وزارة التعليم العالي والبحث العلمي
جامعة المثنى / كلية العلوم
قسم علوم الحياة

التأثير الوقائي للمستخلص المائي لجذور الزنجبيل على التغييرات النسيجية والفسلجية بعد العلاج بالوارفرين في ذكور الفئران *Musculus domesticus*

هذه الرسالة مقدمة كجزء من متطلبات نيل درجة الماجستير في
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1.1 Introduction

Plants play a critical role in delivering vital environmental services, the use of medicinal plants in the treatment of diseases goes back to the beginning of human life (Halberstein, 2005). More than a tenth of the plant species,(over 50,000 species) are used in medical and cosmetic products, the term medicinal plant refers to a severed plants with medicinal properties, plants are a rich source of compounds that can be used for the production of drug synthesis, medicinal plants are used for therapy and they have many features, including synergistic behavior (Fatemeh *et al*., 2018).

The parts of medicinal plants that can be used are various forms of seed, root, leaf, fruit, skin, flowers or even the entire plant, the active compounds in most parts of medicinal plants have overt or indirect beneficial effects and are used as medicinal goods (Phillipson, 2001).

Ginger has been renowned for its many scientific properties and has been appreciated in various parts of the globe for the last 2500 years, ginger has rich phytochemistry and many health promoting viewpoints, ginger is one of the most commonly used species in the ginger family and is found in many foods and beverages(Al-Awwadi, 2017).

Many bioactive compounds have been found in ginger, such as phenolic and terpene compounds, phenolic compounds are primarily gingerols, shogaols and paradols, which are responsible for the different bioactivities of ginger (Stoner, 2013).

Ginger is a strong source of critical micronutrients, such as potassium 410.91 mg/100g, magnesium 45.02 mg/100g, phosphorus 32.56 mg/100g, calcium 15.76 mg/100g, manganese 0.70 mg/100g, copper 0.58 mg/100g, iron 0.54 mg/100g, zinc 0.33mg/100g and silicone (Tanweer *et al*., 2014).

Potassium and manganese help develop infectious resistance and protect the lining of the heart , blood vessels and urinary tract, silicon promotes healthy skin, hair, teeth and nails and helps assimilate calcium, tiny levels of vitamins A , E and certain quantities of vitamin B and vitamin C are also present in ginger rhizomes (Adel and Prakash, 2010).

Ginger root is used to attenuate and treat a variety of chronic illnesses, such as headaches, colds, nausea, and emesis, also, proved as an anti-diabetic, anti-carcinogenic anti-tumor activity, and effect against pregnancy-induced vomiting and nausea, and showed for the treatment of arthritis and motion sickness (Mele, 2019).

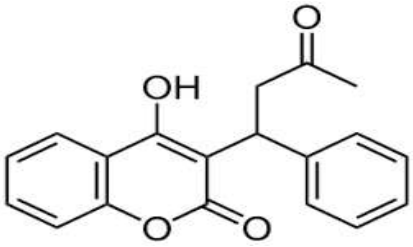
Ginger increases blood circulation in the body by relaxing the heart muscle and diluting flowing blood, this increases cell metabolism and helps to reduce cramping and tension(Mahmood, 2019), ginger is effective in lowering blood glucose levels when it has been taken in a dried form(Mahluji *et al*., 2013).

It also lowers cholesterol and triglyceride levels, long-term use helps increase the concentration of high-density lipoprotein cholesterol(Alizadeh *et al*., 2008), ginger is

having powerful antioxidant activity due to its oil which has aprotective effect on DNA damage, they have demonstrated this effect in many cell culture due to phenolic compounds, ginger has shown excellent antimicrobial properties and effective in controlling the virus, bacteria, fungal disease (Chaiyakunapruk *et al*., 2006; Kim *et al*., 2007).

Ginger contained an amount of minerals especially potassium at the rate of (410.91±13.97mg/100g) (Tanweer *et al*., 2014). Potassium used for the prevention of vascular calcification and aortic stiffness (Blau and Team, 2017). Raise the chance or cause vascular calcification in certain individuals may be due to warfarin (Han and O'Neill, 2016). Seversl studies have found that vascular calcification can be caused and accelerated in patients undergoing long-term warfarin therapy (Zhang and Tang, 2014).

Warfarin is a commonly used anticoagulant in the management and prevention of thrombosis, chronic atrial fibrillation, mechanical valves, pulmonary embolism, and dilated cardiomyopathy, warfarin exerts its action by inhibiting vitamin K epoxide reductase in the liver, which is essential for the synthesis of functional clotting factors II , VII , IX and X, warfarin also influences the synthesis and operation of the Gla Protein Matrix MGP (Palaniswamy *et al*., 2011).



4-Hydroxy-3-(3-oxo-1-phenylbutyl) 2*H*-chromen-2-one

Warfarin-induced hemorrhage is an important risk of anticoagulation treatment, and a study of several studies indicates that overall annual rates of warfarin-related bleeding are as high as 0.8%, 4.9% and 15% for fatal, major and minor bleeding complications, respectively (Palareti *et al*., 1997).

The use of warfarin is associated with a rise in systemic calcification, including coronary and peripheral vasculature. This rise in vascular calcification is due to inhibition of the enzyme gamma-carboxyglutamate Gla protein (MGP) (Poterucha and Goldhaber, 2016). One of the strongest in vivo inhibitors of arterial calcification is MGP, effects of MGP in human vascular smooth muscle cell (VSMC) monolayers undergoing calcification after exposure to elevated concentration of calcium, increased calcium salt deposition was observed in vitamin K antagonist warfarin-treated cells (Schurgers *et al*., 2007).

MGP is a vitamin K-dependent protein that ordinarily prevents systemic calcification by scavenging calcium phosphate in the tissues, warfarin-induced systemic calcification can result in adverse clinical effects, it is mainly produced by vascular smooth muscle cells

and chondrocytes, its function became clear in MGP deficient mice who died within 6 to 8 weeks of birth as a result of rupture of large arteries (Luo *et al*., 1997). High doses of warfarin induce focal calcification of elastic lamellae in the media of major arteries and aortic heart valves in rats (Price *et al*., 1998).

1.2 Aims of the Study

1- To evaluation the role of watery ginger root extract in treating the side effects of warfarin.

2- To determine the role of watery ginger root extract in reduce vascular calcification.

3- To investigate the side effects of warfarin on some histological and biochemical parameters in the male white mice.

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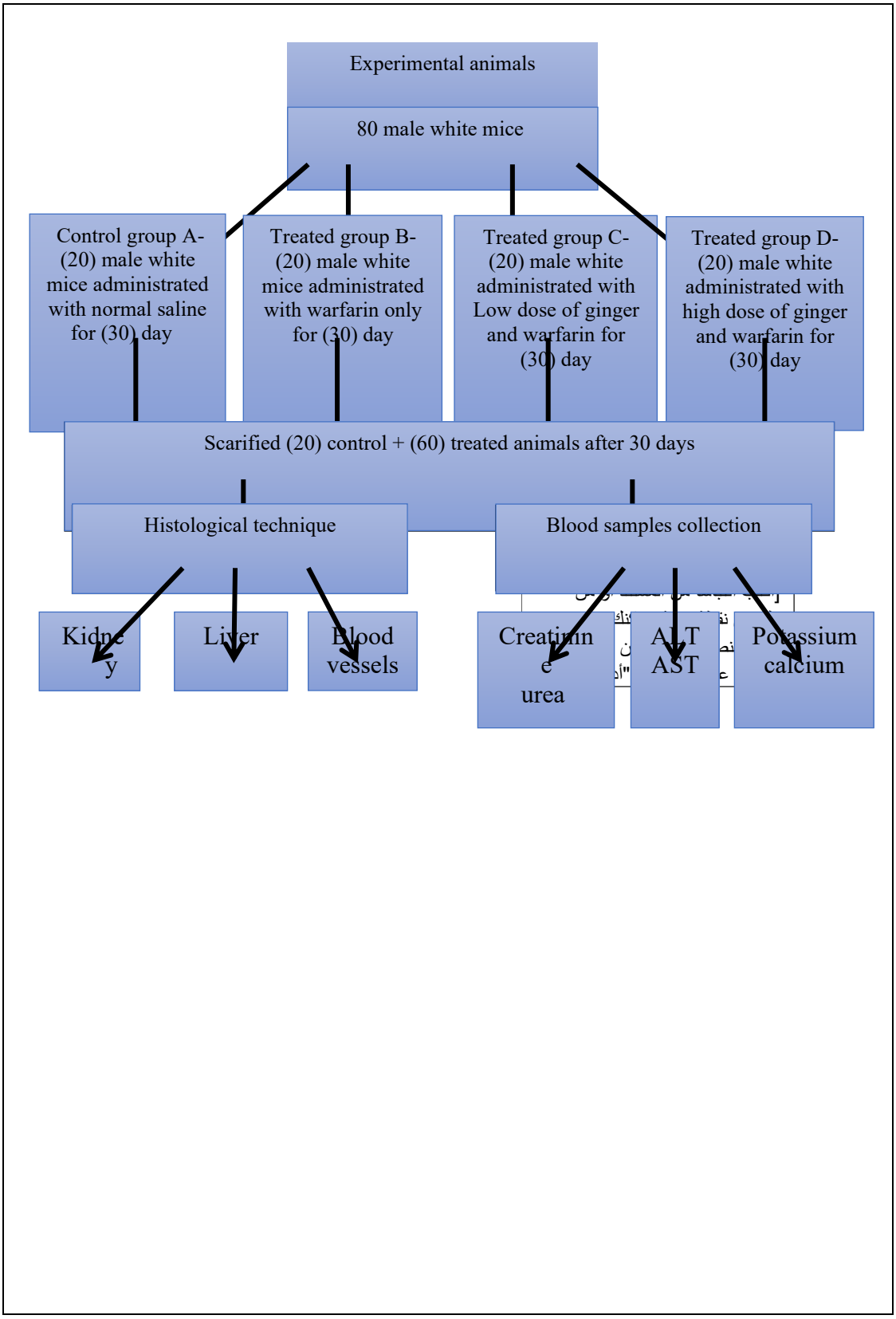
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CVD	Cardiovascular disease
DVT	Deep venous thrombosis
INR	International normalized ratio
CYP	Cytochrome p450
MGP	Matrix gama carboxylase protein
ICH	Intracranial hemorrhage
VSMC	Vascular smooth muscle cell
Gla	gamma-carboxyglutamate protein
ALT	Alanine transaminase
AST	Aspartate transaminase
ALP	Alkaline phosphatase
CRD	Complete random design
PCT	Proximal convoluted tubule
DCT	Distal convoluted tubule
RBCs	Red blood cells

3. Materials and Methods

3.1 Experimental Design



3.2 Materials

3.2.1 Experimental Animals

The present work was carried out on male white mice (*Musculus domesticus*). The experimental animals were collected from the Drug and Health center in Baghdad province, sixty adult male white mice which consider as treated groups that are treated with two types of substance (warfarin and ginger). The twenty male mice treated with warfarin only for 30 days (group B), twenty male mice administrated with Low dose of ginger and warfarin

(group C), and), twenty male mice administrated with a high dose of ginger and warfarin (group D) for 30 days, and twenty male mice as a control group.

The experimental animals living in laboratory plastic cages, all cages put in the animal house of the college of science in Al- Muthanna University. The main weight of experimental animals was (27-30gm), and age ranged three months. Living in lab house under controlling of temperature 25-28ºC and humidity about 40 to 45% with feeding by using standard pellets and water. The animals were left in separate cages for two weeks to experience the adaptation period. The current study beginning at (2019-2020) of experimental time.

3.2.2: Chemicals:

Table.3-1: Chemicals with Their Productions

No.	Chemicals	Productions
1	Formalin 37 %	Linear chemicals, Barcelona-Spain
2	Chloroform	India
3	Absolute Alcohol	Japan
4	Glycerin	England
5	Paraffin wax	England
6	Hematoxylin crystals	England
7	Eosin	England
8	Oil emersion	China
9	Xylene	England

Table.3-2: Instruments with their Productions

NO.	Tools	Productions
1	Filter paper	China
2	Disposable surgical Gloves	Malaysia
3	Cotton	Jordon
4	Refrigerator	Concord
5	Warring Blender	Crown
6	Gel tube	Celeco- Jordon
7	Slides	China
8	Cover Slide	China
9	Ependroff tubes	Germany
10	Beakers	China
11	Disposable syringe	China
12	sterile bottle	Jordon
13	Petri dishes	China
14	Electrical balance	Shimadu company- Japan
15	Centrifuge	GMBH- Germany
16	Microscope camera	OMAX-China
17	Microtome	Mycrom-Germany
18	Oven	Memmert- Germany
19	Cobas 111	Roche- Germany
20	Light compound Microscope	GENEX-USA
21	Micropipette	Huawei –China
22	Water path	Germany
23	Surgical set	England
24	Processer	Cyan-Germany
25	Bluking	Thermo-England
26	Container	China

Table.3-3: The stains

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

Table.3-4: The Biochemical Kits and Their Production

NO	Test Kits	Productions
1	ALT kit	Roche-Germany
2	AST kit	Roche-Germany
3	Urea kit	Roche-Germany
4	Creatinine kit	Roche-Germany
5	Potassium kit	Roche-Germany
6	Calcium kit	Roche-Germany

3.3: Methods

3.3.1: Plant material

3.3.1.1: plant collection

Ginger were purchased from the local market in Najaf city, the rhizomes were cleaned and dried at room temperature and then crushed by a blender at the same day of preparation of the extract.

3.3.1.2: preparation of watery extract of ginger

According to Hernandez *at el* (1994), used (10 gm) of plant powdered added to (200ml) of distilled water in a sterile glass beaker and left 24 hours with a shaking ,then passed on the layers of sterile soft cloth for its candidacy and then separation of the filtrate by using a centrifuge (3000/rpm) and then dried the liquid in an electric oven degree (40 c) for drying the extract, then collected and placed in a sterile bottle and preserved in the refrigerator to until use.




Fig.3.1:Conversion of ginger into powder after dehydration.

3.3.1.3 Lethal dose of the ginger extract (LD)

Lethal dose,50 percent kill of aqueous extract of ginger was (11.75) g/kg when administered orally to mice (Shalaby and Hamowieh, 2010).

3.3.1.4 Concentration preparation (stock) is

Dissolving (500) mg active ingredient (ginger) in (50) ml DW.

Mean: (500) mg /(50) ml (DW) → (500,000µg /50) ml DW = (10,000)µg /ml.

Mean: each (0.1) ml of stock solution equal to(1000)µg active ingredient.

3.3.1.5 Concentration prepares of the low dose of ginger

Dose (150)mg/kg convert to µg/gm. equal (150) mg/kg.

Example: mouse weight = (30) gm. →(150) µg ×(30) gm. =(4500) µg

Mean: mouse dosing is about (0.45) ml.

3.3.1.6 Concentration prepares of a high dose of ginger

Dose (300)mg/kg convert to µg/gm. equal (300) mg/kg.

Example: mouse weight =(30) gm. →(300) µg ×(30) gm. =(9000) µg

Mean: mouse dosing is about (0.9)ml.

3.3.1.7 Concentration prepares of warfarin (stock) is

acute LD50 of warfarin was (60) mg/kg when administered orally to mice (Seyler,1994).

Dissolving (30) mg active ingredient (warfarin) in (10) ml DW.

Mean: (3) mg / ml DW → (3000)µg /ml DW.

Mean: each (0.1) ml of stock solution equal to (300) µg of an active ingredient.

Dosage : (30) µg/gm.

mouse weight = (30) gm.→ (30)µg × (30) gm. = (900)µg.

Mean: mouse dosing is about (0.3)ml.

3.3.2 Administration of experimental animals

The animals were administrated with warfarin and aqueous extract of Zingiber officinale with two different doses (high dose and low dose) to period of 30 days:

Group (B) was administered with (0.3ml) of warfarin.

Group (C) were administered with both warfarin(0.3ml) and Low dose of ginger(0.45ml).

Group (D) were administered with both warfarin(0.3ml) and high dose of ginger(0.9ml).

3.4.Collecting blood studied samples and members of the laboratory animals

3.4.1 Blood samples collection

The mice were anesthesia after the administration after 30 days from administration period; two milliliters of blood was attained by cardiac puncture and transferred to gel tubes. The blood was left at room temperature for 15 minutes after centrifuged with (3000 rpm) for 15 minutes the serum was collected ependrof tubes and divided into equal amount (50µl) and frozen until used for laboratory assessments, to purpose examine ALT ,AST, creatinine, urea, calcium and potassium.

The tissue samples were taken with $1cm^3$ after sacrificed of experimental animals and taken the kidney , Liver and blood vesseles, the tissue samples were passed through the histological technique which including many stages.

3.4.2 Histological technique

1. Fixation: The samples (kidneys, liver and blood vessels) were put in a labeled container contain 10% formalin for 48 hours, and shaky the container gently several times to ensure that the fluid reached all surface and that pieces were not sticking to the bottom or sides (A shank of glass wool placed in the container will aid in keeping the tissue free of the bottom).

2. Washing: The kidney, liver and blood vessels are 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 (Vacca,1985; Luna,1991).

3. Dehydration: the samples were done to remove all extractable water by dehydrated diffusing through the tissue, alcohol was used. The dehydration procedure was continued by upgrading alcohol from 50% to absolute alcohol (50%, 60%, 70%, 80%, 90%1, 90%2, 100%1, 100%2).

4. Clearing: this stage is considered as a middle step, it is obligatory 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. 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 (Luna, 1991).

5. Embedding (Blocking): Paraffin wax is considered to be either soft (melting point 50-52 c) or 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 is already filled with malted paraffin. Orientation of the tissue is important in order to limit the proper surface for sectioning (Edward, 1962).

6. Cutting: Cutting was done by using the rotary microtome. The thickness is (5 µm). The samples were put in warm water path with (50 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: (H & E) stain: For the demonstration of the general structure of the tissue (Luna, 1991).

3.5 Statistical analysis

Experimental items of study were designal using complete random design (C.R.D) with one way (ANOVA) . The results were analyzed using (SPSS) (SPSS, 2012), to compared between the control and treated mice groups results, The comparation means was implemented using Duncan test for multiple range (Duncan, 1955), Data were expressed as mean ± standard deviation, the differences were considered to be significant when P value was <0.01.

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2.1 Medical plants

The herbs are used in every part of the world not only as food but also as potent medicines for thousands of years, they do not act as chemical medicines (Horne, 2013). medicinal plants are used by 80% of the world population, mostly due to the common belief that plant derived medicines are without any side effects along with being economical and locally available and consider as a source of many chemical extracts that have essential action as an antioxidant factor (Hashim *et al*., 2010).

The use for medicinal plants is for the treatment of diseases which have deep roots in human history, used since ancient times for the treatment of diseases, burns, illnesses injuries and dermatophytes, because the plants contain some of their sections of chemical compounds of interest and significant importance for the career impact and therapeutic function of humans and animals and used as catalysts for growth also antibacterial and fungi, stimulation the digestive system by production of digestive enzymes, liver action, pancreas and small intestine effectiveness (Murlidhar and Goswami, 2012).

