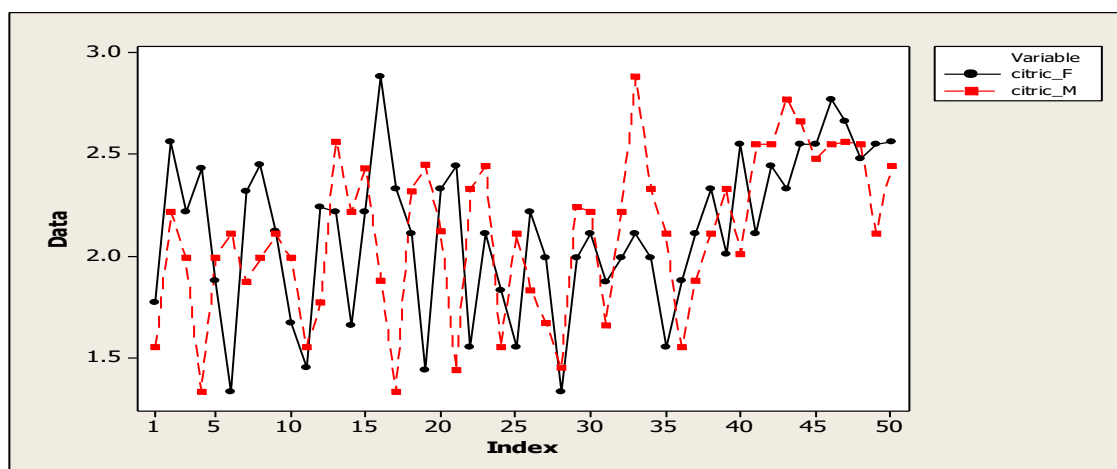
(Appendix -45) Thickness of the epithelial layer in treated groups with formalin (Male and Female)



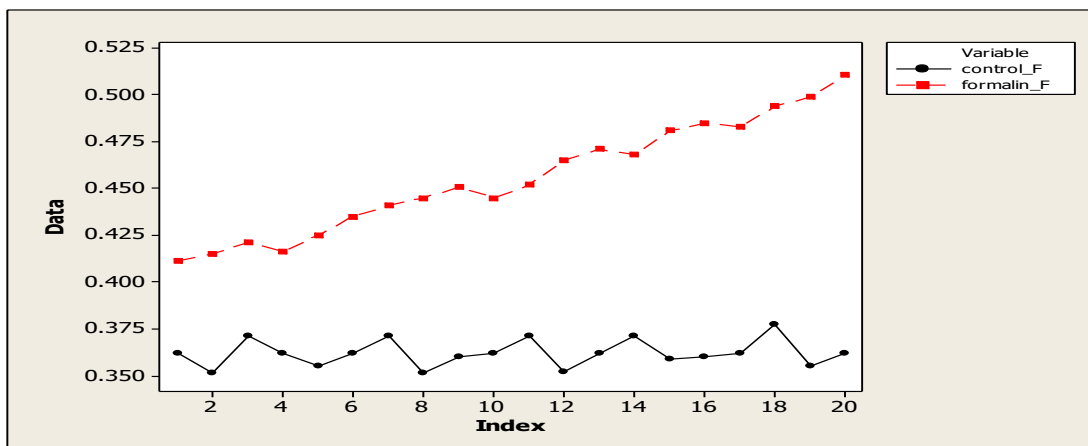
(Appendix -46) Thickness of the epithelial layer in treated groups with citric acid (Female).



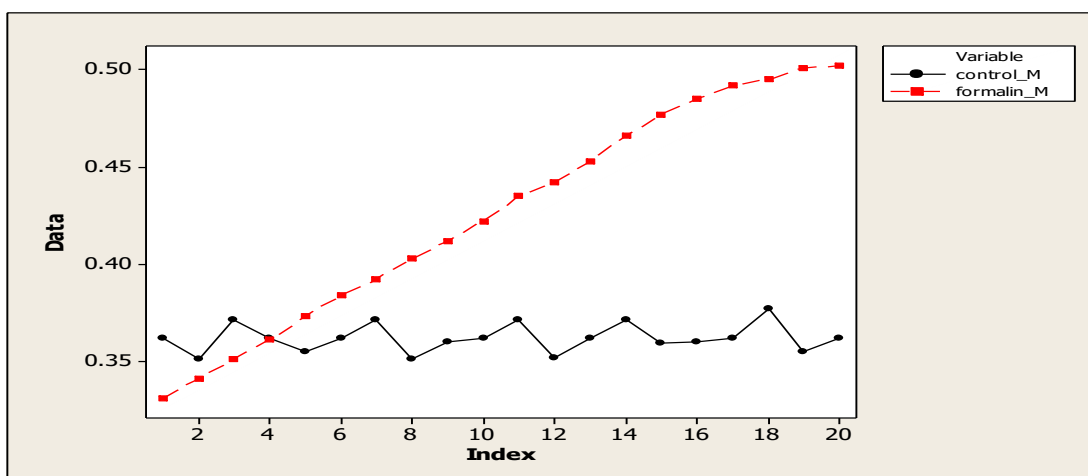
(Appendix -47) Thickness of the epithelial layer in treated groups with citric acid (Male).