Extracts of plants used over hundreds of years with few side effects, Ginger (*Zingiber officinale Roscoe*) is one of the most important medicinal plants in the world and is commonly used in food as a spice, it has been an essential ingredient in Chinese, and Tibb-Unani herbal medicines for the treatment of catarrh, rheumatism, nervous disorders, gingivitis, toothache, asthma, stroke, constipation and diabetes for centuries. (Tapsell *et al*., 2006).

Ginger contains many cations and anions, such as calcium , magnesium and phosphorus, which have a role in bone growth, muscle contraction and nerve conduction, these ginger minerals are effective for muscle contraction, hypertension, muscle fatigue, seizures, it also contains a significant amount of potassium that plays a role in the control of blood pressure and heartbeat (Shaban *et al*., 2017).

2.1.1 Taxonomy, availability and morphology of ginger plant

Locally name : Ginger

Scientific name**:** *Zingiber officinale Roscoe*

Table 2.1: Position of ginger in plant kingdom taxonomy system:

Kingdom	Plantae
Class	Liliopsida
Order	Zingiberales
Family	Zingiberaceae
Genus	*Zingiber*
Species	*Zingiber officinale var. Roscoe*

(Marx *et al*., 2015).

Ginger is a perennial herbaceous rhizomatous, up to 90 cm tall under cultivation. Rhizomes are aromatic, thick lobed, pale yellowish, with simple alternating distichous, narrow, oblong, lanceolate leaves (Mishra *et al*., 2012).

Ginger is cultivated in the whole of South East Asia, China and parts of Japan, Austria, Latin America, Jamaica and Africa. India is the top producer of Ginger, followed by China, Indonesia, Nepal and Thailand, but the most expensive and high-quality varieties come from Jamaica, Australia and South India (Ali and Gilani, 2007).

2.1.2 Active ingredient of ginger

2.1.2.1 ginger rhizomes

The major constituents are carbohydrates (50–70%), lipids (3–8%), terpenes, and phenolic compounds, 1.5%-3% essential oil, 2–12% fixed oil, 40–70% starch, 6–20% protein, 3–8% fiber, 8% ash, 9–12% water, pungent principles, other saccharides, cellulose, coloring matter and trace minerals (Chan et al.,2009). They also contain amino acids and vitamins such as nicotinic acid and vitamin A (Shukla and Singh, 2007).

2.1.2.1.1 phenolic components

Include gingerol, paradols, and shogaol, these gingerols (23–25%) and shogaol (18–25%) are present in higher concentrations than others(Shukla and Singh, 2007).

2.1.2.2 ginger root components

Includes gingerols, sugar, protein, shogaol phenol (gingeol, zingerone), proteolytic enzyme (zingibain), vitamin B6 , vitamin C, calcium ,magnesium, phosphorus, potassium, linoleic acid, gum, lignin, vegetable matter, acetic acid, potash acetate, sulphur (Dhanik *et al.,* 2017).

2.1.2.2.1 Minerals of ginger root

Ginger root consists of Calcium (16 mg/100 g), Iron (0.6 mg/100 g), Magnesium (43 mg/100 g), Phosphorus (34 mg/100 g), Potassium (415 mg/100 g), Sodium (13 mg/100 g), and Zink (0.34 mg/100 g) (Shahrajabian, 2019).

2.1.3 Traditional medicin uses of ginger

2.1.3.1: cardiovascular protection

Ginger is protected from Cardiovascular diseases that cause premature mortality, and 17.9 million people die every year (Du *et al*., 2016), A variety of studies have shown that ginger can lower blood pressure and lipid levels, which contribute to the protection against cardiovascular disease (Akinyemi *et al*., 2016).

Dyslipidemia and hypertension are considered to be risk factors for cardiovascular diseases, including stroke and coronary heart disease (Akinyemi *et al*., 2015).

Ginger was described as a great heart tonic, it protects the heart in various ways, by decreasing blood clotting that can lead to plaque formation or thrombosis, by opening blockages in the blood arteries to reduce peripheral artery resistance and thus blood pressure, by lowering the elevated blood cholesterol level to keep the heart healthy (Akoachere *et al.,* 2002). An early study showed that gingerol activated the ATPase pumping activity of the skeletal and cardiac muscles of Ca2+ (Kabayashi *et al.,*1987).

2.1.3.2 Antioxidant Activity

several studies have shown that ginger protects against oxidative stress (Akinyemi *et al*.,2013). Ginger extract demonstrated antioxidant effects in human chondrocyte cells, with oxidative stress induced by interleukin-1β, enhanced the expression of many antioxidant enzymes and decreased the production of lipid peroxidation (Hosseinzadeh *et al*., 2017).

The antioxidant action of ginger has been proposed as one of the major possible mechanisms for the protective actions of the plant against toxicity and lethality of radiation(Haksar *et al*., 2006) and as an anti-ulcer drug (Gull *et al*., 2012).

Lately, It has been shown that (6)-gingerol has a strong anti-oxidant activity both invivo and in vitro, as well as a strong anti-inflammatory and anti-apoptotic function (Kim *et al*., 2007).

2.1.3.3 Protective Effects against Respiratory Disorders

Ginger induced significant and rapid relaxation in the isolated human airway smooth muscle.In results from guinea pig and human tracheas models, gingerol, and shogaol could lead to the rapid relaxation of precontracted airway smooth muscle. The nebulization of gingerol attenuated airway resistance via a reduction in Ca2+ influx in mice (Townsend *et al*., 2013).

According to Townsend *et al* (2014), gingerol and shogaol mediated β-agonist-induced relaxation of human airway smooth muscle by inhibition of 4D phosphodiesterase. Bioactive ginger compounds have demonstrated broncho dilating and antihyper active effects in some trials (Mangprayool *et al*., 2013).

2.1.3.4 Food Preservative

Ginger is used as a food preservative that can improve the food protection which shelf life of fatty and refined food products, ginger contain essential oils that can be used to prevent cell damage due to spoilage by bacteria and fungi, oils and bioactive ingredients may be used as natural food preservatives to resist lipid peroxidation, which causes food spoilage (El-Baroty *et al*., 2010).

2.1.3.5 Rheumatologic effect and headache of ginger

Ginger is anti-inflammatory effects using pathways that clarify the function of inhibition of pro-inflammatory factors such as prostaglandin and leukotriene biosynthesis, which may alleviate pain associated with rheumatoid and osteoarthritis, ginger is used for the treatment of headache which has a positive effect on the relief of signs of pain, this result is due to a reduction in the synthesis of prostaglandin(Ha *et al*., 2012).

2.1.3.6 Ginger anti-nausea effects due to chemotherapy

Chemotherapy is considered to induce extreme nausea and vomiting, ginger is effective in reducing nausea and vomiting caused by chemotherapy, gingerols have been found to have apharmacological impact on the primary ingredients responsible for the activity, it is also used to relieve anxiety after surgery and is the same in many randomized clinical trials(Krim *et al*., 2013).

2.1.3.7 Gastro-protective activity

Ginger is very helpful in the treatment of many gastrointestinal disorders, including peptic and duodenal ulcers, the ulcer is usually caused by the mismatch between defensive and aggressive influences such as acid, pepsin and Helicobacter pylori, and in this case ginger is beneficial due to its anti-inflammatory properties, ginger acts and protects the gastric mucosa against several ulcerative agents, ginger is also very effective in cases of ulcerogenic due to its antioxidant activity (Gull *et al*., 2012).

2.1.3.8 Anti-hyper cholesteraemic effects

Ginger extracts interact with the biosynthesis of cholesterol contributing to lower levels of cholesterol, ginger extracts have anti-lipid effects by reducing thermogenesis and elevated levels of lipids, it also helps to improve HDL-cholesterol serum, it also reduces cholesterol and triglyceride amounts, long-term use helps increase the concentration of high-density lipoprotein cholesterol, ginger is very effective in reducing blood glucose levels when the same thing has been taken in a dry shape (Chaiyakunapruk *et al*., 2006; Kim *et al*., 2007).

2.1.3.9 Ginger–drug interactions

Ginger–drug interactions have been reported in the literature, ginger does not interact with the anticoagulant drug warfarin in rats or men (Weidner and Sigwart, 2000). According to (Jiang *et al* . ,2006) ginger administered orally at a dosage of 400 mg (three times daily for 1 week) before warfarin, and continued for another 1 week after that, ginger was found to have no major impact either on clotting status or on kinetics and dynamics.

2.2 Warfarin uses in cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of death worldwide, and prevention plays a key role in stemming the global epidemic (Huffman and Bhatnagar, 2012).

Blood clots that develop in the arteries may cause the heart to develop, stroke, severe leg pain and difficulty walking, blood clots in veins or venous systems can cause deep venous thrombosis (DVT) in the pelvic, leg, and upper extremity veins, as these DVTs break down and pass across the bloodstream to the heart and then to the lung blood vessels, they induce acute pulmonary embolism (Goldhaber and Morrison, 2002).

Treatment of arterial clots may include aspirin and clopidogrel (oral antiplatelet agents), intravenous antiplatelet agents, heparin (a blood thinner and anticoagulant), and clot busters (thrombolytic agents), addition to other medications that used to remove or compress these arterial clots, one of the most important medication is warfarin (Goldhaber and Grasso Correnti, 2002).

Warfarin is a vitamin K antagonist, widely used to suppress coagulation by inhibiting vitamin K-dependent coagulation factors, it has recently become apparent that warfarin also affects vascular calcification by inactivating the Gla protein matrix (Krüger *et al*., 2013).

Warfarin is a 4-hydroxycoumarin analog that was presented as a rodenticide in the 1940s, warfarin has been extensively used as an anticoagulant for the prevention and treatment of thrombotic and thromboembolic diseases in the world since the 1950s (Holbrook *et al*., 2005)

Warfarin dosing is very complex between patients and needs to be individualized, normal doses are about 5 mg / day, but can be as low as 0.5 mg / day in some patients or as low as 50 mg / day in others, factors such as race , gender , age, anticoagulation indication, vitamin K intake, albumin body weight and interactive medication may all contribute to this variability (Ansell *et al*., 2004).

Warfarin is a drug that is closely bound to proteins (Schurgers *et al*., 2010), the decrease International Normalised Ratio (INR) in patients following initiation of a high-protein, low-carbohydrate diet may be associated with effect of protein intake on albumin level., an rise in

albumin level appears to occur within ten days of starting a high-protein, low-carbohydrate diet, the increase in albumin level is attributed to an increase in warfarin binding to albumin, which leaves warfarin less free available for anticoagulant effect (Beatty *et al*., 2005)

The mechanism is believed to be that high protein diets lead to increased albumin stores resulting in additional warfarin binding and eventually increased warfarin dosage requirements, more fat is also eaten in the Atkins diet, which will improve the absorption of vitamin K, since high protein diets restrict carbs, more vegetables are eaten, resulting in a rise in vitamin K consumption and eventually altering warfarin dosage requirements (Nutescu *et al*., 2006).

Patients undergoing warfarin treatment require routine blood checks to assess how long blood clots take, called the International Normalised Ratio (INR), vegetables such as broccoli , spinach, parsley, kale, etc. are rich sources of vitamin K and consuming large amounts or making sudden changes in the consumption of these vegetables can interfere with the effectiveness and safety of warfarin therapy, Changes in the use of related drugs, diet, acute illness, alcohol consumption, liver disease, and unexplained causes can often interfere with treatment, so frequent INR and dosage changes in warfarin are also needed (Hebert *et al*., 2004).

2.2.1:Warfarin interaction:

Anticoagulation is the mainstay of treatment to avoid thromboembolic complications in people with atrial fibrillation, prosthetic heart valves, venous thromboembolism and coronary artery disease, for long-term clinical care, oral anticoagulation is favoured to intravenous or subcutaneous routes due to patient convenience and cost (Ansell *et al*., 2004).

2.2.1.1: Interactions of warfarin and dietary vitamin K

Vitamin K is a cofactor for the carboxylation of glutamate residues to γ- carboxyglutamates on the N-terminal regions of vitamin K-dependent coagulation factors II, VII, IX, and X, these coagulation factors require γ-carboxylation by vitamin K for their biological function, warfarin inhibits vitamin K epoxide reductase, resulting in insufficient generation of vitamin K hydroquinone to support full carboxylation and therefore full function of the vitamin K-dependent coagulation factors (Shearer and Newman, 2008).

Vitamin K is involved in the blood coagulation process playing a central role in the synthesis of many coagulation factors such as prothrombin (Factor II), and Factors VII, IX and XII in the liver as well as in the formation of circulating anticoagulants (proteins c, s and z), vitamin K also participates in the production of several proteins involved in bone and soft

tissue mineralization, vitamin K dependent proteins are also present in extra hepatic tissues such as bone (osteocalcin) and the vascular wall (matrix Gla protein) (El Asmar, 2014).

2.2.2 Adverse effects of warfarin

2.2.2.1 bleeding

The risks of severe bleeding intracranial hemorrhage (ICH) or other severe site bleeding associated with all anticoagulants are of interest to physicians (Gattellari *et al*., 2008).

Numerous studies have shown that the incidence of major bleeding in patients on warfarin ranges from 0.4%–7.2% per year. Minor bleeding rates can be as high as 15.4% per year (DiMarco *et al*., 2005). This wide range is thought to be a result of the numerous patient-specifc factors that can alter metabolism (Holbrook *et al*., 2005).

The most important parameter used to monitor its effect on the clotting system during follow up of patients is the international normalized ratio (INR), usually, the dose of warfarin is frequently adjusted to maintain the INR level between 2 and 3.5 depended on the underlying condition, because of its narrow therapeutic index, patients using warfarin may have minor and major bleeding especially in those with poor drug compliance (Witt *et al*., 2009).

High INR levels are an important risk factor for bleeding (Garcia *et al*., 2006). Most bleeding related to the use of warfarin occurs in the gastrointestinal tract, urinary tract, soft tissues and oropharynx with gastrointestinal haemorrhage being the most severe (Pautas *et al*.,2006). Patients with extra cranial hemorrhage and warfarin are less likely to die from the initial event or the first month after discharge and are also less likely to have long-term functional deficits than those with intracranial hemorrhage (Fang *et al*., 2007).

Several conditions raise the risk of over-anticoagulation genetic polymorphisms influencing metabolic enzymes, impaired liver function, drug interactions, congestive heart failure, diarrhea, fever, and vitamin K rich diets (Makris and Watson, 2001).

2.2.2.2 Osteoporosis

After initial reports that warfarin could reduce bone mineral density, several studies have demonstrated a link between warfarin use and osteoporosis-related fracture. A 1999 study in 572 women taking warfarin for deep venous thrombosis, risk of vertebral fracture and rib fracture was increased; other fracture types did not occur more commonly. A 2002 study looking at a randomly selected selection of 1523 patients with osteoporotic fracture found no increased exposure to anticoagulants compared to controls (Pilon *et al*., 2004).

2.2.2.3 Purple toe syndrome:

Another rare complication that may occur early during warfarin treatment (usually within 3 to 8 weeks of commencement) is purple toe syndrome. This condition is thought to result from small deposits of cholestrol breaking loose and causing embolisms in blood vessels in the skin of the feet, which causes a blueish purple colour and may be painful (O'Keeffe *et al*., 1992).

It is typically thought to affect the big toe, but it affects other parts of the feet as well, including the bottom of the foot (plantar surface). The occurrence of purple toe syndrome may require discontinuation of warfarin (Talmadge and Spyropoulos, 2003).

2.2.2.4 Vascular calcification:

Vascular calcification plays a key role in the pathophysiology of coronary artery disease, ischemic stroke, and peripheral arterial disease. Commonly recognized risk factors for vascular calcification include hypertension, diabetes, aging, chronic kidney disease, cigarette smoking, and systemic inflammation. Treatment with vitamin K antagonists such as warfarin is associated with vascular calcification, even when other risk factors are controlled (Weijs *et al*., 2011).

Vascular calcification has been defined as a consequence of tightly regulated processes that culminate in the creation of an organized extracellular matrix deposition of osteoblast-like cells. These cells are either derived from circulating stem cells or from cells located in the vascular wall, the osteoblast-like cells may also have differentiated from smooth muscle cells or pericyte (Johnson *et al*.,2008).

Vascular calcification consists of calcium salt precipitates, mostly in apatite form, similar to the hydroxyapatite found in bone, several risk factors which are associated with the presence or progression of vascular calcification have been identified (Newman *et al*., 2001).

In the past vascular calcification was seen as an inert end-point of atherosclerosis, however, recently it has become clear that it is a actively regulated process already occurring in the early stages of atherosclerotic lesions (Demer and Tintut, 2003).

Numerous inhibitory defensive agents, such as osteopontin and osteoprotegerin, are used to avoid tissue calcification, all of these defensive factors is the matrix gamma-carboxyglutamate Gla protein MGP, a relatively insoluble protein synthesized by smooth muscle cells (Johnson *et al*., 2006).

MGP is one of the strongest inhibitors of arterial calcification, which function depends on the presence of vitamin K. MGP is a local inhibitor of vascular calcification, and it has been demonstrated that circulating MGP has no biological function (Murshed *et al*.,2004). However, circulating MGP may reflect calcification processes and inhibition of those processes in the vascular wall (Snegarov and Galunska, 2019).

Mechanism of action and function in regulatory processes involving calcium deposition in the vascular walls has been intensively studied in recent years. (Rennenberg *et al*., 2010).

Calcification of the arteries, aorta, celiac axis, renal arteries and iliac arteries is normal in mice, this calcification occurs in the internal elastic laminae of the coronary arteries as well as in the elastic fiber and collagen fibrillate media of the aortic wall, further study has shown that the administration of MGP inhibits calcification in these in vitro models (Schurgers *et al*., 2007).

vascular calcification caused stiffening of the vascular wall, which may result in decreased arterial compliance, development of left ventricular hypertrophy and decreased coronary perfusion leading to an increased risk of fatal complications. Calcification is common in the elderly population, and in patients suffering from diseases such as chronic kidney disease, diabetes, aortic stenosis, and atherosclerosis (Price *et al*., 2006).

Patients with chronic kidney disease develop vascular calcification due to a number of MGP-independent pathways, including impaired calcium-phosphate handling and hypertension. In the chronic kidney disease population, vitamin K deficiency has been linked to an increase in vascular calcification (Schurgers *et al*., 2010).

2.3 Warfarin effects on the kidney

The kidney is bean-shaped, firm, reddish - brown in color, and is located in the posterior cavity of the abdomen. It begins in most animals almost symmetrically one on each side of the vertebral column and dominantly in the lumbar region, although often extending forward under the last ribs (Glodny *et al*., 2009).

The parenchyma of the kidney is enclosed in a hard fibrous and thin, strong capsule, the kidney consists of two regions, the outer cortex of a dark region under the capsule, a cortex that is granular, reddish-brown in color like most nephrons, the inner medulla of the light area under the cortex, which was very dark in color (Dunnill and Halley, 1973)

The role of the kidney in the purification of blood, electrolyte balance and regulate fluids of the body and the introduction of harmful substances such as food products (urea, creatinine and toxins) that the body and the site of producing hormones such as erythropoietin and rennin (Rayner *et al*., 2016).

Several adverse effects of warfarin therapy on the kidney which is characterized by hematuria and acute kidney injury, these adverse effects are collectively referred to as warfarin-related nephropathy (Brodsky *et al*., 2011).

During warfarin drug pathological observations in renal biopsy specimens of 9 patients with warfarin overdose in each biopsy specimen noted acute glomerular hemorrhage and tubular injury, red blood cells RBCs in the Bowman region and multiple occlusive RBCs in

the tubules, each biopsy showed chronic kidney damage, in most cases of warfarin related nephropathy, red blood cells in the renal tubules block the flow of urine, resulting in acute kidney damage (Brodsky *et al*., 2009).

Patient was receiving warfarin because of a history of deep venous thrombosis, renal biopsy showed massive occlusion of renal tubules by red blood cells because tubular cell damage consistent with acute tubular necrosis (Abt *et al*., 2000).

Warfarin-related nephropathy, in which acute kidney damage is caused by glomerular hemorrhage and renal tubular blockade by red blood cells, warfarin therapy for chronic kidney failure, macroscopic hematuria and acute kidney damage, a renal biopsy revealed a large occlusion of red blood cells and casts in the renal tubules, the potential association between warfarin treatment, intratubular hemorrhage and acute kidney injury is explored (Santos *et al*., 2013).

2.4 Warfarin effects on the liver

The liver is the largest organs in the body, located in the upper right half of the abdominal cavity under the diaphragm. The liver is conical, brownish-red in color, and surrounded by a connective tissue called Glasson's capsule (Petcoff *et al*., 2006).

The liver located mainly in the upper right half of the abdominal cavity, covered by the thoracic cage and diaphragm, liver composed of two main lobes right and left lobe, each lobe is divided into smaller lobes called lobules which is the basic functional unit of the liver, each lobule consisting of parenchymal cells called hepatocytes ,central vein, Portal area, liver sinusoids that run from the central vein to the portal triads, hepatic macrophages (Kupffer cells) (Ozougwu, 2017).

Hepatocytes make up 60 percent of the liver cells and approximately 80 percent of the overall liver cell mass, the key role of the hepatocytes is to contribute to carbohydrate , protein and lipid metabolism, as well as to generate serum proteins such as coagulation factors and albumin (Jeejeebhoy and Phillips, 1976).

Hepatocytes produce and secrete bile, detoxification and excrete cholesterol, steroid hormones and xenobiotic drugs, they are also involved in excretory and secretory activities along with other hepatic cells (Hall and Cash, 2012).

Hepatocytes are produced for most liver functions such as digestion, bile secretion, detoxification, excretion of cholesterol and steroid hormones, and storing of proteins , carbohydrates, vitamins and fats, they are also active in secretive and excretory roles along with other hepatic cells (Hall and Cash, 2012).

The hepatic disorder is considered by changes in serum levels of liver enzymes and metabolic products, hepatic damage is usually observed as a rise in serum alanine

transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP)(Arora *et al*., 2009).

Liver damage related to warfarin treatment is uncommon, but clinically evident several studies have demonstrated intrahepatic hemorrhage is a very unusual occurrence of warfarin, and patients suffered intrahepatic and subgaleal hemorrhage, but there were no risk factors for bleeding or dysfunction of the International Standardized Ratio Regulation (Park *et al*.,2013). According to (Geçmen *et al*., 2016) demonstrated that warfarin therapy cause liver enlargement and massive liver hematoma, raised serum alanine aminotransferase was more when patients receiving warfarin drug.

2.5 Warfarin effects on Blood vessels

There are two types of vessels, arteries and veins. Arteries are brighter, since they transport blood rich in oxygen to the organs of the body. The veins aftewards transport the blood, which is at a low oxygen level and thus darker, to the lungs and the liver (Kondermann *et al*., 2007).

Arteries and veins are all composed of inner endothelial tissue (tunica intima) surrounded by internal elastic tissue, smooth muscle cell layer (tunica media), external elastic tissue, and fibrous connective tissue (tunica adventitia), larger caliber arteries have thicker smooth muscle cell layers while larger veins possess specialized structures such as valves, the two networks of tubes are completely separate at the level of the larger vessels but are linked together distally, in a system of fine capillaries found throughout all tissues, as well as proximally, at the heart (Cleaver and Krieg, 1999).

Warfarin tends to be associated with an improvement in systemic calcification, including coronary and peripheral vasculature, this rise in vascular calcification is due to inhibition of the gamma carboxyglutamate gla protein MGP enzyme matrix, MGP is a vitamin K dependent protein that normally inhibits systemic calcification by scavenging calcium phosphate in the tissues (Poterucha and Goldhaber, 2016).

A significant number of studies have demonstrated that vascular calcification can be caused and increased in patients undergoing long-term warfarin therapy, contributing to several serious complications, such as hypertension, atherosclerosis, valvular calcification, and coronary calcification, especially in the atrial fibrillation, hemodialysis, and chronic kidney disease populations (Zhang and Tang, 2014).

High doses of warfarin induce focal calcification of elastic lamellae in the media of major arteries and aortic heart valves in rats, aortic calcification was first seen after 2 weeks of warfarin therapy and progressively in density at 3, 4 and 5 weeks of treatment, the calcification of warfarin-induced arteries is close to that seen in the matrix Gla-protein (MGP)-deficient mouse, which indicates that warfarin causes artery calcification by

inhibiting γ-carboxylation of MGP and thereby inactivating the putative calcification-inhibitory function of the protein(Price *et al*., 1998).

The relationship of warfarin and arterial calcification according to (Schori and Stungis, 2004), coronary arteries in a stable man with no complications who has had long-term warfarin therapy are heavily calcified, long-term warfarin therapy doctors deem arterial calcification to be one of its possible effects.

2.6 The effect of potassium on blood vessels

Potassium is necessary for the proper functioning of all cells , tissues and organs in the human body, it is also essential for cardiac function and plays a key role in skeletal and smooth muscle contraction, making it critical for normal digestive and muscular function (Pohl *et al*., 2013).

Reduced potassium is associated with cardiovascular diseases such as hypertension and chronic heart failure, and appropriate dietary potassium intake improves those pathological conditions (WHO, 2003).

Reduced dietary potassium intake has been linked to cardiovascular diseases such as hypertension and incidental stroke, in model mouse reduced dietary potassium (0.3%) promoted atherosclerotic vascular calcifcation and increased aortic stiffness, compared with normal (0.7%) potassium–fed mice. In contrast, increased dietary potassium (2.1%) attenuated vascular calcifcation and aortic stiffness. (Sun *et al*., 2017).

Low serum potassium levels are associated with a slightly higher risk of cardiovascular mortality relative to patients with usual serum potassium concentrations (Lai *et al*., 2015).

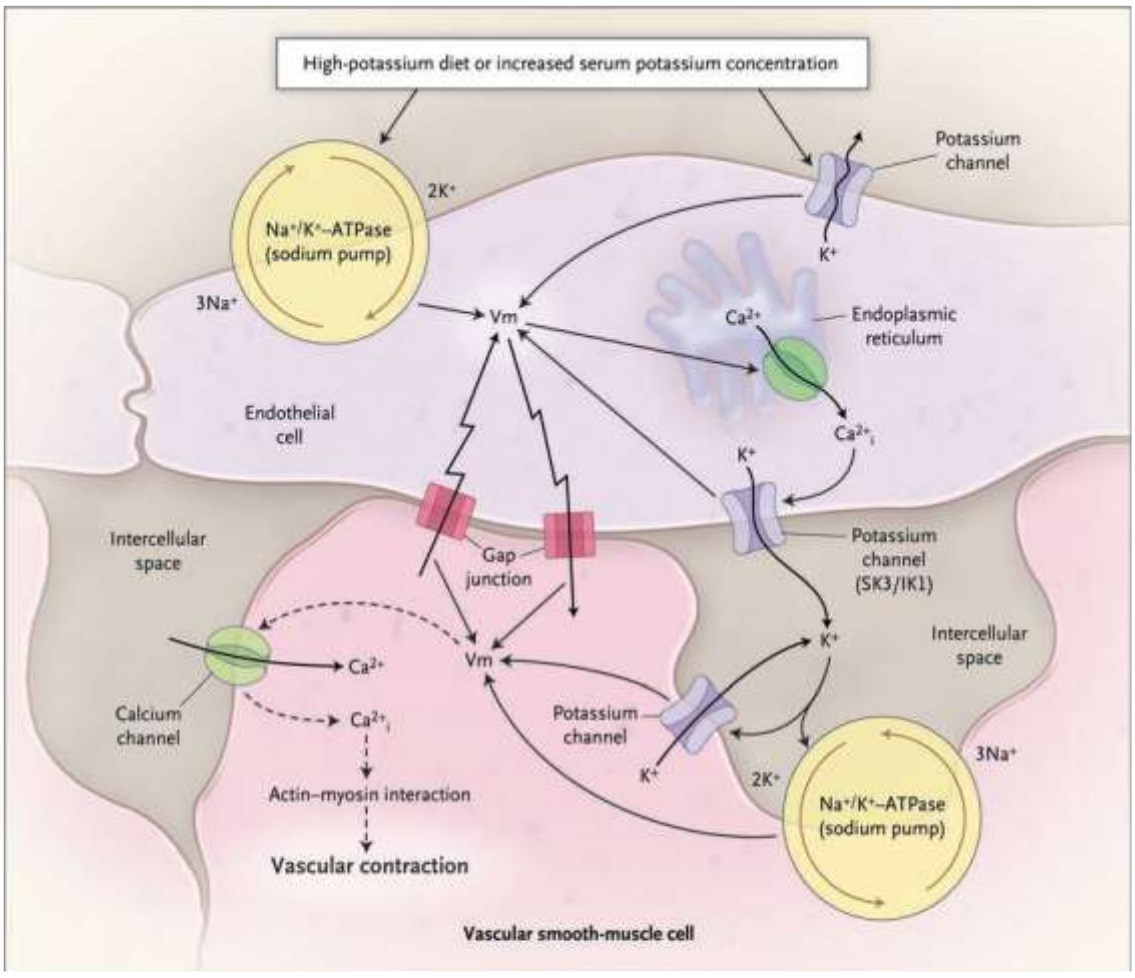
Experimental findings show that a potassium-rich diet lowers cardiovascular risk by inhibiting arterial thrombosis, atherosclerosis, and medial arterial wall hypertrophy (McCabe and Young, 1994).

Good quality data indicates that elevated potassium consumption decreases blood pressure in persons with hypertension and does not negatively influence blood lipid concentrations, catecholamine concentrations or renal function in adults. Higher potassium consumption was associated with a 24 % lower risk of stroke, these results showed that increased potassium consumption is potentially beneficial to most people without impaired renal handling of potassium for the prevention and control of elevated blood pressure and stroke (Aburto *et al*., 2013).

A high-potassium diet and increases in serum potassium, even within the physiologic range, cause endothelium-dependent vasodilatation by hyperpolarizing the endothelial cell through stimulation of the sodium pump and opening potassium channels (Haddy *et al*., 2006). Endothelial hyperpolarization is transmitted to the vascular smooth-muscle cells, resulting in decreased cytosolic calcium, which in turn promotes vasodilatation.

Experimental potassium depletion inhibits endothelium dependent vasodilatation (Haddy *et al*., 2006).

The stimulation of the sodium pump and the opening of the potassium channels hyperpolarize the endothelial cell (with membrane potential shifting to more negative values). Endothelial-cell hyperpolarization is transmitted to the vascular smooth-muscle cell by means of myoendothelial gap junctions and also by increasing the intracellular calcium concentration(Ca2+). Change activates potassium channels of small (SK3) and intermediate (IK1) conductance localized to the cell membrane, causing the potassium to exit the cells and to accumulate in the myoendothelial intercellular space. This accumulation of potassium adds to vascular smooth-muscle hyperpolarization by activating membrane potassium channels and stimulating the sodium pump. Vascular smooth-muscle hyperpolarization lowers Ca2+, resulting in vascular relaxation(Haddy *et al*., 2006 ; Amberg *et al*., 2003).



(Figure2.1) Molecular Pathways of Potassium-Induced Endothelium-Dependent Vasodilatation.
(Adrogué and Madias, 2007).

5.1 Conclusions

1- Warfarin effect the kidney which leads to damage in the renal corpuscle, acute hemorrhage, cystic dilation filled with blood in the parenchyma of kidney, sever destruction between renal tubules, also effect on liver which included hepatocyte hypertrophy, cystic dilation, and necrosis in the parenchyma of the liver.

2- Warfarin have prominent effects on the blood vessel wall when used for several days, which leads to calcification in the wall of the blood vessel, used the warfarin for a long time without supplements that lead to damage the internal and external elastic lamina in the arterial wall, so degeneration the tunica intima.

3- The high dose of ginger root extract lead to decreased histological changes on the renal corpuscle, renal tubules, liver and blood vessels when gives with warfarin.

4- Warfarin has a prominent effect on the biochemical parameters which lead to increased ALT, AST, urea, creatnine, in the treated group with warfarin only.

5- The ginger root extract treated the biochemical differences when used with warfarin drug during the treated period.

5.2 Recommendations

1- Immunohistochemical studies of blood vessels wall and the nervous system after the treated with warfarin to detect the density of calcification in the blood vessel wall.

2- Ultra-structural studies of blood vessel wall after treatment with warfarin in lab animals.

3- Histochemical studies of the endocrine gland in the body after treating with warfarin in male white mice.

The warfarin drug have prominent side effects on the body organs when warfarin is used for treatment of the cardiovascular system diseases, One of the important side effect is blood vessel wall calcifications. According the present study focused to investigation the role of watery ginger root extract in processing the histological and biochemical changes in the some organs of male white mice after being treated with warfarin for 30 day. The present work carryout 80 males white mice, the average age was 3 months, all laboratory animals were housed in the animal house of the sciences college / AL-Muthanna University. All environmental parameters in the animal house were under control.

The experimental animals were divided into four main groups which including (A, B, C, and D), each group composed of 20 males white mice, A group was consider as control group, B group was treated with warfarin only, C group was treated with both warfarin and low dose of ginger root extract, and D group was treated with both warfarin and high dose of ginger root extract. The histological results of the kidney have significant histological deference's in renal corpuscle and renal tubules and have prominent histological changes after treated with warfarin only (B group) compared with control (A group), while the histological results after treated with both warfarin and low dose of watery ginger root extract (C group) were similar in something to histological findings of B group, while have significant histological changes when compared with control groups, so the statistical measurements were non-significant with B group and significant compared with A group.

The histological findings after treated with both warfarin and high dose of watery ginger root extract (D group) were something similar to A group, so have some statistical analyses were significant when compared with B and C groups, but non-significant compared with A group. The biochemical results after treated with warfarin for 30 days noted significant increase in the level of ALT,AST, urea, crietinine and potassium, while the level of calcium have significant decrease compared with control group, the treated group with low dose of water ginger root extract and warfarin have significant increase in the level of ALT, AST, urea, creatinin, potassium, calcium, while in the treated group with high dose of ginger and warfarin have non-significant in the level of ALT, AST, urea, creatninie, while have significant increase in the level of potassium and calcium compared with the control group.

4.1 The Histological Results

4.1.1 The Histological Results of Kidney

4.1.1.1The histological results of the control group

Macroscopically the kidney in mouse have bean in shape and reddish brown in color, the kidneys have two surfaces where hilum is found in the convex surface when renal artery inlet and exit the renal vein ureter. The histological study noted the kidney surrounded by a thin layer of connective tissue capsule. This capsule is consisted of two layers, an outer layer which composed of irregular dense connective tissue mostly collagenous fibers and a small amount of elastic fiber, the inner layer is consist of mainly smooth muscle cells(Fig.4.1).

The tissue section of the kidney showed the cortical region consists of spherical structures called the renal corpuscles and tubular structures called renal tubules. The renal corpuscle have a prominent diameter (16.38±0.348µm) in (Table.4.1) the renal corpuscle composed of a tuft of blood capillaries called glomerular capillaries. The renal corpuscle is surrounded by a connective tissue capsule that composed of two layers (parietal and visceral layers) called bowman's capsule, the parietal layer of the capsule supported by a prominent basement membrane, the visceral layer enveloped the glomerular capillaries in the renal corpuscle, the tissue section noted prominent space between two layers of Bowman's capsule called Bowman's space. The podocytes and mesangial cells were located between the glomerular capillaries(Fig.4.2).

The renal tubules were distributed between the renal corpuscle, some of the renal tubules have narrow diameter with limited cells numbers in the wall, these tubules called proximal convoluted tubules (P.C.T.) which have (2.54±0.114µm) in diameter, (Table.4.2), the tissue section noted the wall of (P.C.T) was consist of a limited number of cells about 4-5 cells in numbers, the internal surface lined by a high simple cuboidal epithelia, the apical portions of epithelial cells have prominent tinny cytoplasmic processes as brush border (Fig.4.2).

The tissue section in an inner portion of the kidney have very narrow tubular structures called the Henley loop, the Henley loop have two branches descending and ascending branches of Henley loop, the descending branch of Henley loop have lumen with (1.90±0.082µm) in diameter, (Table.4.3), and lined by flat simple squamous epithelia, the epithelial cells have prominent spherical nuclei which protrude into the lumen of Henley loop(Fig.4.3).

The ascending branches of Henley loop have a prominent diameter in width was (3.43±0.116µm), (Table.4.3), the wall of ascending arm have 2-3 cells numbers and the internal lumen lined by simple squamous epithelia(Fig.4.3).

The other prominent tubular structures that located beside the P.C.T. which have a very prominent diameter, the wall of these tubules composed of the high number of cells, the tubules called distal convoluted tubules D.C.T. have (5.09±0.130µm) in diameter, (Table.4.2), The luminal surface of distal convoluted tubules lined by simple cuboidal epithelia without brush border (Fig.4.2).

The tubular structures that arrangement in the medullary region of the kidney which have a prominent lumen and thick wall called collecting ducts which have a very wide lumen with (5.65±0.122µm) in diameter (Table.4.1). The inner surface of the collecting duct is lined by simple cuboidal epithelia(Fig.4.3).

4.1.1.2 The histological results of a kidney after treated with warfarin

The renal corpuscle of the kidney in the treated group have a prominent diameter (17.64±0.382µm) (Table.4.1), the diameter of renal corpuscle have significant increased compared with the control group, the tissue section of the kidney showed broken some glomerular capillaries in the renal corpuscle, the meningeal have irregular dark nuclei which located between glomerulus capillaries, tissue section showed a prominent defect in the partial layer of bowman's capsule, so noted parenchymal degenerated of the kidney with prominent hemorrhage, the hemorrhage noted may be due to the side effect of warfarin on the tissue structures of the kidney, aggregation of inflammatory cells around blood hemorrhage and cystic dilation (Fig.4.4). These results coincided with (Lim, 2013) were explained that warfarin cause acute kidney damage and hemorrhage in the kidney.

The present study showed abnormal wide cystic dilation filled with blood, also noted aggregation of inflammatory cells near the cystic dilation, tissue section appeared prominent blood vessels congested with blood and some lesions of necrosis, this may be due to rupture of the wall of blood vessels caused by increase blood pressure because the role of warfarin and broken of blood vessels that lead to blood flow to outside of blood vessels(Fig.4.5).