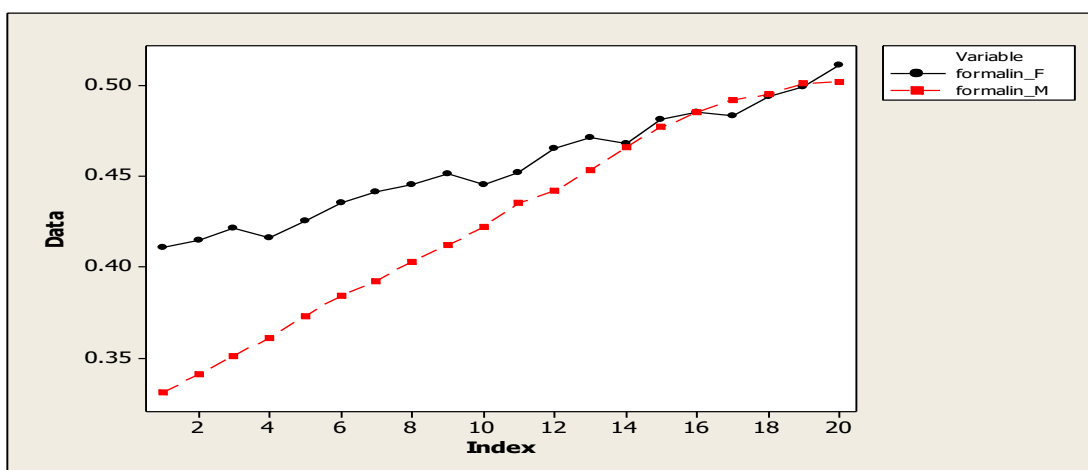
(Appendix -48) Thickness of the epithelial layer in treated groups with citric acid (Male and Female)



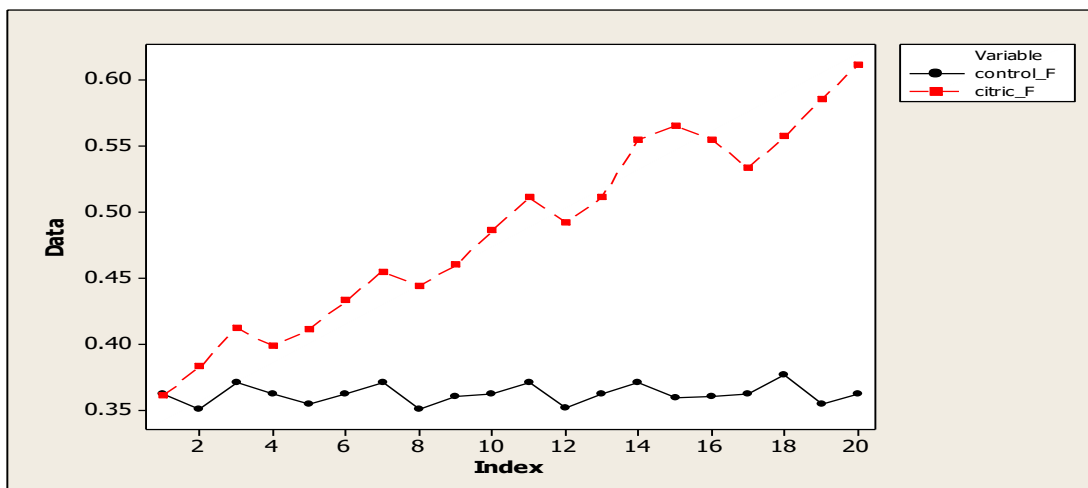
(Appendix -49) Level of Creatinine in the serum of treated group with formalin (female).



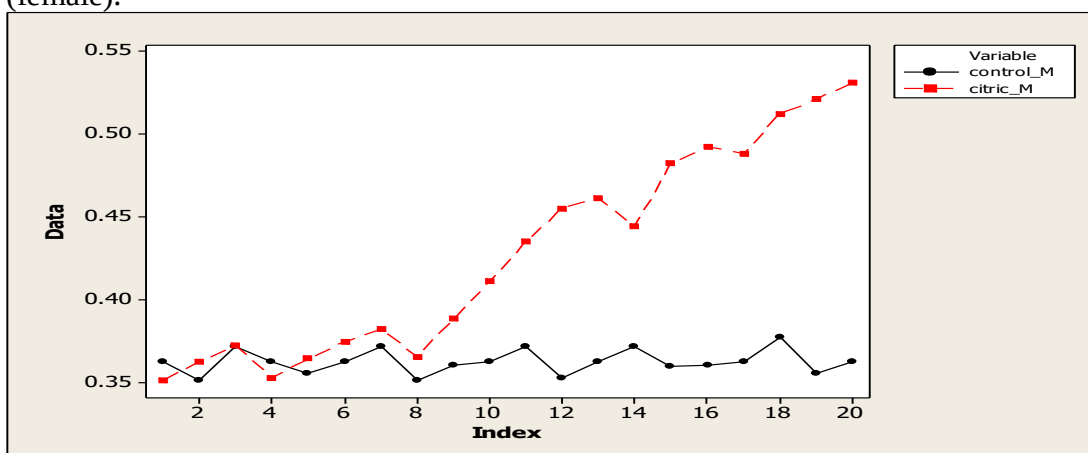
(Appendix -50) Level of Creatinine in the serum of treated group with formalin (male).



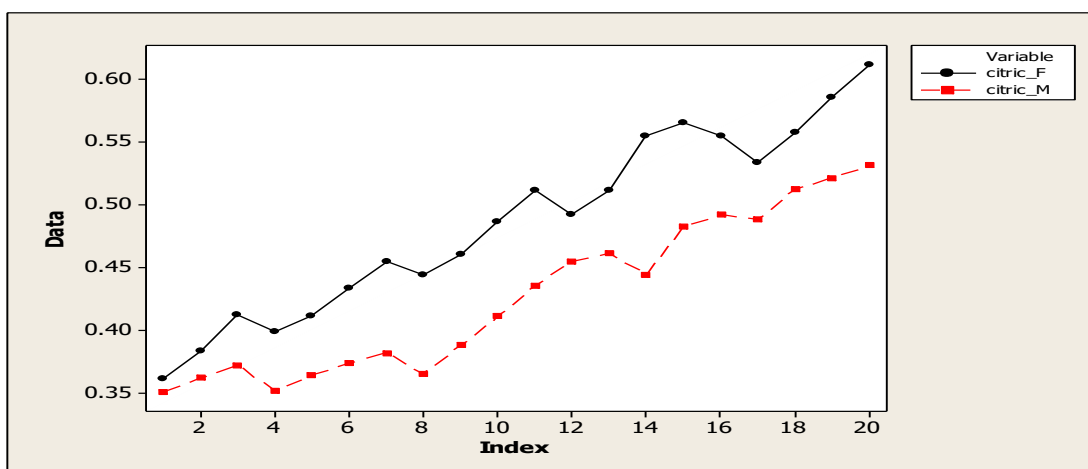
(Appendix -51) Level of Creatinine in the serum of treated group with formalin between female and male.