The present findings (Fig.4.6) showed prominent damage in renal corpuscle, prominent degeneration in bowman space, abnormal cellular proliferation of mesangeal cells, so, noted abnormal cystic dilation filled with secretion and blood, and spot of hemorrhage in parenchyma of the kidney, this may be cause according to (Brodsky et al., 2010), who demonstrated the over-anti coagulation due to warfarin

overdose and INR greater than 3.0 can be found to cause bleeding in glomerular of the kidney.

The current results of kidney after treated with warfarin showed the diameter of P.C.T was (4.98±0.141µm) (Table.4.2) which have a significantly increased compared with the control group, the tissue section showed acute degeneration in proximal convoluted tubules wall, tissue section noted exfoliated the epithelial layer that line the internal lumen of P.C.T, the prominent isolated epithelial layer of the P.C.T without brush border (Fig.4.7), this acute degeneration in tubules may be due to the high toxicity of warfarin, this result agreement with (Conjeevaram *et al*., 2019) which noted that warfarin cause degeneration in renal tubules and tubules toxicity that lead to worsening kidney damage.

The descending branches of Henley loop in the kidney of the treated group have normal structures with (3.05±0.123µm) in diameter(Table.4.3), the diameter of descending arm of Henley loop has significantly increased compared with control group. The diameter of ascending branches of Henley loop have (6.20±0.206µm) (Table.4.3), which have asignificant increase in diameter compared with control group. Henley loop arms have destroyed epithelial layers that lining both branches of Henley loop, both branches were filled with a thick fluid, the tissue section noted prominent blood congestion and hemorrhage between the arms of Henley loop and disappeared cells from epithelial layers that lining both arms of Henley loop (Fig.4.8), this may be due according to (Conjeevaram *et al*., 2019) who explained obstruction of red blood cell renal tubules and direct hemoglobin-released tubular toxicity caused by extravagant red blood cell hemolysis lead to worsening of renal disorder.

The distal convoluted tubules(D.C.T.) have prominent lumen with (7.69±0.192µm) in diameter (Table.4.2), which have significantly increased compared with the control group, the histological finding showed prominent effects in the wall of D.C.T, the D.C.T. lose the cells that formed the D.C.T. wall, so noted exfoliated epithelial layer,(Fig.4.5,7). The histological result of the kidney after treated with warfarin noted the collecting ducts have prominent diameter with (8.72±0.213µm) (Table.4.1) which have significantly increased compared with the control group, this results may be due to the high toxicity of warfarin that lead to blood pressure on tubules, this result agreement with (Abt *et al*., 2000) who explained showed massive occlusion of renal tubules by red blood cells because tubular cell damage consistent with acute tubular necrosis.

4.1.1.3 The histological results of a kidney after treated with a low concentration of ginger with warfarin:

The current result of kidney in the mice after treated with both warfarin and a low dose of watery root ginger extract for thirty days of experimental time, the tissue section showed congested glomerular capillaries, So, noted many spot of blood congestion in limited regions through the kidney parenchyma, these histological effects were may be due to elevated in the international normalized ratio (INR) caused by the effects of warfarin, the tissue field noted of irregular spaces filled with blood exactly located between renal corpuscle in the cortical region of the kidney, (Fig.4.9). This results agree with (Garcia *et al*., 2006) who noted high INR levels were an significant risk factor for bleeding associated with the use of warfarin.

The renal corpuscle have prominent diameter was (17.45±0.497µm) (Table.4.1), which have significantly increased compared with control and non-significant compared with previous study, the tissue section showed high cellular proliferation as large clusters beside the peripheral border of cystic dilation, the renal corpuscle have abnormal wide bowman's spaces, so the result showed progressive glomerular capillaries, the glomerular capillaries have a wide lumen and some of them were congested with blood, this may be due to a reduction in blood flow in the outer medulla(Fig.4.10). This result consonant with (Mendonca *et al*., 2017) was explained that warfarin causes congested glomerulus in the capillary vessels with red blood cells.

The histological finding of proximal convoluted tubules (P.C.T) have (4.23±0.146µm) in diameter (Table.4.2) so, have significantly increased compared with the control group, and slightly decrease compared with warfarin group, (Fig.4.11) showed prominent abnormal lumen of P.C.T filled with the dark fluid contained on the many vacuoles, the inner surface of P.C.T was lined by simple cuboidal epithelia the brush border disappeared in the tissue section of P.C.T, some of P.C.T have prominent destruction in their wall and isolated epithelial cells and accumulation in the lumen of P.C.T, the tissue section showed abnormal aggregation of inflammatory cells between the tubules, aggregation of inflammatory cells may be due to immune response, the tissue section showed disappeared the epithelial layer from the inner surface of P.C.T.

The tissue section of the cortical region of the kidney noted the distal convoluted tubules(D.C.T.) have a diameter with (8.21±0.326µm) (Table.4.2), which have significant in increased compared with the control group, but non-significant with diameter of D.C.T. in the group that treated with warfarin only, the tissue section noted abnormal dilated of D.C.T filled with secretion and aggregation of inflammatory around D.C.T., tissue section showed irregular wide cystic dilation in parenchyma of kidney(Fig.4.13), this may be due to the toxicity of warfarin after the

treated time that leads to degeneration some renal tubules, these tissue findings were similar something in the treated group with warfarin only. This histological change constant with (Ware *et al*., 2013) which explained warfarin caused glomerular hemorrhage with degeneration tubular red blood cell (RBC) casts and severe kidney damage.

The tissue section appeared the descending and ascending branches of Henley loop have significant diameters were (3.66±0.079µm), (7.23±0.362µm) respectively(Table.4.3), (Fig.4.14) both branches have significantly increased compared with the control group and previous treated group, the lumen of Henley loop arms were something filled with fluid, the cells in the wall of Henley loop have prominent dark spherical nuclei, the inner surface of Henley loop branches were lined by simple squamous epithelia, the histological result of kidney noted destruction of the epithelial layer and exfoliated epithelial cell from the basal line of the inner surface, this histological change may be due to acute toxicity of warfarin.

Also, showed prominent blood congestion and blood hemorrhage between the branches of Henley loop (Fig.4.14). This histological change in the tissue section of the kidney may be due to an increase in the rate of excretion or elimination the toxic material through the kidney which increased the blood congestion in the renal tubules which lead to these changes, These histological result of kidney were coincided with (Ware *et al*., 2011) which explained renal tubular disruption by casts of red blood cells by warfarin.

These histological changes were different in structures compared with the control group, but similar in something with the group that treated with warfarin only, but have slight histological changes in comparison with group that treated with warfarin and high concentration of watery root ginger extract, tissue section of collecting ducts have significant in diameter (7.24±12.307µm) (Table.4.3) compared with the control group but significant decrease with warfarin group.

4.1.1.4 The histological results of a kidney after treated with a high concentration of ginger with warfarin

The kidney in the mice which treated with both warfarin and high concentration of watery root ginger extract have tissue structures with slight histological changes compared with the previous group that treated with warfarin only, the cortical region of kidney have tissue structure without histological degeneration.

The renal corpuscle have diameter was (16.87±0.458µm) (Table.4.1), the renal corpuscle diameter showed no significant compared with a control group, but have

significant decreased compared with warfarin group and group that treated with both the low concentration of ginger root extract and warfarin.

The histological finding of kidney noted, the renal corpuscle have spherical in shape, prominent Bowman's capsule, the partial layer of Bowman's capsule was clear with prominent smooth cells, have elongated dark, the Bowman's space was prominent and filled with thick secretion, clear visceral layer of Bowman's capsule that surrounded the glomerular capillaries, the tissue section of renal corpuscle showed the mesangial cells that located between glomerular capillaries in the renal corpuscle, these cells have large irregular dark nuclei, (Fig.4.13,14). This result constant with (Mustafa *et al*., 2016) Which explained ginger caused decrease in the nephrotoxic effect resulted from oxidative damage induced by monosodium glutamate because ginger is a source of excellent antioxidant substance that enhanced and prevented the toxic effect.

The tissue section appeared proximal convoluted tubules (P.C.T) have (3.80±0.132 µm) in diameter (Table.4.2) which significantly increased in diameter compared with the control group, but significant decreased with warfarin group and previous study, the tissue section of P.C.T showed filled with secretion, have a prominent thin wall, the P.C.T were lined by simple cuboidal epithelia but no clear brush border on the apical surface of epithelial layer which lining the internal lumen of P.C.T (Fig.4.14)

The tissue section of the kidney showed abnormal cellular proliferation between renal tubules, prominent blood congestion in different location in the cortical region of the kidney, these tissue finding were variable or different with tissue section of the kidney in the control group, but were fewer histological changes or defect compared with group that treated with warfarin only, these histological finding were may be to the role of the Ginger root extract in the decrease of the warfarin effects on the histological tissue of the kidney (Fig.4.14,15).

The tissue section of distal convoluted tubules (D.C.T.) have a diameter with (7.24±0.215µm)(Table.4.2) that noted a significant increased in diameter compared with the control group but non-significant compared with warfarin treated group and significant decrease compared with the previous treated group, so, noted the inner surface of D.C.T. was lined by low simple cuboidal epithelia, the histological result noted most of D.C.T. filled with thick secretion and have prominent spherical nuclei in the cells that composed of D.C.T. wall (Fig.4.14,15). These result constant with (Al-Qudah *et al*., 2018) which said ginger extract protective kidney tissue in the treatment of diabetic rats.

The tissue section of the kidney noted the descending branch of Henley loop have (3.01±0.122µm) in diameter, (Table.4.3), which was a significant increase compared

with the control group, but non-significant compared with warfarin treated group and slightly significant decrease compared with group that treated with low concentration of ginger and warfarin, the result noted the Henley loop branch have narrow lumen filled with secretion lined by simple squamous epithelia, in some section noted disappeared the nuclei of the cells in the wall of descending branches of Henley loop (Fig.4.16).

The diameter of ascending branches of Henley loop was (6.35±0.271µm) ,(Table.4.3), which have significant increased compared with the control group, but non-significant with warfarin treated group and have significant decrease compared with the previous treated group, the tissue sections noted the ascending branch have wider lumen than the lumen of descending branch, which lined by prominent simple sequames epithelia, so most section of Henley loop branches was filled with secretion(Fig.4.16), the tissue section showed the diameter of collecting duct was (7.15±0.304µm),(Table.4.1), that have significant increased compared with the control group, but significantly decreased compared with warfarin treated group and non-significant compared with the previous treated group. These results constant with (Haksar *et al*., 2006) who said the antioxidant action of ginger was proposed as one of the major mechanisms for the protective actions of the plant against toxicity.

Table.4.1: Diameter of renal corpuscles and collecting duct in mice. µm

diameter / Treatment	Renal corpuscle mean ±S.E	Collecting duct mean ±S.E
Control group	16.38±0.348^{c}	5.65±0.122^{c}
Warfarin group	17.64±0.382^{b}	8.72±0.213^{a}
Warfarin and low dose of ginger	17.45±0.497^{b}	7.24±12.307^{b}
Warfarin and high dose of ginger	16.87±0.458b^{c}	7.15±0.304^{b}

*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.01).

Table.4.2: Diameter of proximal convoluted tubules and distal convoluted tubules in mice. µm

diameter / Treatment	proximal convoluted tubules mean ±S.E	distal convolute tubules mean ±S.E
Control group	2.54±0.114[d]	5.09±0.130[c]
Warfarin group	4.98±0.141[a]	7.69±0.192[ab]
Warfarin and low dose of ginger	4.23±0.146[b]	8.21±0.326[a]
Warfarin and high dose of ginger	3.80±0.132[c]	7.24±0.215[b]

*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.01).

Table.4.3: Diameter of descending branch and ascending branch of Henles loop in mice. µm

diameter / Treatment	descending branch mean ±S.E	ascending branch mean ±S.E
Control group	1.90±0.082[c]	3.43±0.116[c]
Warfarin group	3.05±0.123[b]	6.20±0.206[b]
Warfarin and low dose of ginger	3.66±0.079[a]	7.23±0.362[a]
Warfarin and high dose of ginger	3.01±0.122[b]	6.35±0.271[b]

*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.01).

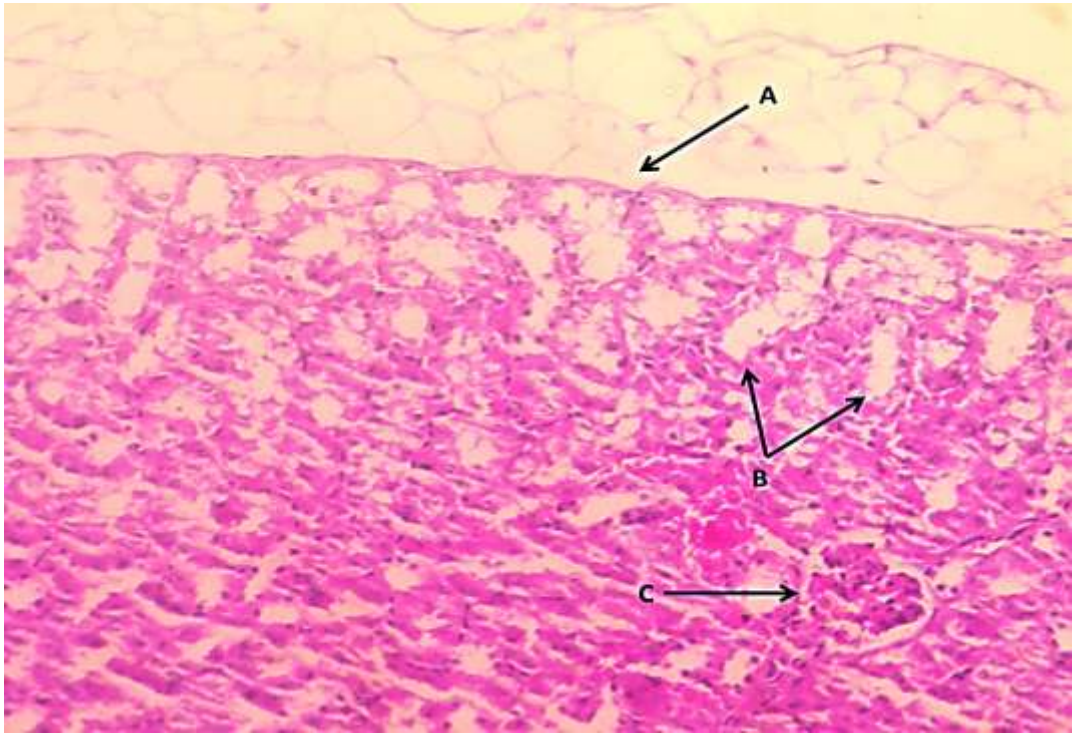


Fig.4.1 : Transverse section of kidney in control group which showed A-kidney capsule, B-renal tubule, C-renal corpuscle. H&E stain 20X.

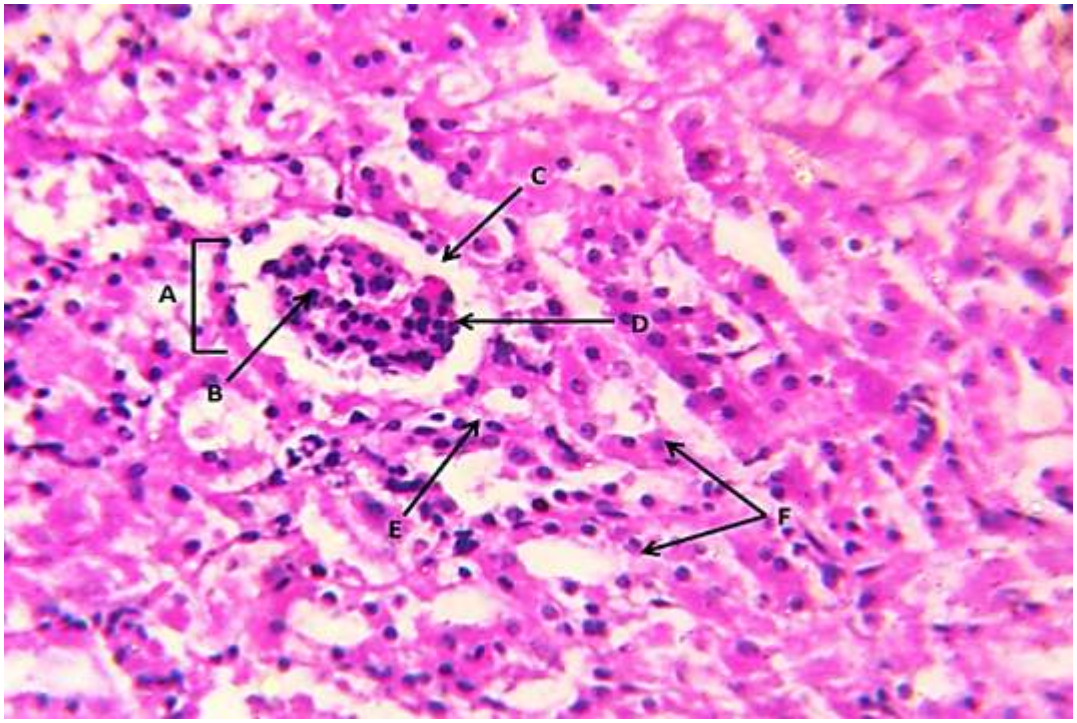


Fig.4.2 : Transverse section of kidney in control group which showed A-Renal corpuscle, B-glomerular capillaries, C-bowman's space, D-mesangeal cells, E-Proximal convoluted tubule, F-Distal convoluted tubule. H&E stain 40X.

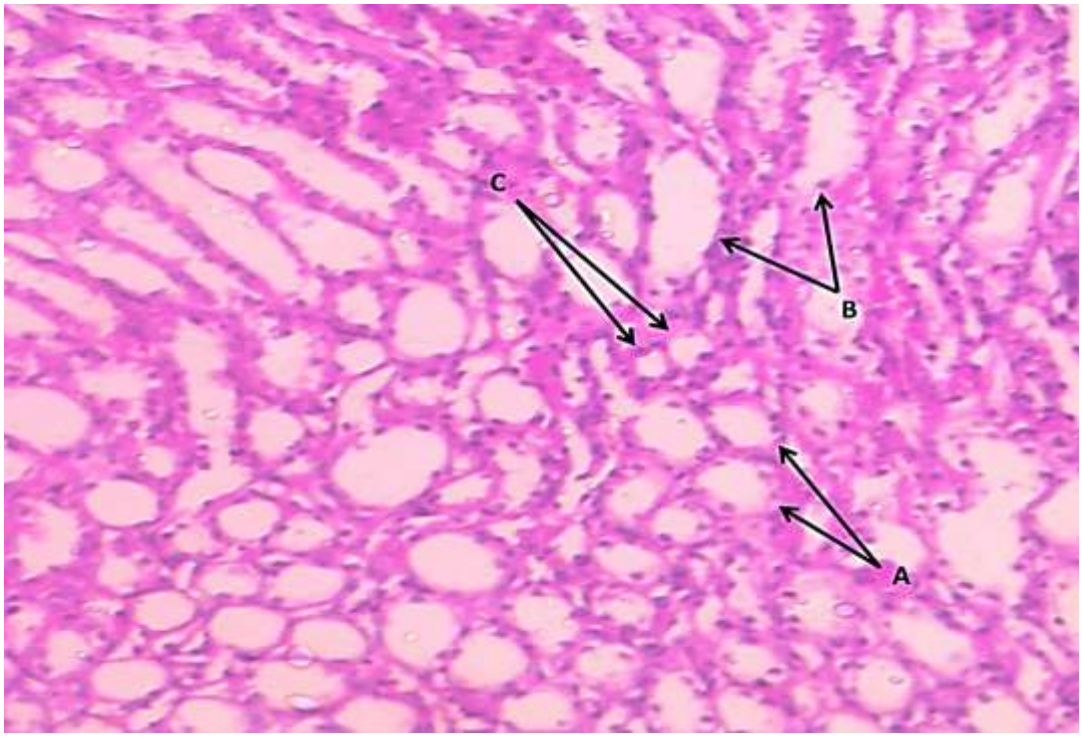


Fig.4.3 : Transverse section of kidney in control group which showed A-ascending branch of Henles loop , B- descending branch of Henles loop, C-collecting duct. H&E stain 40X.

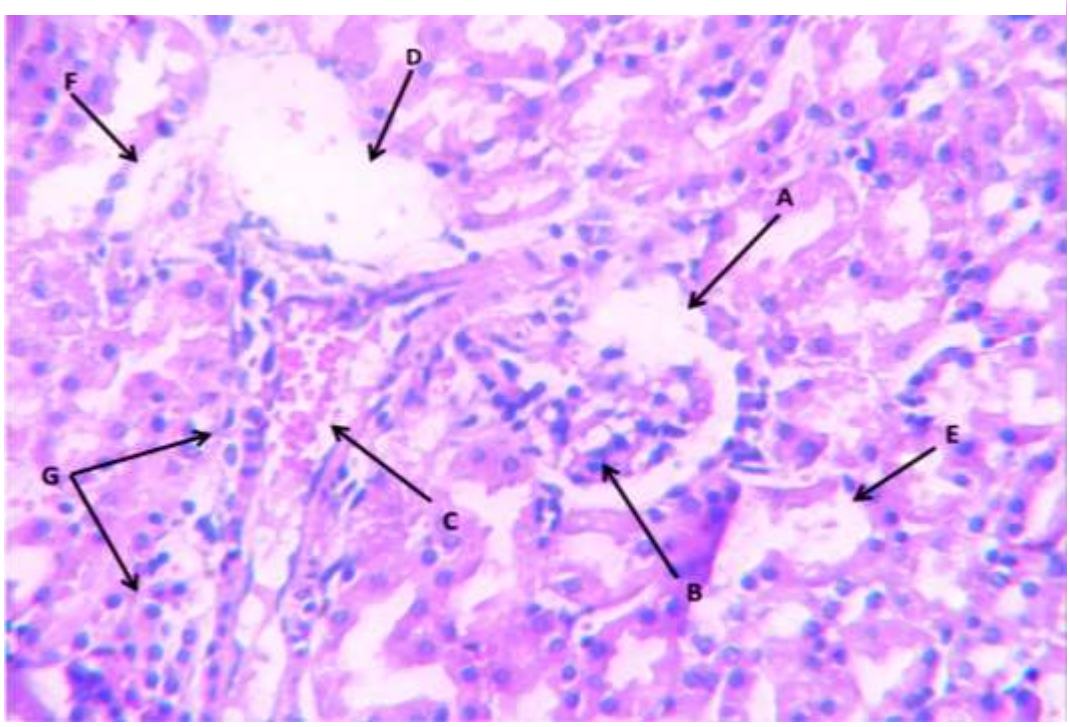


Fig.4.4: Transverse section of kidney after treated with warfarin which showed, A-degeneration bowman's space, B-abnormal glomerular capillaries, C-hemorrhage, D-cystic dilation, E-degeneration in the wall of D.C.T, F-exfoliated epithelial layer, G-inflammatory cells. **H&E** stain X40.

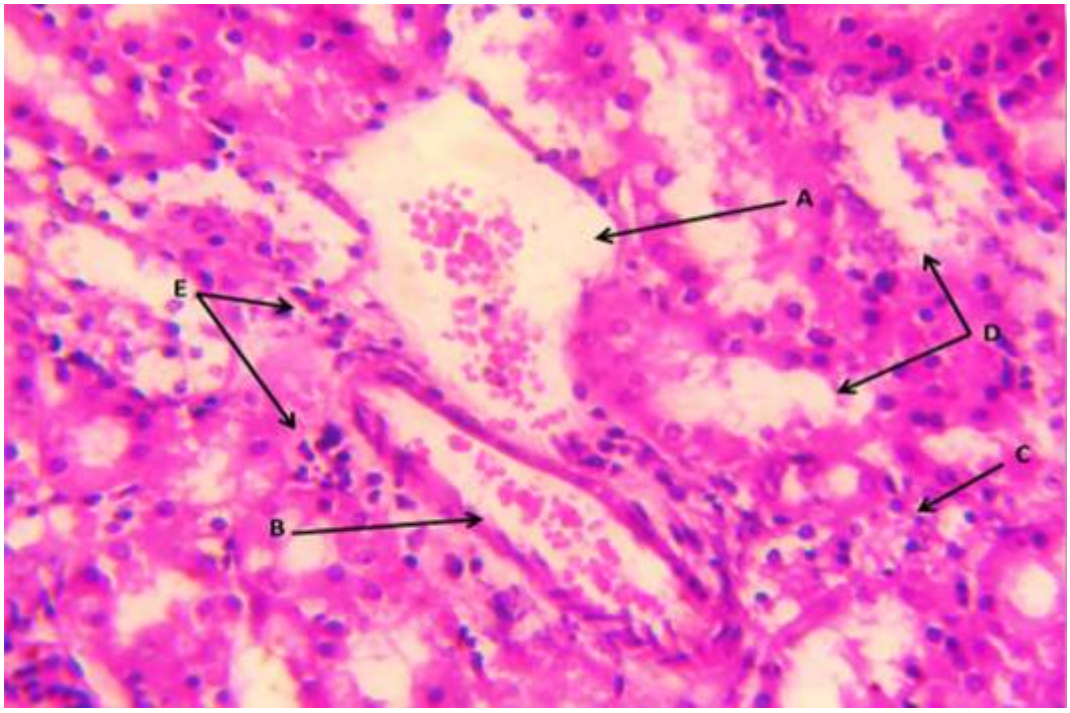


Fig.4.5 : Transverse section of kidney after treated with warfarin which showed A-wide dilation with RBCs, B-blood vessels congested with blood, C-necrosis, D-distraction of D.C.T., E-inflammatory cells . **H&E** stain X40.

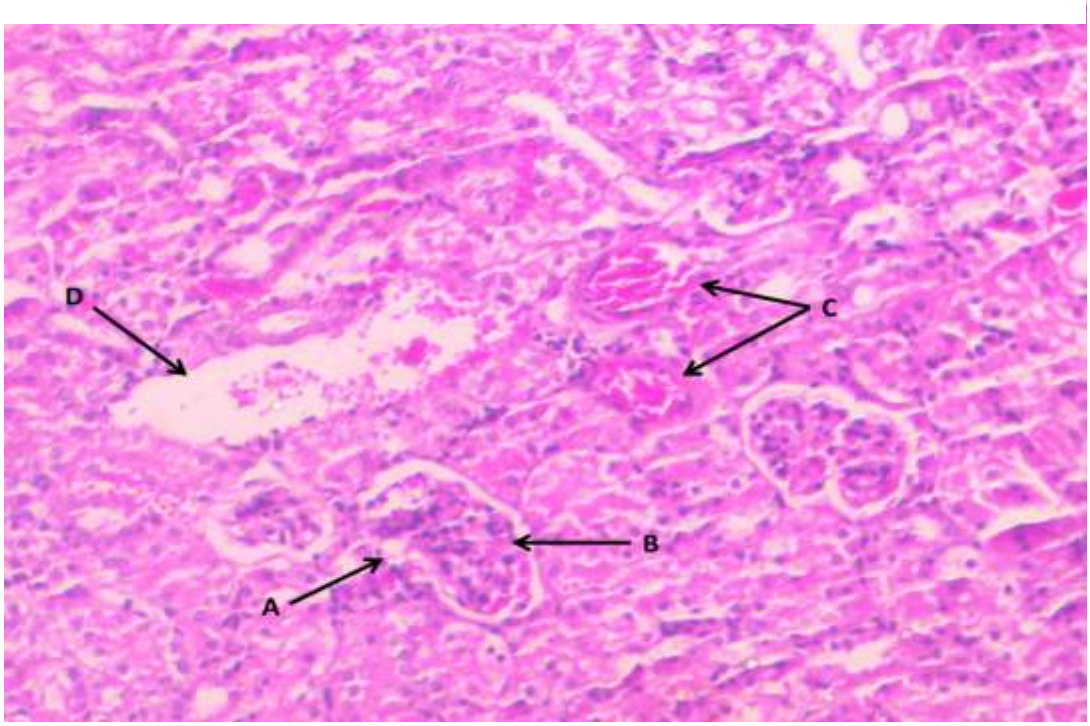


Fig.4.6 : Transverse section of kidney after treated with warfarin which showed A-damage in bowman capsule, B-cellular proliferation of mesangeal cells, C-spot of hemorrhage, D-cystic dilation. **H&E** stain X40.

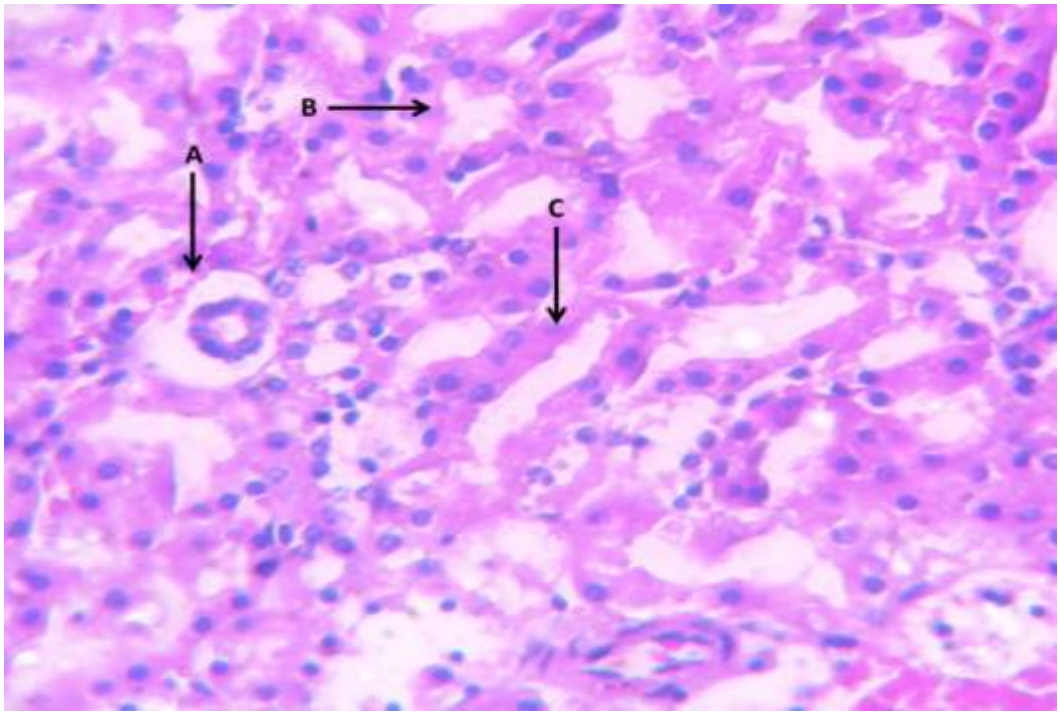


Fig.4.7 : Transverse section of kidney after treated with warfarin which showed, A- isolated epithelial layer of the P.C.T, B-disappeared brush border,C-loose the cells in the D.C.T **H&E** stain X40.

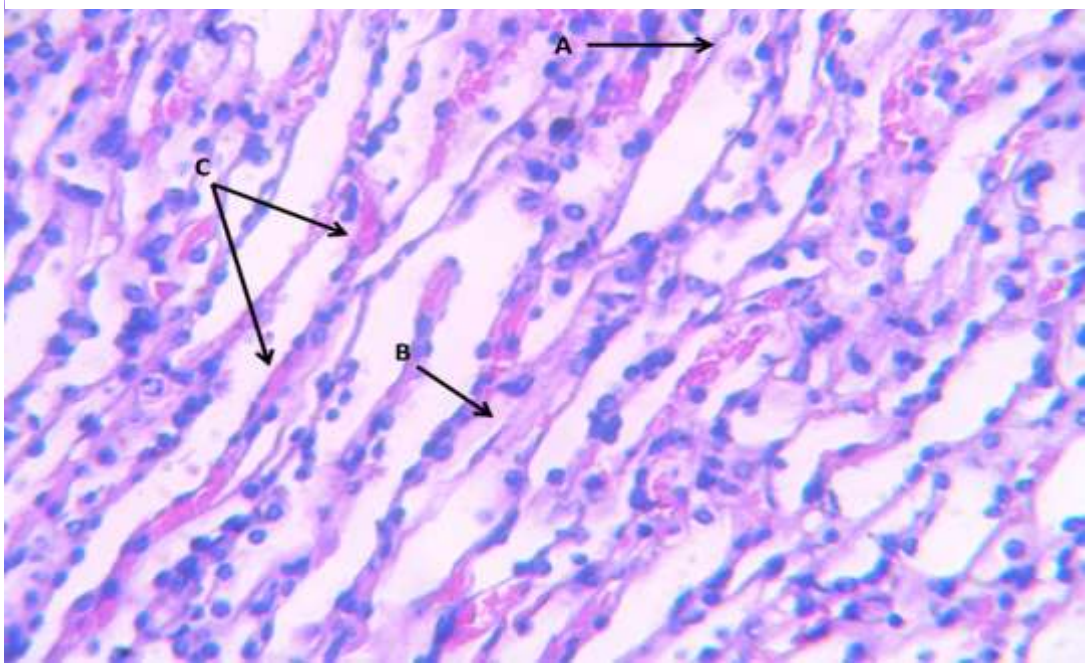


Fig.4.8 : Transverse section of kidney after treated with warfarin which showed A- distruction of Henley loops., B- disappeared of the epithelial cells, C- blood congestion beside Henley arms. **H&E** stain X40.

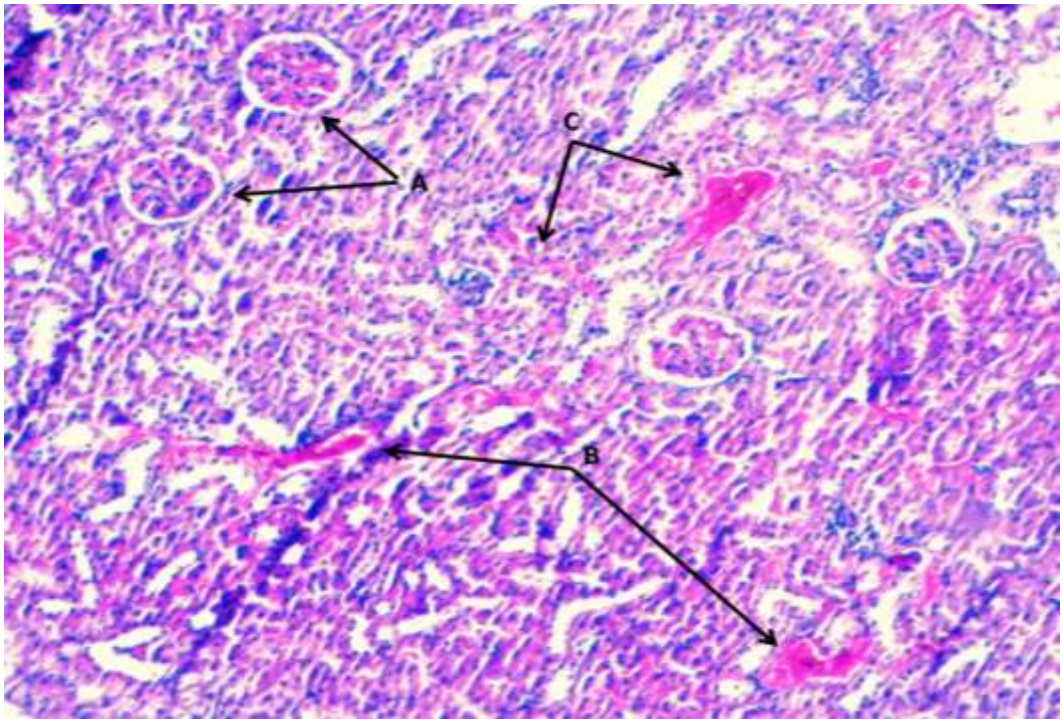


Fig.4.9 : Transverse section of kidney after treated with low dose of ginger with warfarin which showed A-congested of glomerular capillaries, B-hemorrhage, C- spots of blood congestion. **H&E** stain X20.

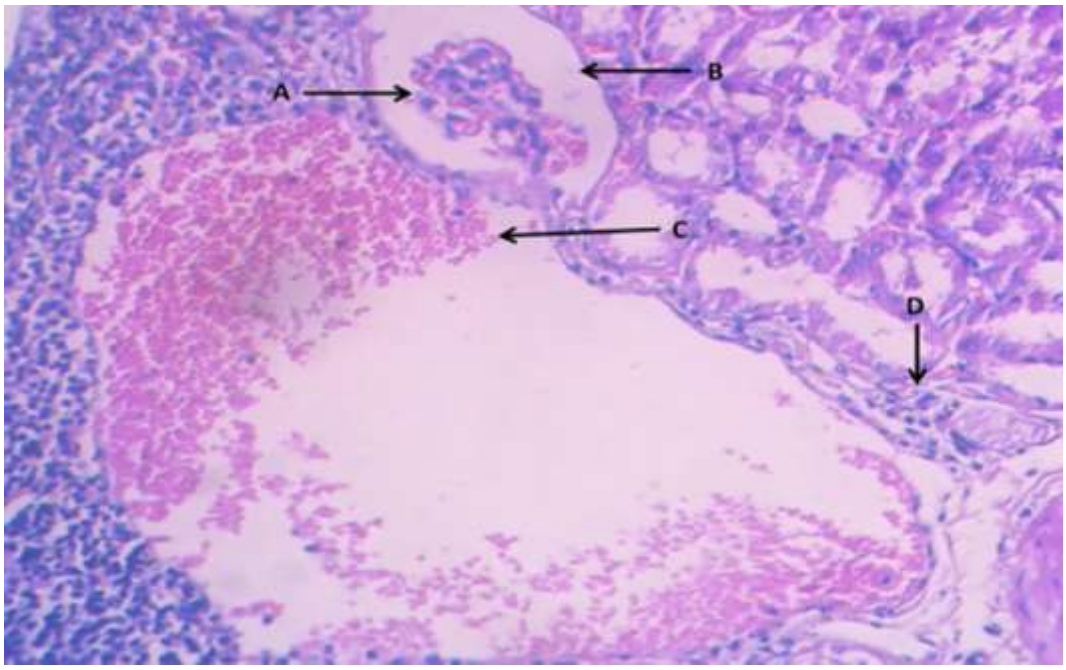


Fig.4.10: Transverse section of kidney after treated with low dose of ginger with warfarin which showed A-progressive glomerular capillaries, B-abnormal wide bowman space, C-wide dilation filled with RBCs, D- inflammatory cells. **H&E** stain X40.