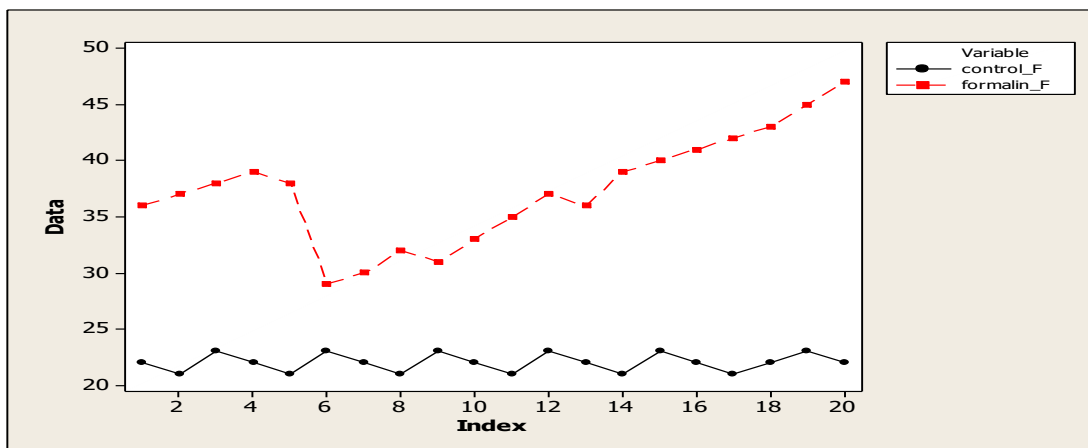
(Appendix -52) Level of Creatinine in the serum of treated group with citric acid (female).



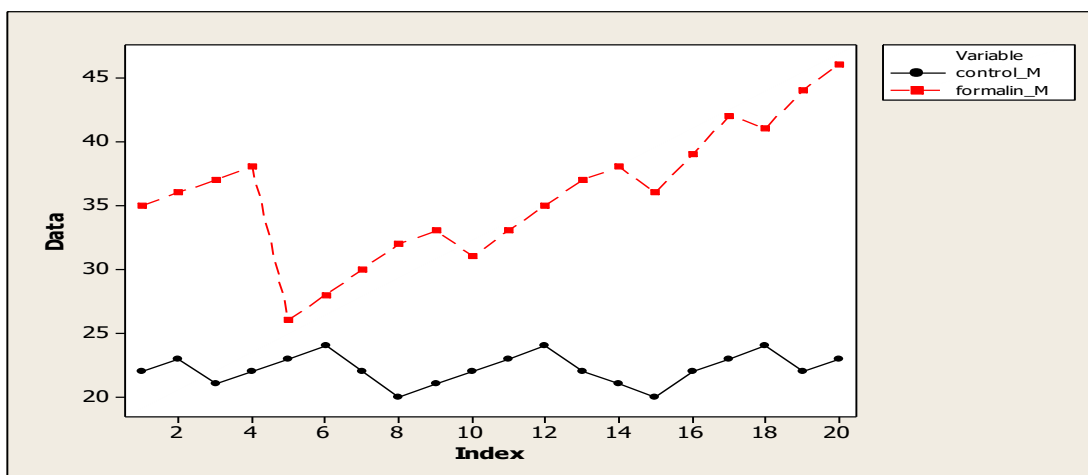
(Appendix -53) Level of Creatinine in the serum of treated group with citric acid (male).



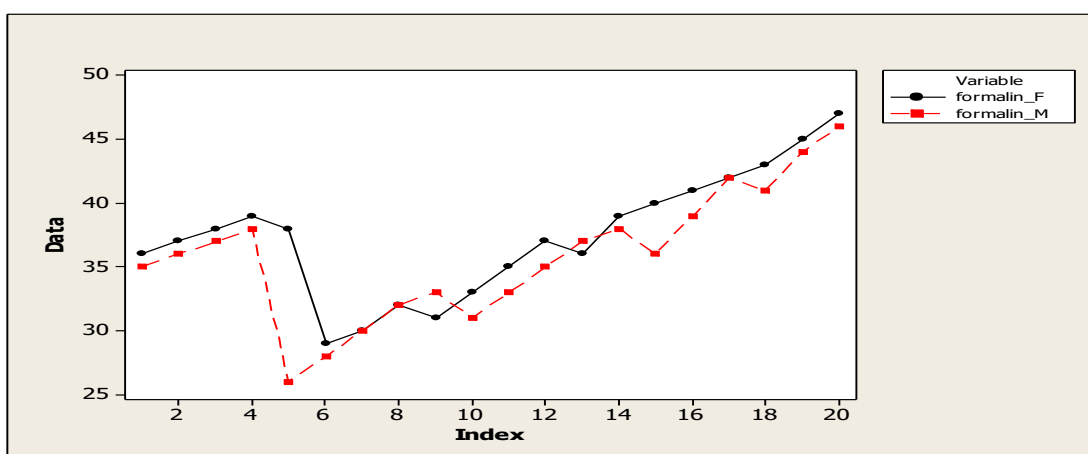
(Appendix -54) Level of Creatinine in the serum of treated group with citric acid between female and male.