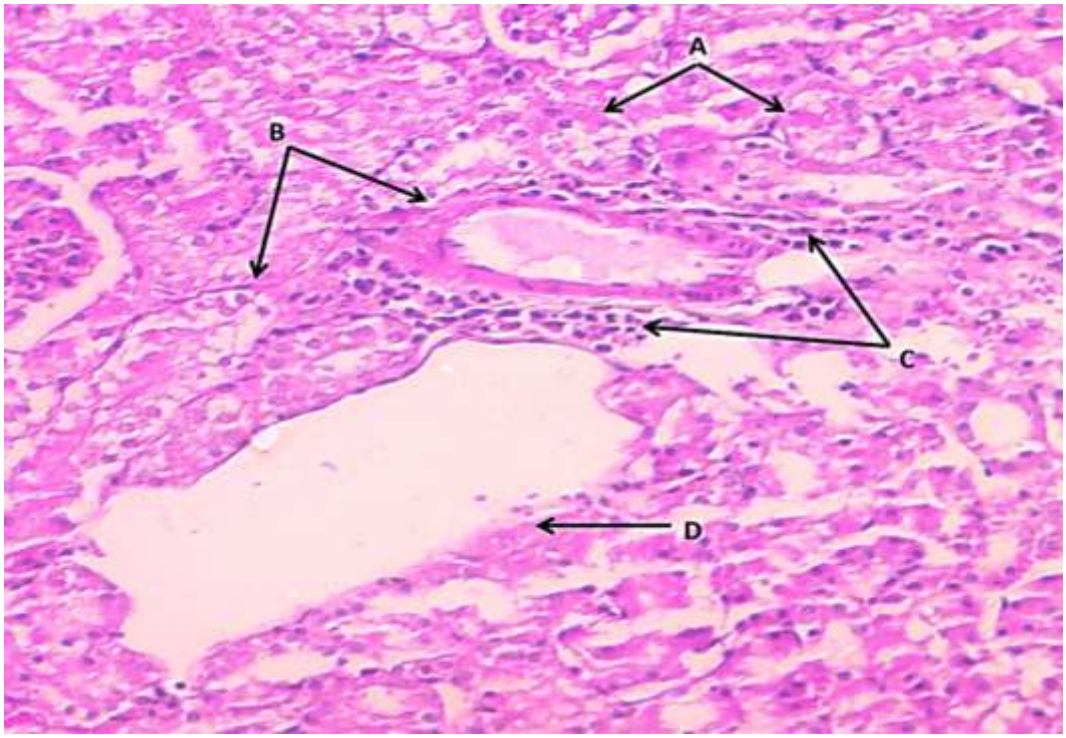


Fig.4.11 : Transverse section of kidney after treated with low dose of ginger with warfarin which showed A- proximal convoluted tubules, B- distal convoluted tubules C- inflammatory cells, D- irregular wide cystic dilation. **H&E** stain X40.

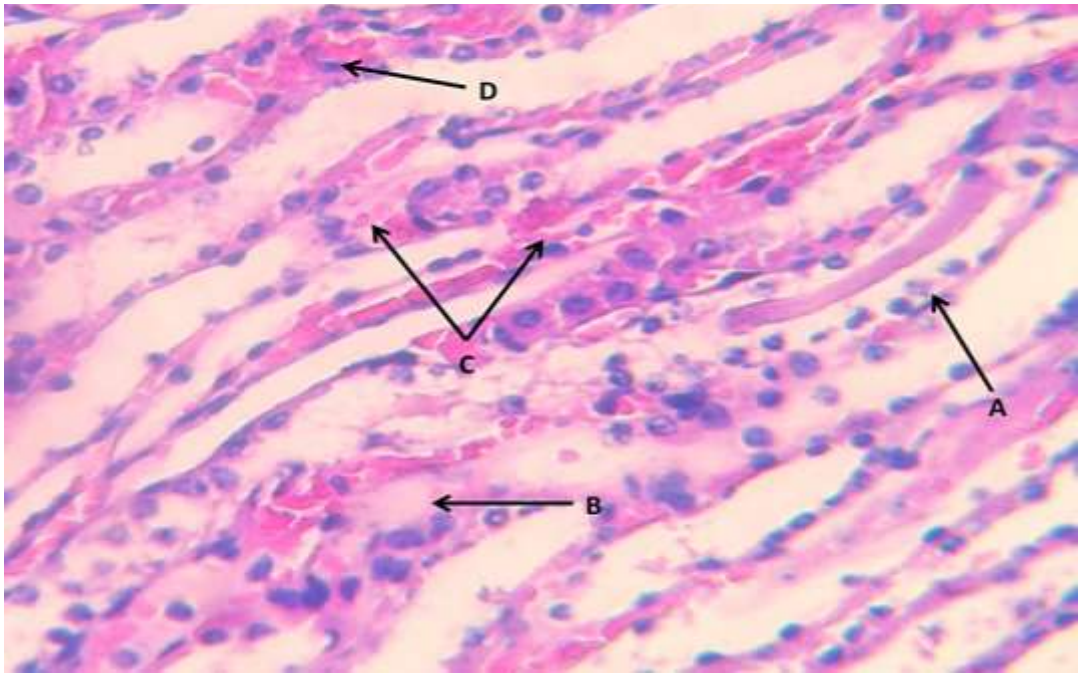


Fig.4.12 : Transverse section of kidney after treated with low dose of ginger with warfarin which showed A-destruction of epithelial layer of Henley loop, B-exfoliated epithelial layer, C-hemorrhage, D-blood congestion. **H&E** stain X40.

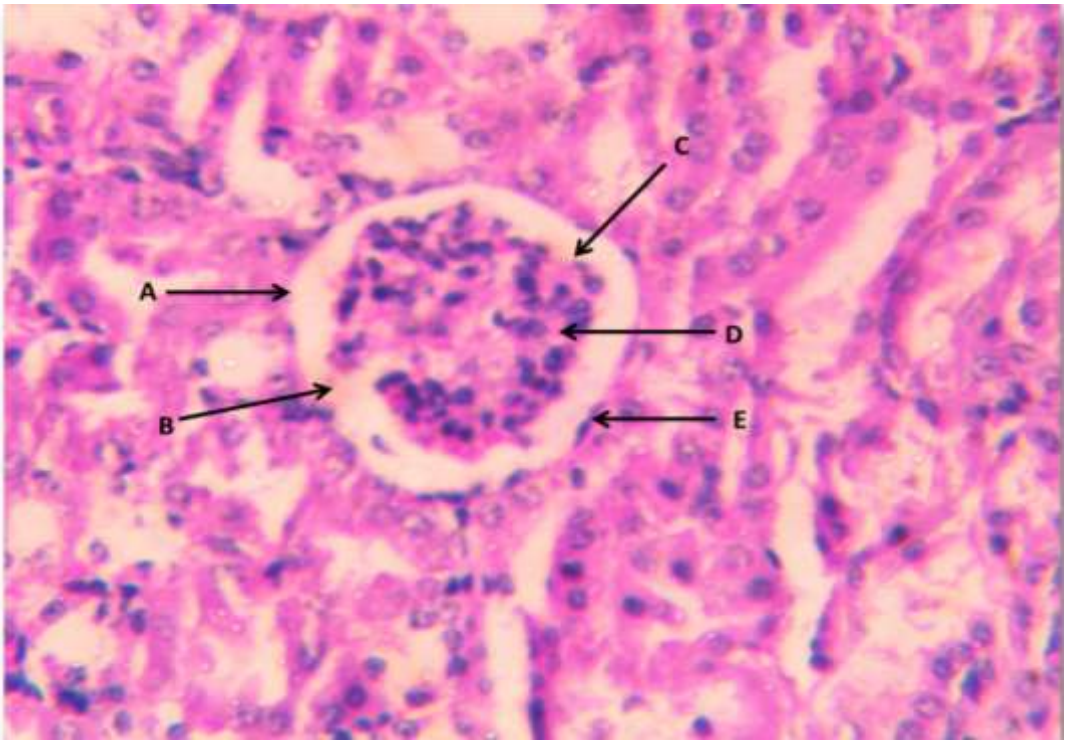


Fig.4.13 : Transverse section of kidney after treated with high dose of ginger with warfarin which showed A-normal renal corpuscle, B- normal bowman space, C- visceral layer D-dark nuclei of mesangeal cells, E-partial layer. **H&E** stain X40.

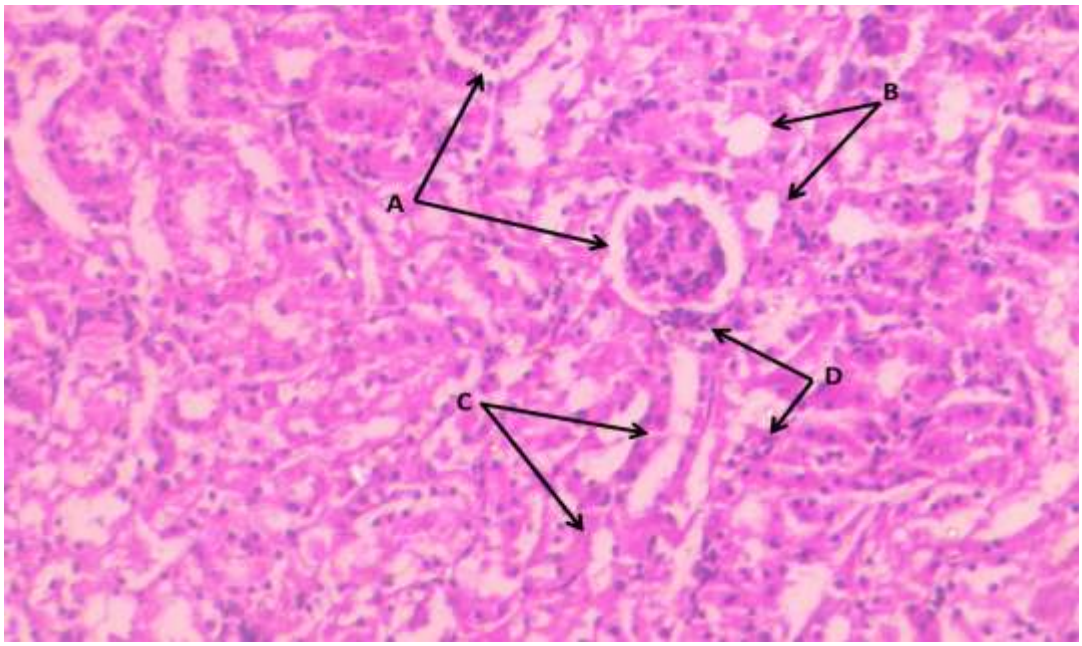


Fig.4.14: Transverse section of kidney after treated with high dose of ginger with warfarin which showed A-normal renal corpuscle, B- normal proximal convoluted tubules, C- normal distal convoluted tubules, D-inflammatory cells. **H&E** stain X20.

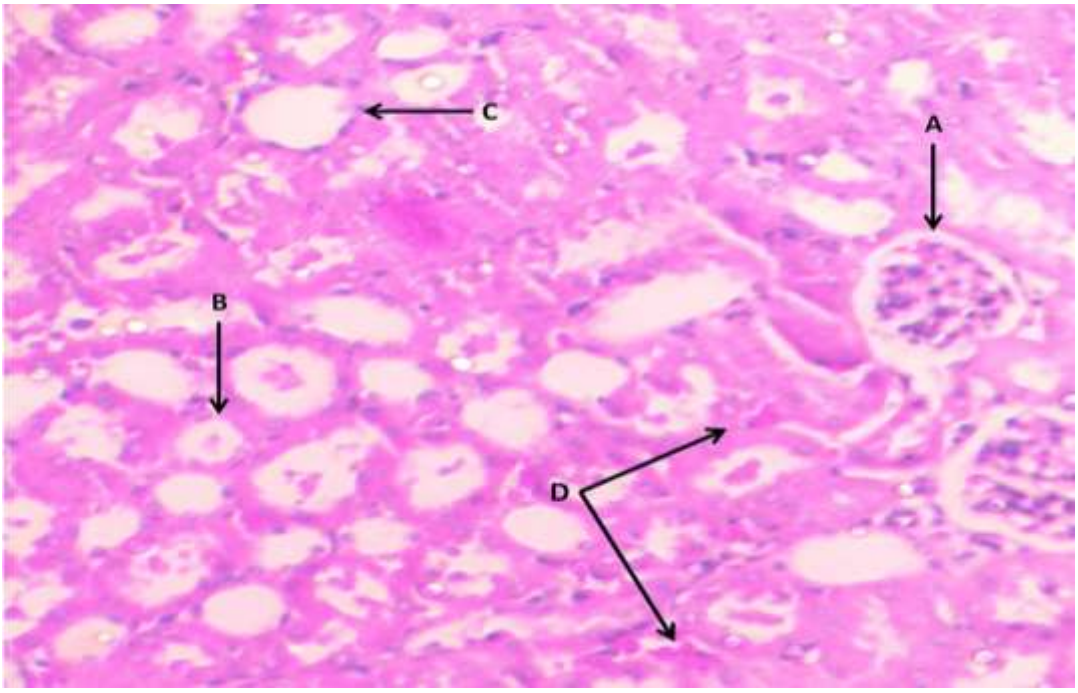


Fig.4.15 :Transverse section of kidney after treated with high dose of ginger with warfarin which showed A-normal renal corpuscle, B- proximal convoluted tubules, C- distal convoluted tubules, D-blood congestion. **H&E** stain X20.

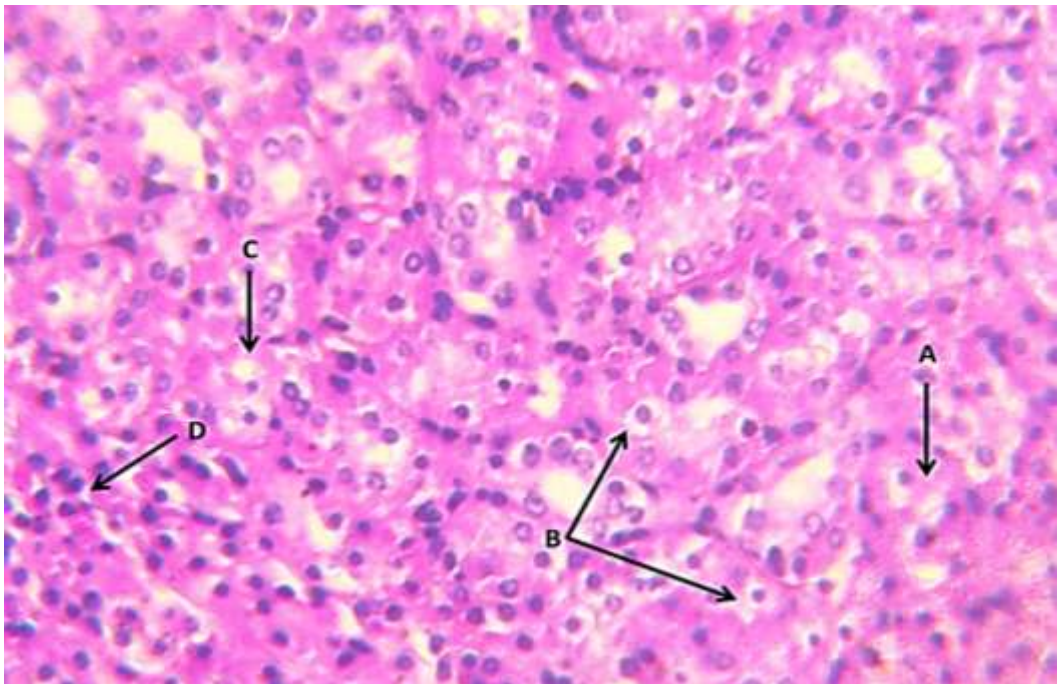


Fig.4.16 : Transverse section of kidney after treated with high dose of ginger with warfarin which showed A-normal ascending branches of Henley loop, B-descending branches of Henley loop, C-normal wall of descending branches, D-cellular proliferation. **H&E** stain X40.

4.1.2 The Histological Results of Liver

4.1.2.1The histological results of the control group

The liver considers the largest visceral organ in the abdominal cavity, reddish brown in color which was surrounded by a connective tissue capsule, the liver is divided into many lobs. Microscopically the liver composed of tiny structures as hexagonal in shape, with a portal area and a central vein in the middle region of liver

parenchyma, the present result noted the liver parenchyma consist of hepatic cord that consists of hepatocytes, hepatocytes were cuboidal in shape having granular cytoplasm with prominent nuclei, among hepatic cord noted the prominent sinusoids (Fig.4.17). The diameter nucleus of hepatocytes was (9.19±0.208µm)(Table.4.4).

4.1.2.2 The histological results of liver the after treated with warfarin:

The histological findings of liver after treated with warfarin showed a significant increased diameter nucleus of hepatocyte was (10.23±0.336µm) (Table4.4) compared with the control group. The liver treated mice with warfarin have clear histological changes in many locations of liver parenchyma, (Fig.4.18) the tissue section of the liver have prominent histological degeneration which appeared the hepatocytes have cytoplasmic vacuoles and progressive nuclei, most hepatocytes was hypertrophy have nuclei with irregular dark nuclei, the tissue section showed clear destruction of the nuclei and appeared as many black spots, hepatocyte swelling due to the imbibition of fluid inside the cell, then, the cells lose their normal form and become unclear from each other, this may be because of increased the activity of hepatocyte of liver response to toxicity.

The histological result noted abnormal hepatic cords, most hepatic cords noted segmented into small pieces irregular in an arrangement through the liver parenchyma, so, noted the prominent proliferation of the kupffer cells in the liver and showed necrosis(Fig.4.18), this increase aggregation of kupffer cells may be due to an activated defense mechanism against warfarin.

The tissue section of the treated liver with warfarin noted irregular portal area, the tissue structures a rounded the portal area was destroyed, so noted abnormal hepatic cords near the irregular portal area, the tissue field noted aggregation of inflammatory cells aggregation as peripheral groups of the portal area, also noted necrosis and inflammation exactly beside the portal area(Fig.4.19), this histological change constant with (Kasahara *et al*., 2017) which said toxic epidermal necrolysis and necrotic keratinocytes caused by warfarin.

The tissue section of the liver noted various spaces filled with thick secretion and have on the inflammatory cells, so, the results noted elongated wide empty cystic dilation contained residual RBCs in the central lumen of cystic dilation and other dilation that filled with thick secretion and blood with prominent vacuoles peripherally location and necrosis(Fig.4.20), this may be due to the toxicity of warfarin metabolism that leads to high pressure on the hepatocyte caused swelling then rapture.

The present study of the liver after treated with warfarin only (Fig.4.21), noted prominent tissue degeneration, tissue section showed hepatocyte destruction and disappeared of nuclei, so, noted very wide hemorrhage in the parenchyma of liver and necrosis beside hemorrhage, this hemorrhage may be due to rupture of vessels wall which leads to the exit of blood to the tissue. These results agreement with (Park *et al*., 2013) who said patients received long-term of warfarin therapy for a recurrent deep vein thrombosis, it can cause intrahepatic hemorrhage and subgaleal hemorrhage although not any risk factors for hemorrhage or variability of the international normalized ratio control.