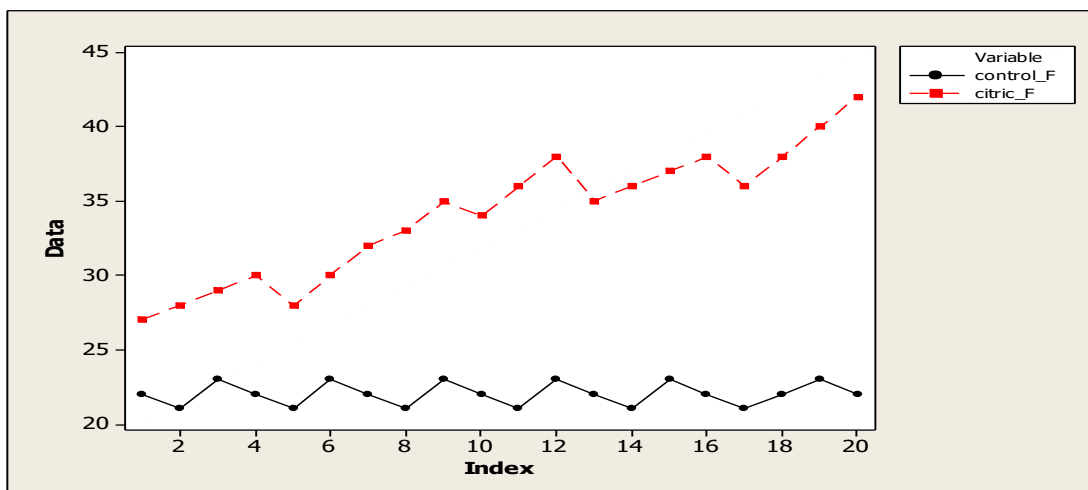
(Appendix -55) Level Urea of in the serum of treated group with formalin (female).



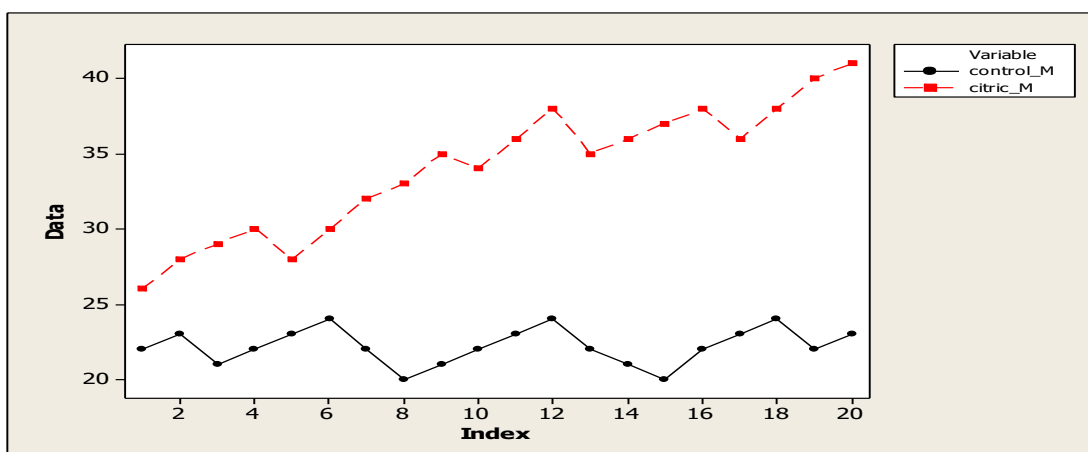
(Appendix -56) Level Urea of in the serum of treated group with formalin (male).



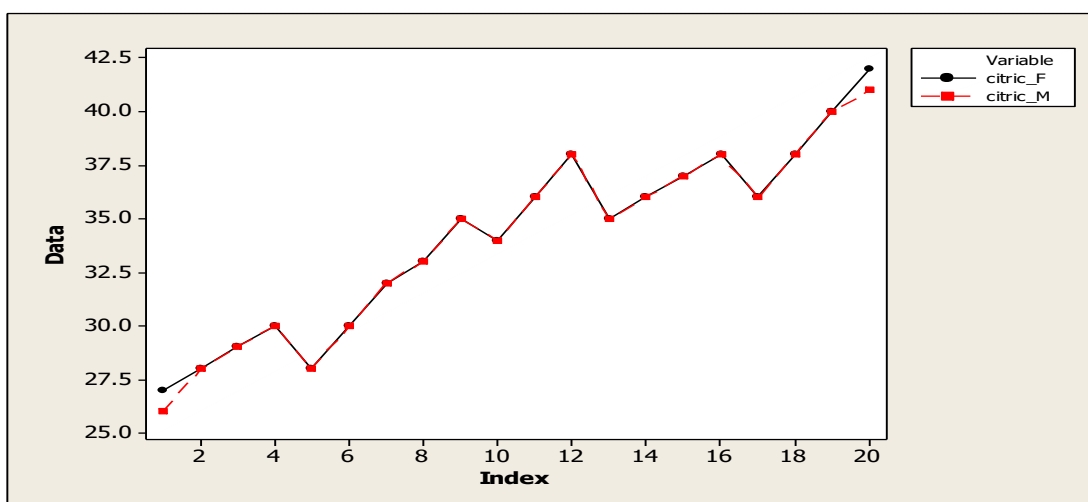
(Appendix -57) Level Urea of in the serum of treated group with formalin between female and male.



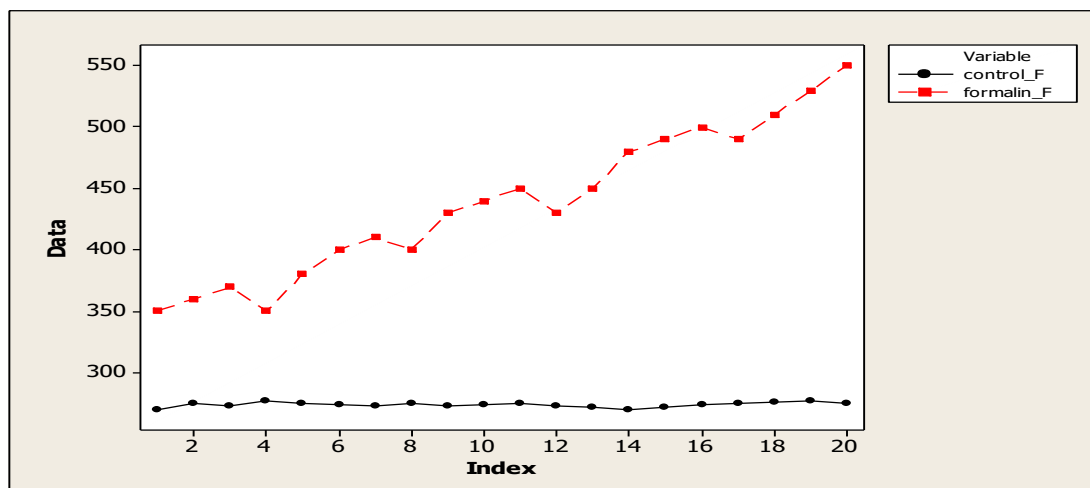
(Appendix -58) Level Urea of in the serum of treated group with citric acid (female).



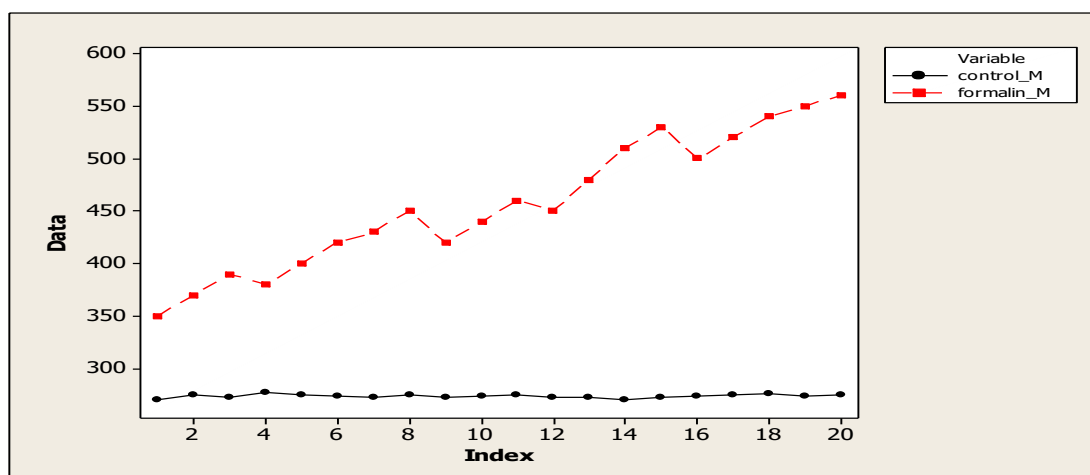
(Appendix -59) Level Urea of in the serum of treated group with citric acid (male).



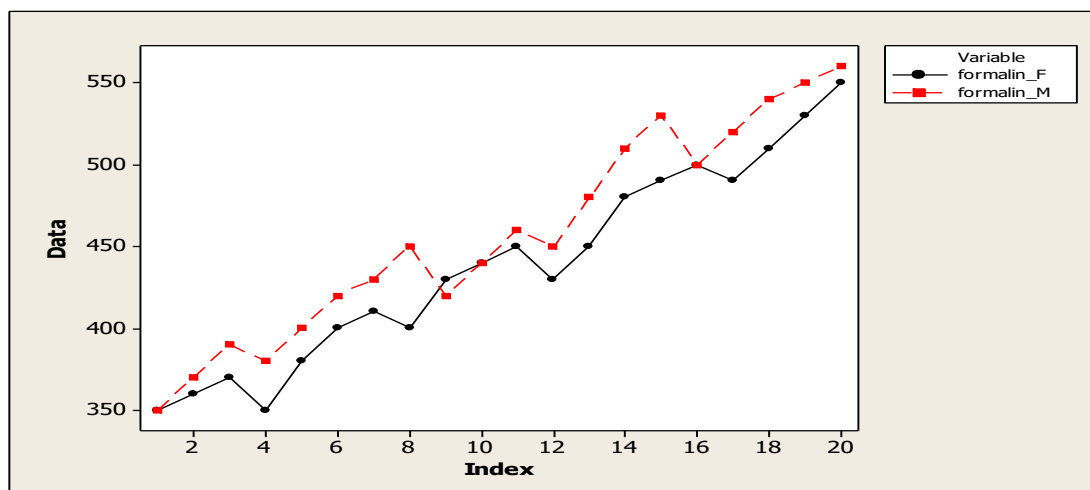
(Appendix -60) Level Urea of in the serum of treated group with citric acid between female and male.



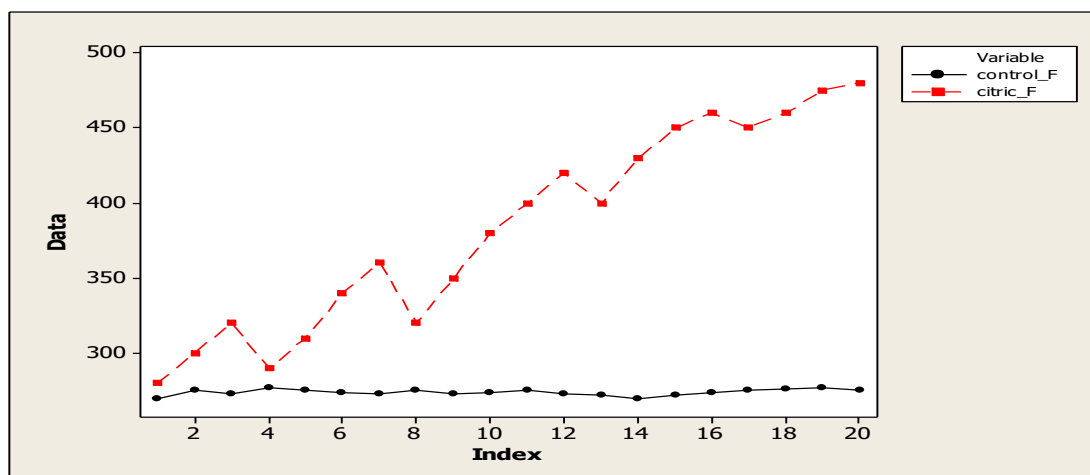
(Appendix -61) Level of AST in the serum of treated group with formalin (female).