The tissue section of the liver after treated with warfarin showed very wide tissue degeneration and the irregular border of residual parts of parenchymal destruction which homogenous with RBCs, most of these degenerations were that filled with thick secretion, the destruction area has prominent irregular empty space, the main histological lesions were hemorrhage and necrosis, this changes were may be due to high toxicity of warfarin on the liver tissue that leads to acute parenchymal destruction and destroyed the small blood vessels walls and capillaries in different regions of the liver(Fig.4.22). This change constant with (Clarke, 2016) which said taking anticoagulant drugs induced hepatocellular injury, liver enzyme elevation and increased in INR.

4.1.2.3 The histological results of liver after treated with a low concentration of ginger with warfarin:

The histological results of the liver in the mice after treated with both warfarin and low concentration of watery ginger root extract showed significantly increased the diameter nucleus of hepatocyte (11.57±0.327μm)(Table4.4), compared with the control group and warfarin group, tissue section of liver parenchyma have prominent tissue changes may be similar to those treated with warfarin only, the tissue changes in the liver after treated with compound warfarin and low concentration of ginger root extract noted many irregular cystic dilation, all cystic dilations were empty from any secretion, the tissue section noted prominent necrosis lesions in different location of the liver parenchyma and hepatocyte hypertrophy, the results showed hemorrhage and the portal area was abnormal in shape compared with control group(Fig.4.23). This histological change constant with (Arora and Goldhaber, 2006) which said anticoagulant drug lead to acute necrosis, inflammatory in the parenchyma of the liver.

The tissue field noted prominent degeneration regions in the liver parenchyma which appeared the destruction of hepatocytes, and different mitotic division figures

of hepatocytes, so, noted abnormal irregular wide dilation filled with blood in the liver parenchyma, may be portal area, can be seen with abnormal cellular proliferation of inflammatory cells around the dilation, the tissue section has prominent lesions of necrosis nearly from the cystic dilation(Fig.4.24,25), These results may be due to materials toxicity of warfarin metabolism caused injury hepatic cord and swelling the hepatocyte which leads to rupture.

The tissue section of the liver in the (Fig.4.26,27) has abnormal parenchyma spaces and distraction of the hepatic cord, the tissue section showed prominent blood vessels that congestive with blood and wide distribution of inflammatory cells exactly around the hemorrhage.

4.1.2.4 The histological results of the liver after treated with a high concentration of ginger with warfarin

The important histological changes in the liver after treated the mice with both warfarin and a high concentration of watery ginger root extract showed the diameter nucleus of hepatocyte was(11.21±0.214 μm) (Table.4.4), which have significantly increased compared with the control group and warfarin treated group, but non-significant with the previous treated group, these changes included the parenchyma of the liver after treated have normal hepatic cords and normal elongated of hepatic cord arrangement through the liver parenchyma, normal duct, the kupffer cells have normal cellular proliferation(Fig.4.28), these results constant with (Akinyemi *et al*.,2013) who showed ginger have protective effect against oxidative stress.

The tissue section showed hepatocytes have acidophilic cytoplasm and prominent nucleus, the chromatin material appeared as dark granules which distributed peripherally in the nucleus, the tissue section didn't showed sever or prominent blood hemorrhage, no aggregation of inflammatory cells(Fig.4.29,30), these results were similar to control group. This result constant with (Haniadka *et al*., 2013) which explained ginger have protected action in the liver against the xenobiotic compound.

The histological results of the liver after treatment showed the hepatocytes normal distribution have hexagonal in shape, their cytoplasm has prominent vacuoles. The tissue field noted milled cellular proliferation besides the portal area, most tissue section of the liver didn't have prominent tissue degeneration and no prominent cystic dilation, compared with the previous group that treated with warfarin only(Fig.4.31), this results agreement with (El-Kott *et al*., 2010) who proved ginger extract has a significant protective effect on liver tissue against alloxan induced diabetic mellitus of rats.

The histological changes in the liver after treated the mice with both warfarin and a high concentration of watery ginger root extract showed normal central vein, normal elongated of sinusoids and the kupffer cells have normal cellular proliferation through sinusoids, the tissue section showed hepatocytes have prominent nucleus and the chromatin material which normal distributed in the nucleus(Fig.4.32) this results may be due to ginger is a potent antioxidant agent that can help reduce or eliminate the production of free radicals.

Table.4.4: Diameter of hepatocytes nuclei in liver in mice. µm

diameter / Treatment	Hepatocyte nucleus mean ±S.E
Control group	9.19 ± 0.208^{c}
Warfarin group	10.23 ± 0.336^{b}
Warfarin and low dose of ginger	11.57 ± 0.327^{a}
Warfarin and high dose of ginger	11.21 ± 0.214^{a}

*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.01).

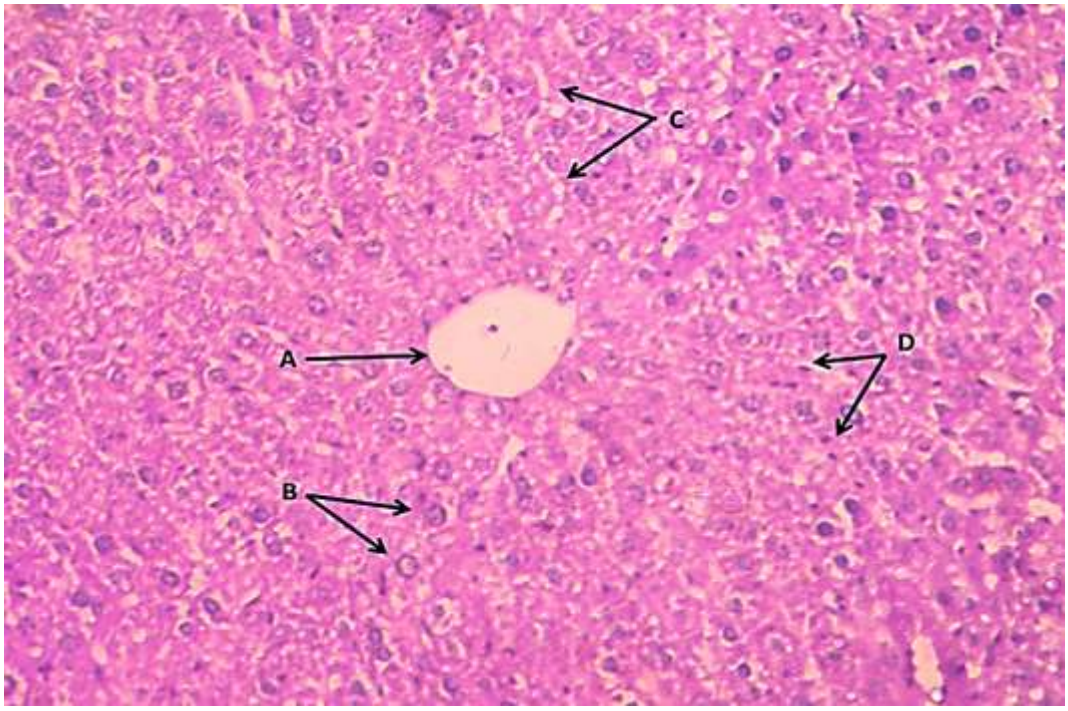


Fig.4.17 : Transverse section of liver in control group which showed A-Central vein, B-Hepatocytes C-Sinusoid D-kupffer cells. H&E stain 20X.

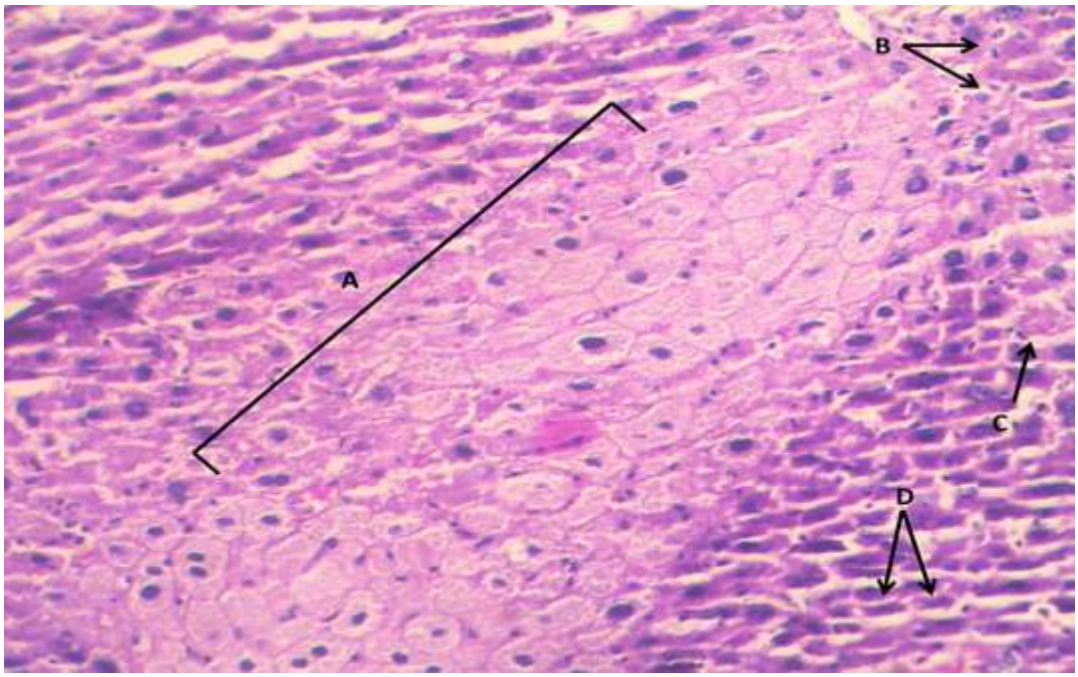


Fig.4.18 : Transverse section of liver after treated with warfarin which showed A-hepatocyte hypertrophy, B-proliferation of kupffer cells, C-necrosis, D-hepatic cord. **H&E** stain X40.

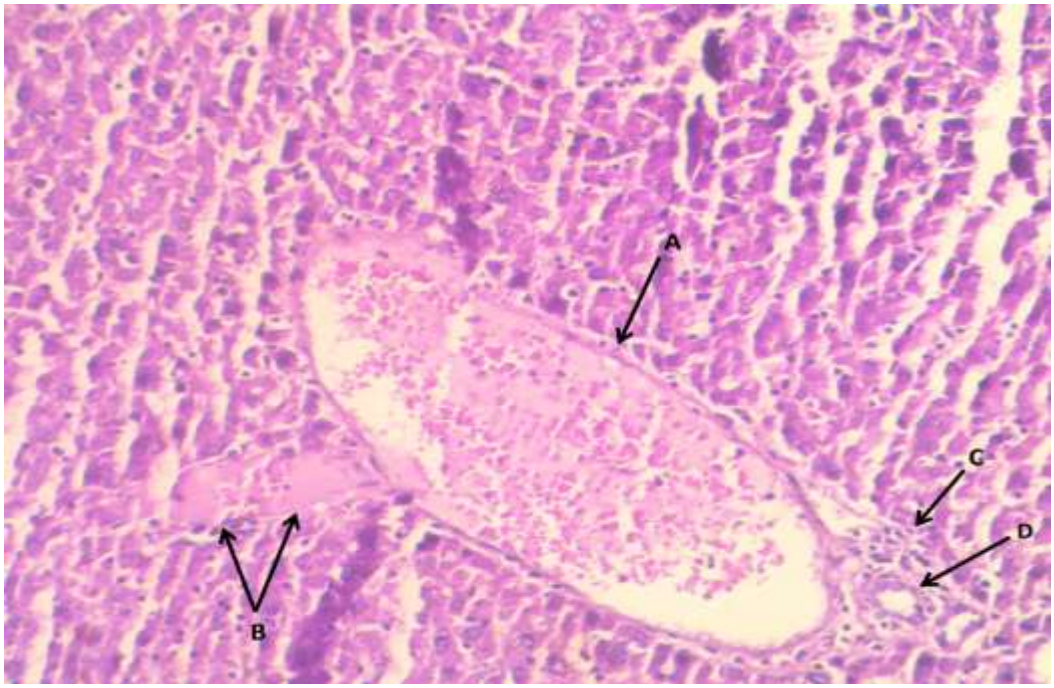


Fig.4.19 : Transverse section of liver after treated with warfarin which showed A-wide portal area, B- lesions of necrosis, C- abnormal inflammatory cells, D-bile duct. **H&E** stain X40.

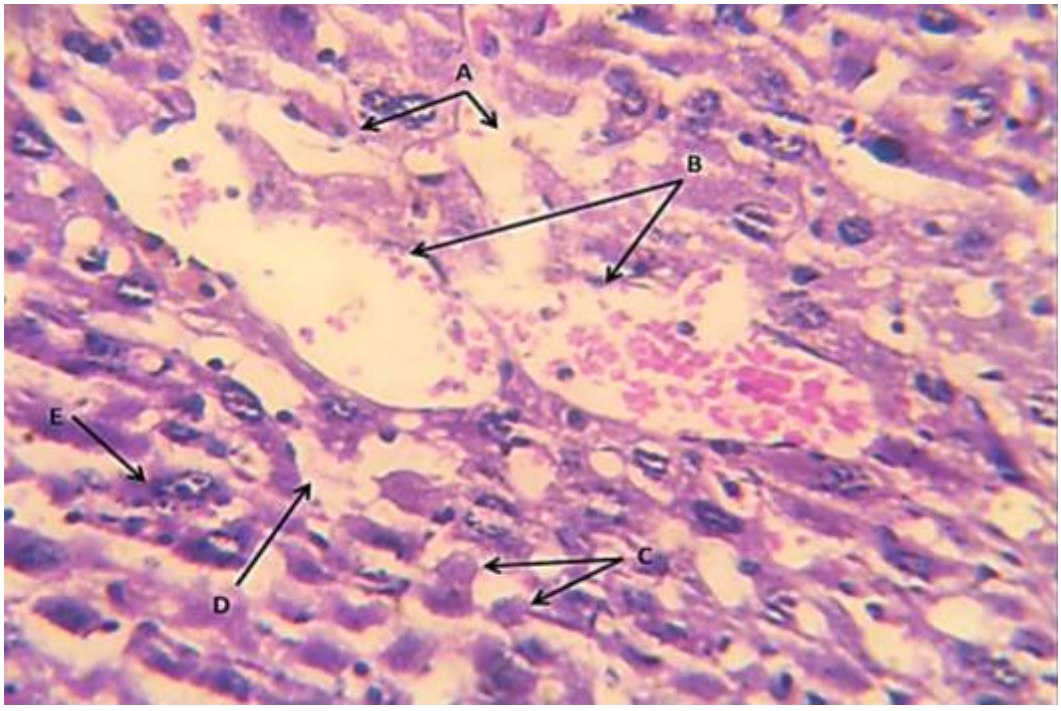


Fig.4.20 : Transverse section of liver after treated with warfarin which showed A-space filled with thick secretion, B-cystic dilation filled with blood, C-destroyed hepatocytes, D-inflammatory cells, E-necrosis. **H&E** stain X40.

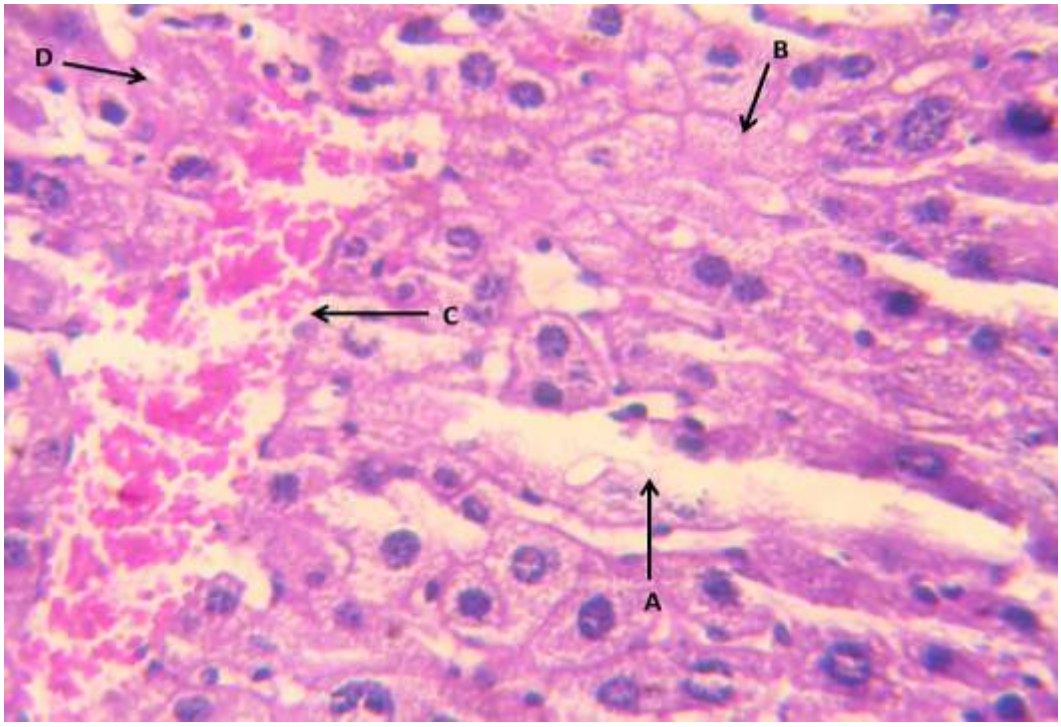


Fig.4.21 : Transverse section of liver after treated with warfarin which showed A- tissue degeneration, B-destroyed hepatocytes, C-blood hemorrhage, D-necrosis. **H&E** stain X40.

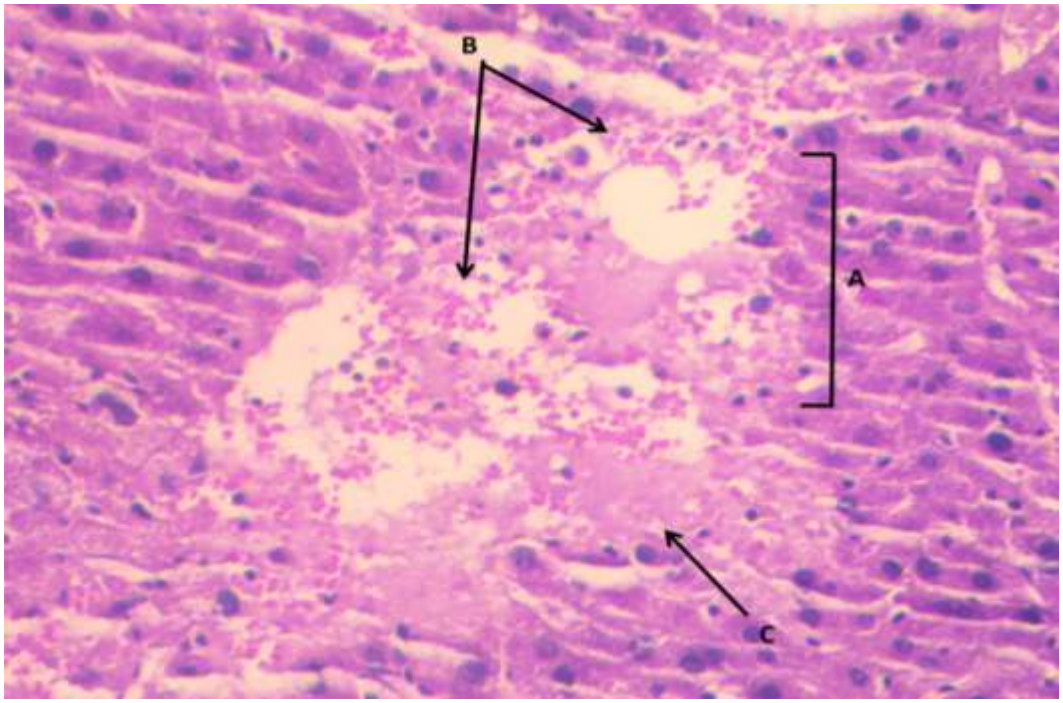


Fig.4.22 : Transverse section of liver after treated with warfarin which showed A-acute degeneration, B-hemorrhage, C-necrosis. **H&E** stain X40.