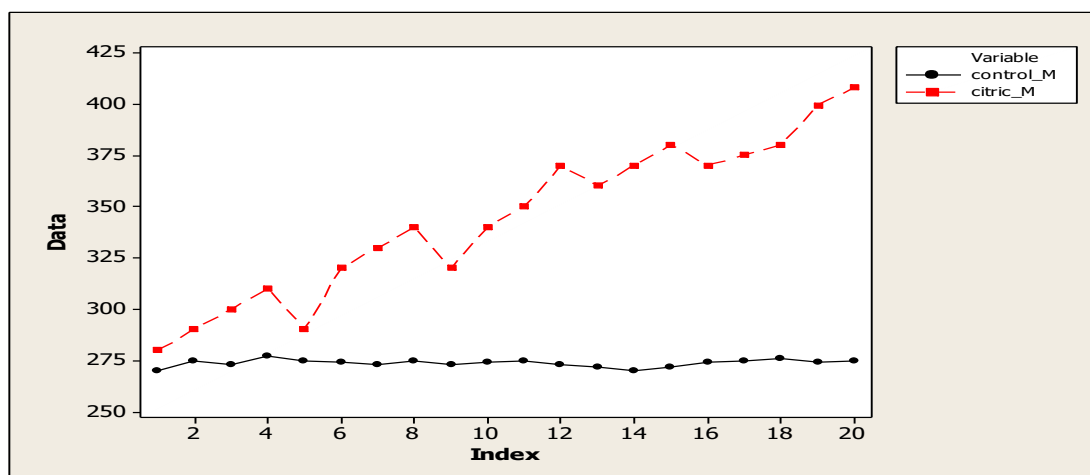
(Appendix -62) Level of AST in the serum of treated group with formalin (male).



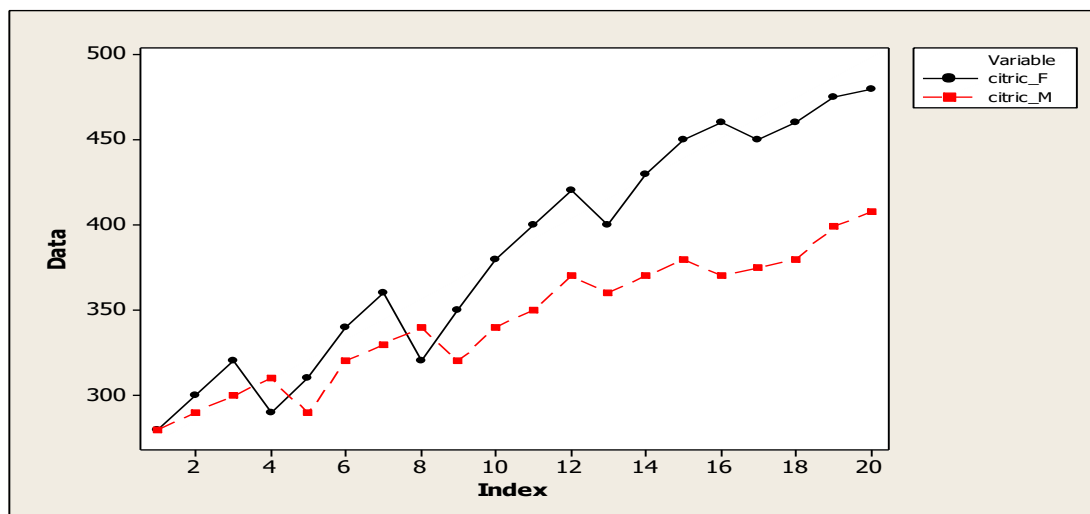
(Appendix -63) Level of AST in the serum of treated group with formalin between male and female



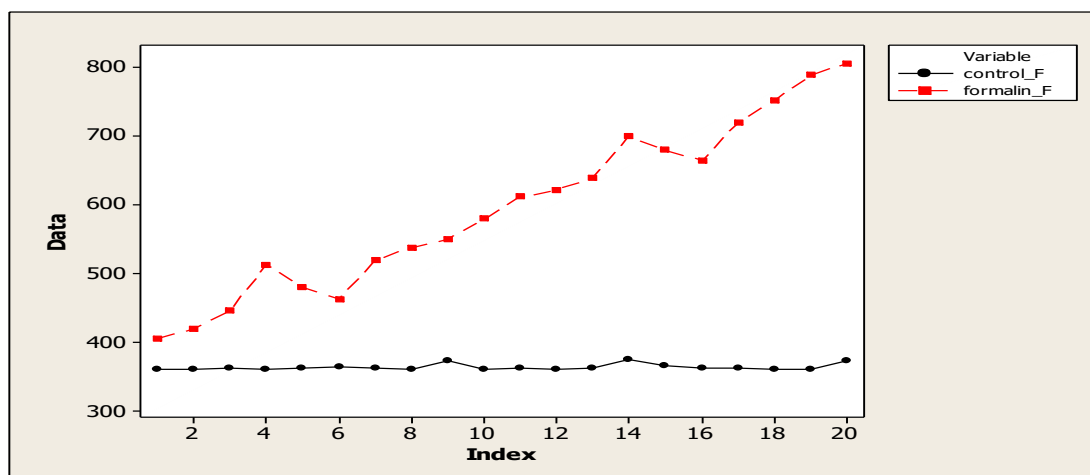
(Appendix -64) Level of AST in the serum of treated group with citric acid (female).



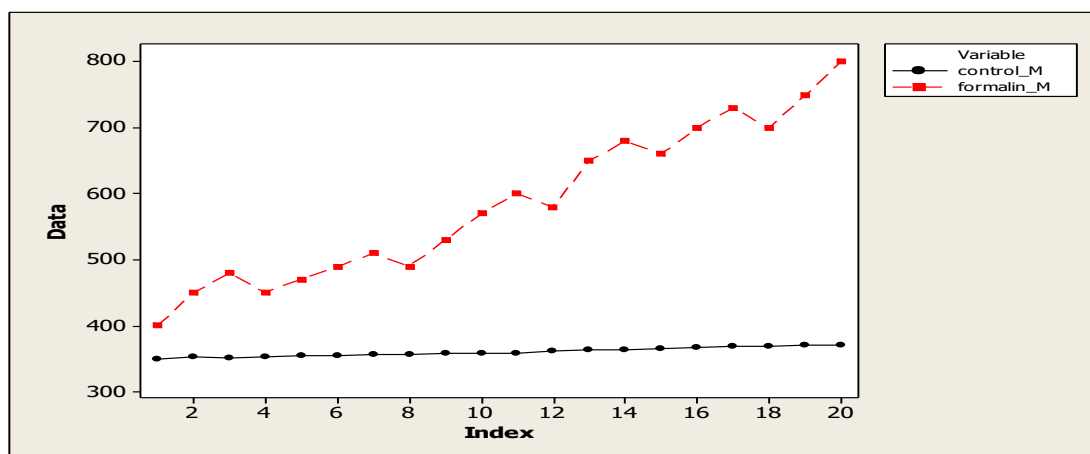
(Appendix -65) Level of AST in the serum of treated group with citric acid (male).



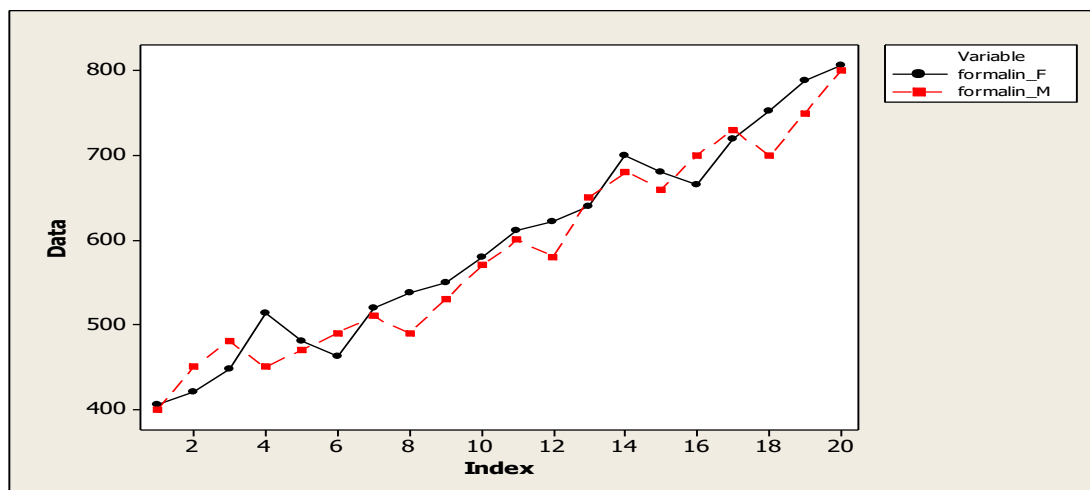
(Appendix -66) Level of AST in the serum of treated group with citric acid between male and female.



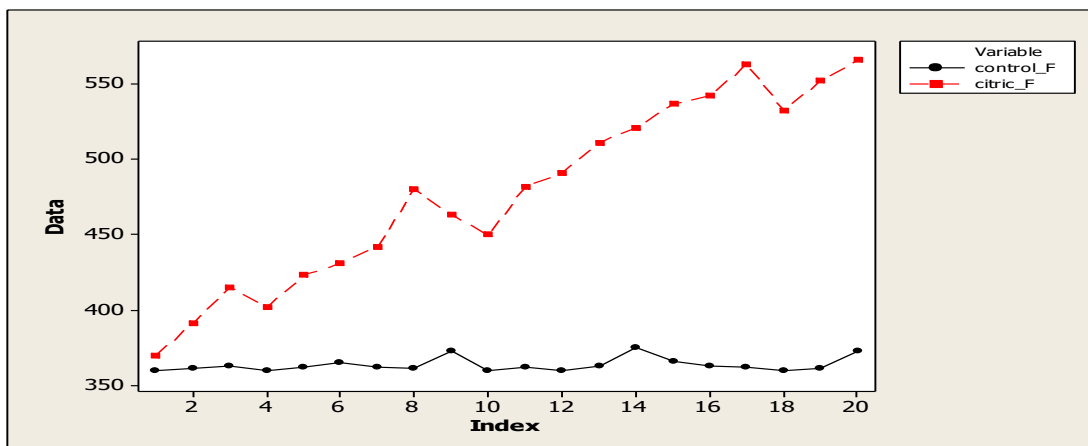
(Appendix -67) Level of ALT in the serum of treated group with formalin (female).



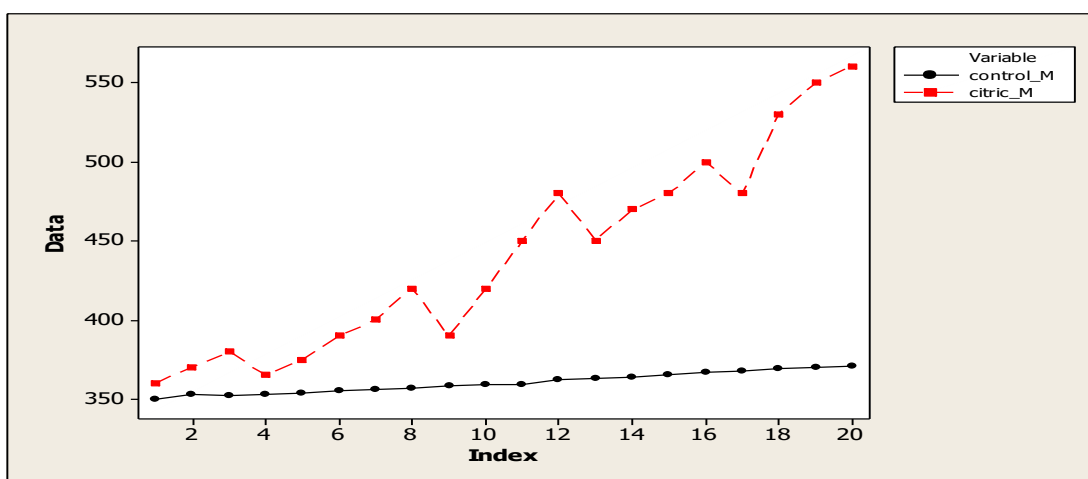
(Appendix -68) Level of ALT in the serum of treated group with formalin (male).



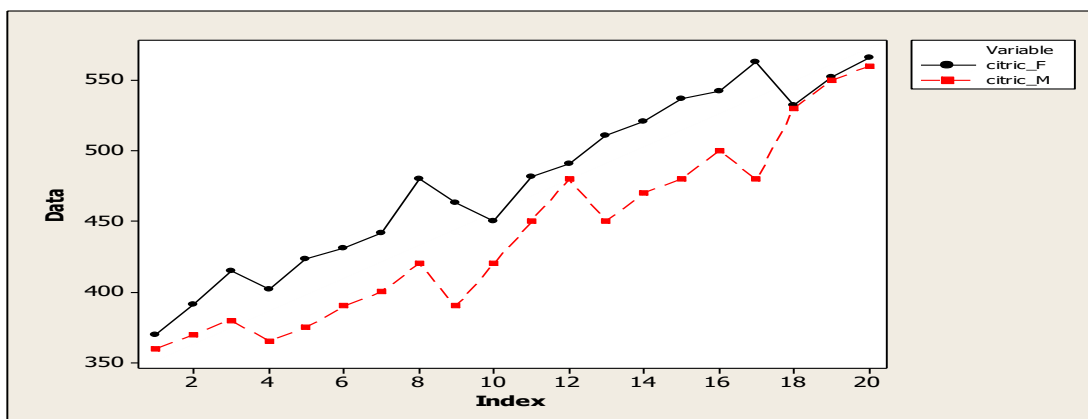
(Appendix -69) Level of ALT in the serum of treated group with formalin between female and male.



(Appendix -70) Level of ALT in the serum of treated group with citric acid (female).



(Appendix -71) Level of ALT in the serum of treated group with citric acid (male).



(Appendix -72) Level of ALT in the serum of treated group with citric acid between female and female.

الخلاصة

ركزت الدراسة الحالية على التغيرات النسجية والكيميائية الحيوية على أنسجة بعض أعضاء الفئران البيضاء بعد معالجتها بالفورمالين وحامض الستريك كمواد حافظة في منتجات الألبان. أظهرت الدراسة التأثيرات الرئيسية بعد المعالجة بالفورمالين (0.2 %) وحامض الستريك (3 %) بعد 30 يوما من التجريع الفموي. النتائج الحالية تحدد التغيرات النسجية والكيميائية الحيوية. تم تقسيم حيوانات التجربة إلى مجموعتين رئيسيتين (أ) إناث و ب ذكور) ومجاميع السيطرة. كل واحد من المجاميع الرئيسية أ و ب قسمت إلى مجموعتين ثانويتين (أ₁، ب₁، أ₂، ب₂) مجموعة أ₁ (إناث) و ب₁ (ذكور) التي جرعت فمويًا 0.5 مل من الفورمالين و أ₂ (إناث) ، ب₂ (ذكور) جرعت ب 1 مل من حامض الستريك ، وأخيرًا مجموعتين هما السيطرة (الإناث والذكور). المقاييس النسجية تضمنت مقاطع نسجية من الكلية والكبد والاثنى عشري ،بينما الفحوصات الكيميائية الحيوية حددت مستوى AST،ALT ، الكرياتينين واليوريا. أظهرت النتائج لسمية للذكور والإناث بعد معالجتها ب 0.5 مل من الفورمالين زيادة في أقطار كل أجزاء النيفرون (تضخم) واحتقان دموي بين الأنابيب الكلوية و انكماش الشعيرات الدموية الكبيبية، في حين أن التغيرات النسجية في الكلى بعد المعالجة ب 1 مل من حامض الستريك أيضا زيادة في قطر الكبيبات الكلوية ونزيف واضح بين الكبيبات الكلوية، تجمع الخلايا الالتهابية وتقرش الطبقة الظهارية للأنابيب.

لوحظ أن التغيرات النسجية في مقاطع الكبد لكل من الذكور والإناث بعد تجريعها فمويًا 0.5 مل من الفورمالين لمدة 30 يومًا تتضمن زيادة ملحوظة في كل من سمك الكبسول وقطر نواة خلايا الكبد، وأيضًا حدوث توسعات كيسية ممثلة بالدم في مواقع مختلفة في نسيج الكبد ومناطق تتخر واسعة، في حين أظهرت النتائج النسجية بعد تجريع 1 مل من حامض الستريك أيضًا زيادة طفيفة في كل من سمك الكبسول وقطر نواة خلايا الكبد ، وتضخم الخلايا الكبدية مع وجود فجوة بارزة في السيتوبلازم وتوسع بالجيوب مع نزيف.

لوحظ من خلال النتائج النسجية الحالية لجدران الاثنى عشر لكل من الذكور والإناث بعد معالجتها ب 0.5 مل من الفورمالين لمدة 30 يومًا زيادة ملحوظة في سمك الطبقة الظهارية، والعديد من الأضرار في طبقة الغشاء المخاطي واحتقان دموي، بينما أظهرت النتائج النسجية بعد تجريع ب 1 مل من حامض الستريك زيادة كبيرة في سمك الطبقة الظهارية ، تقشير الطبقة الظهارية التي تغطي الزغابات المعوية إلى التجويف المعوي وانفصال بين الطبقات.

النتائج الرئيسية الكيميائية الحيوية للذكور والإناث لكلا المعاملتين (فورمالين وحامض الستريك) أظهرت زيادة كبيرة في مستويات AST ،ALT ، كما لاحظت زيادة كبيرة في مستوى الكرياتينين واليوريا مقارنة مع مجموعات السيطرة. ومن الجدير بالذكر إن هناك تغير طفيف في النتائج بين الذكور و الإناث، كما إن الفورمالين يمتلك تأثير أكبر من حامض الستريك في أغلب النتائج



وزارة التعليم العالي والبحث العلمي

جامعة المثنى / كلية العلوم

قسم علوم الحياة

دراسة تأثير بعض المواد الحافظة في منتجات الألبان على بعض التغيرات النسجية والكيموحيوية في الفئران البيضاء

هذه الرسالة مقدمة كجزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل

ياسمين صبار نعيمة الفلاحي

بكلوريوس علوم الحياة/ 2016

بإشراف

أ.م.د. باسم عبدالله جاسم الخزاعي