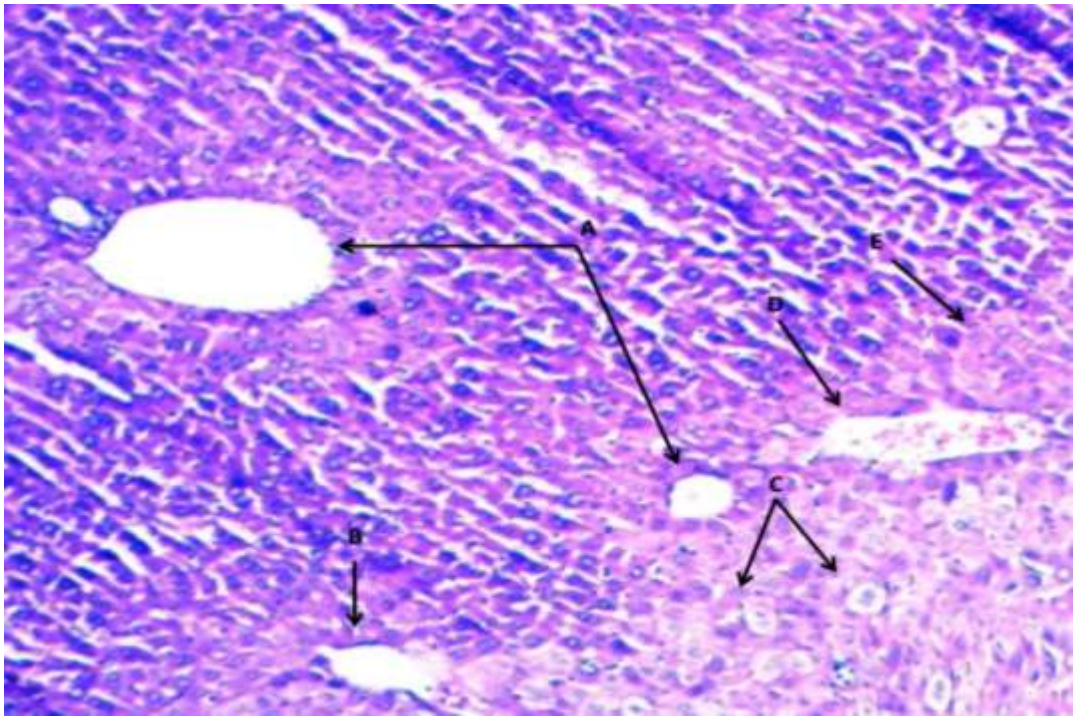


Fig.4.23 : Transverse section of liver after treated with low dose of ginger with warfarin which showed A- cystic dilation,, B-abnormal portal area, C-hepatocyte hypertrophy, D-hemorrhage, E-necrosis. **H&E** stain X20.

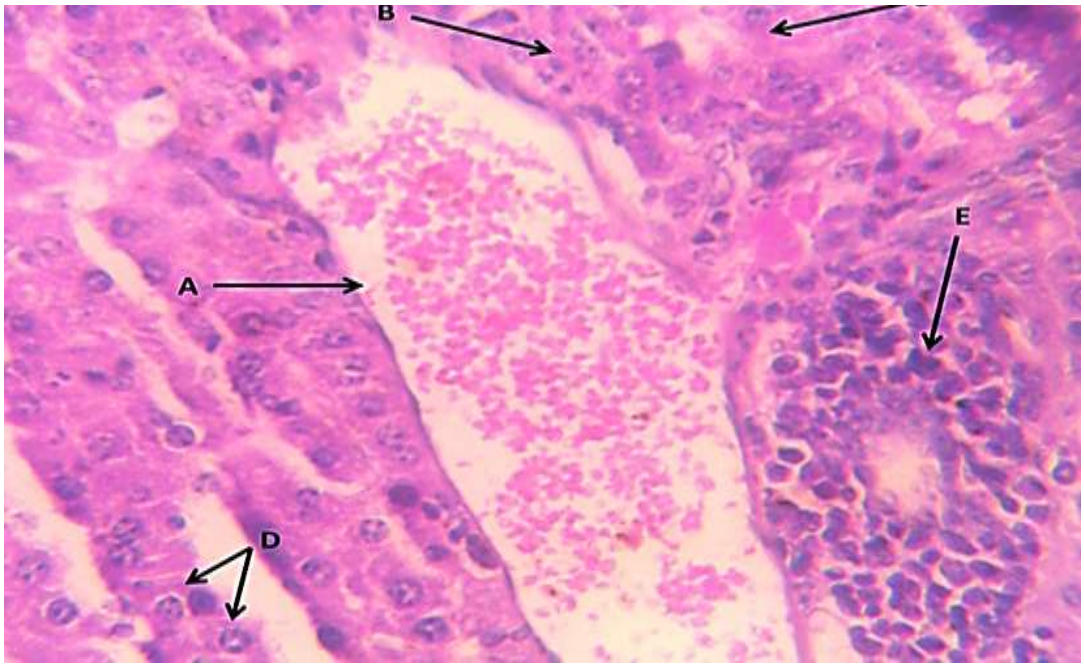


Fig.4.24 : Transverse section of liver after treated with low dose of ginger with warfarin which showed A- irregular cystic dilation filled with blood, B-hepatocytes, C-necrosis, D-different figure of hepatocyte in mitotic division, E-cellular proliferation of inflammatory cells. **H&E** stain X40.

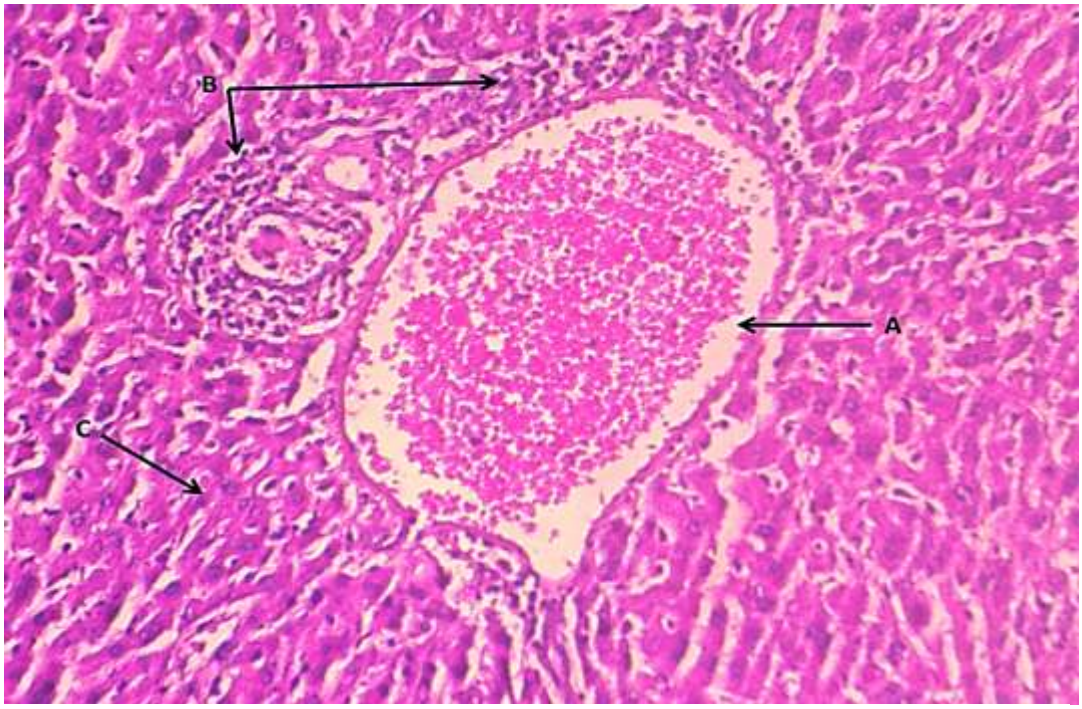


Fig.4.25 : Transverse section of liver after treated with low dose of ginger with warfarin which showed A- irregular portal area, B- cellular proliferation of inflammatory cells, C-necrosis. **H&E** stain X40.

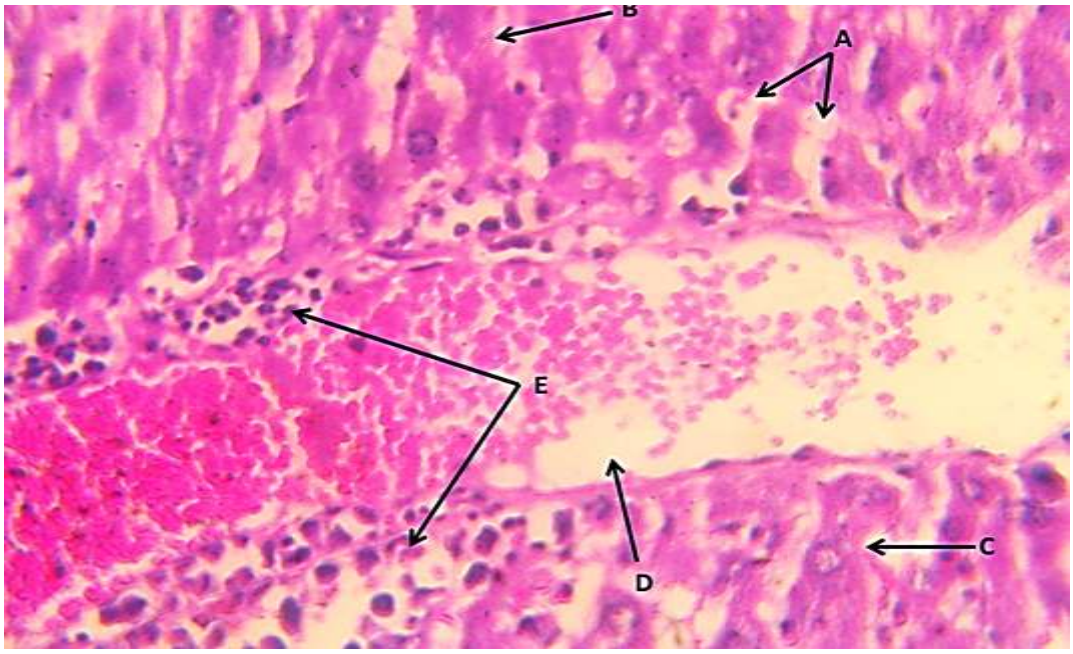


Fig.4.26 : Transverse section of liver after treated with low dose of ginger with warfarin which showed A- abnormal parenchyma space, B-hepatic cord, C-hepatic tissue destruction, D-blood vessels congestion, E-inflammatory cells. **H&E** stain X40.

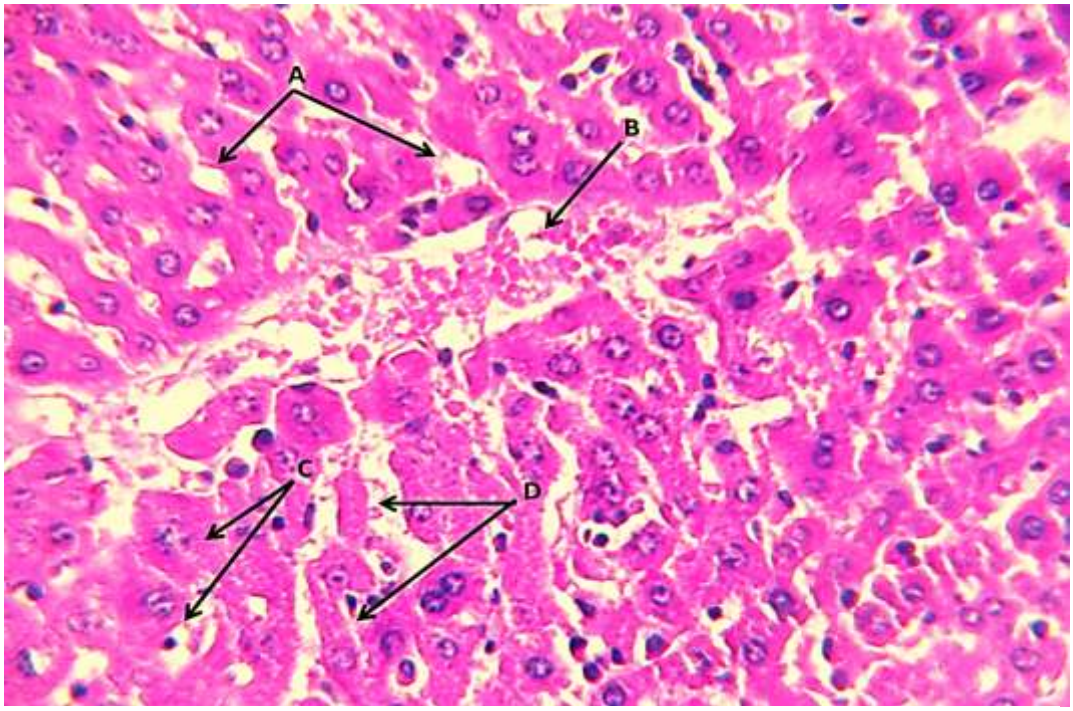


Fig.4.27 : Transverse section of liver after treated with low dose of ginger with warfarin which showed A- abnormal parenchyma space, B-hemorrhage, C-hepatocytes , D-hepatic cord. **H&E** stain X40.

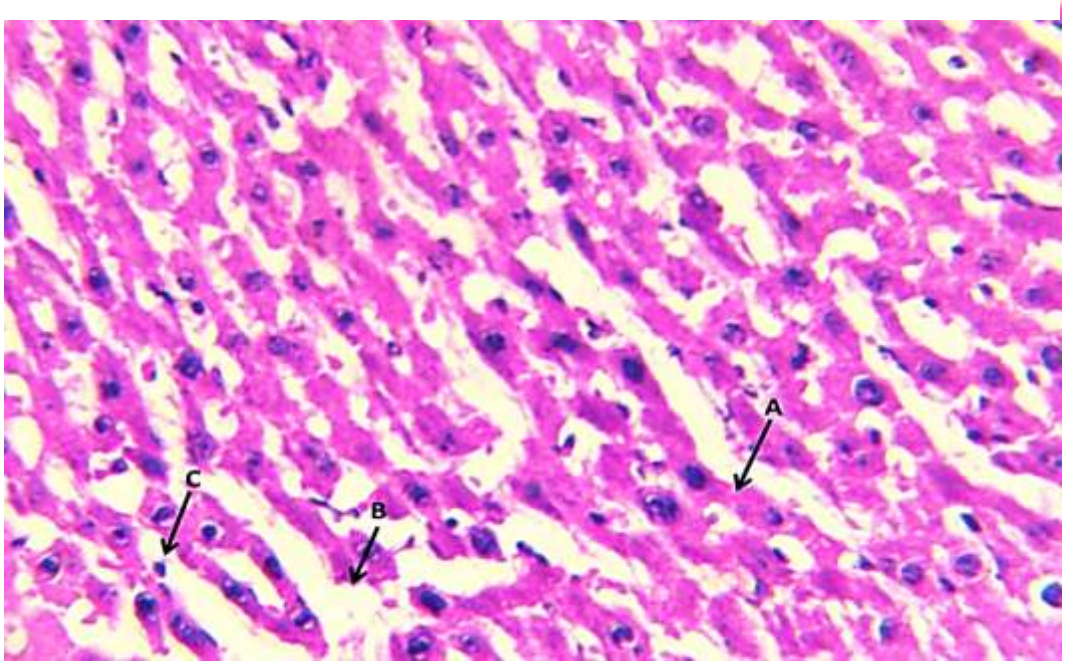


Fig.4.28 : Transverse section of liver after treated with high dose of ginger with warfarin which showed A- normal hepatic cord, B-normal duct, C-normal proliferation of kupffer cells. **H&E** stain X40.

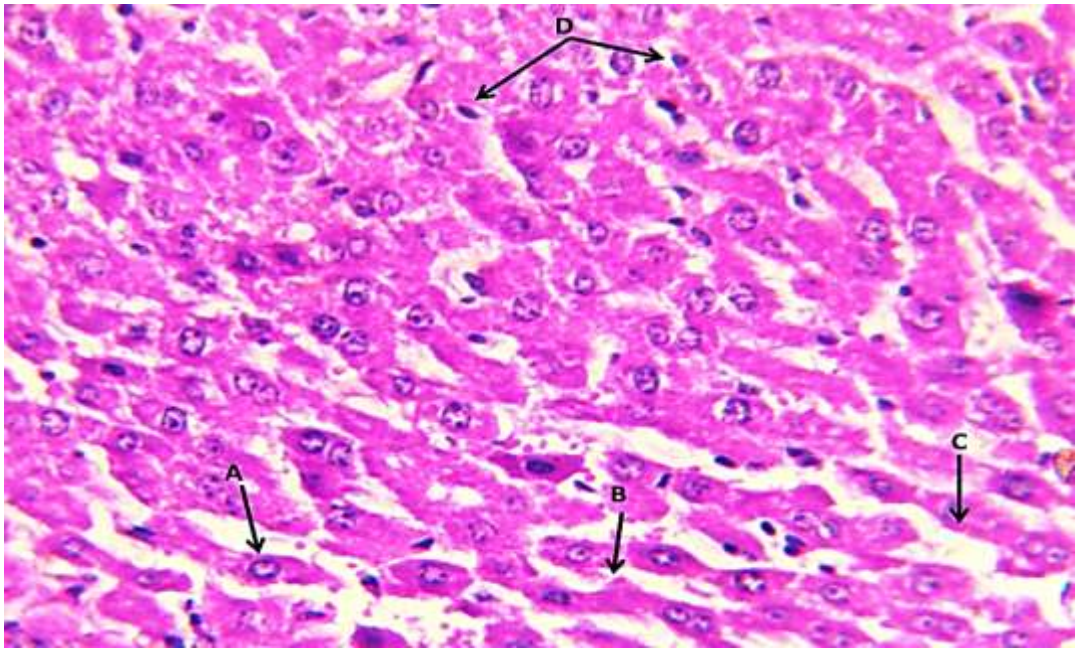


Fig.4.29 : Transverse section of liver after treated with high dose of ginger with warfarin which showed A- hepatocyte nuclei, B-normal hepatic cord, C-normal inter lobular duct, D-normal distribution of kupffer cells. **H&E** stain X40

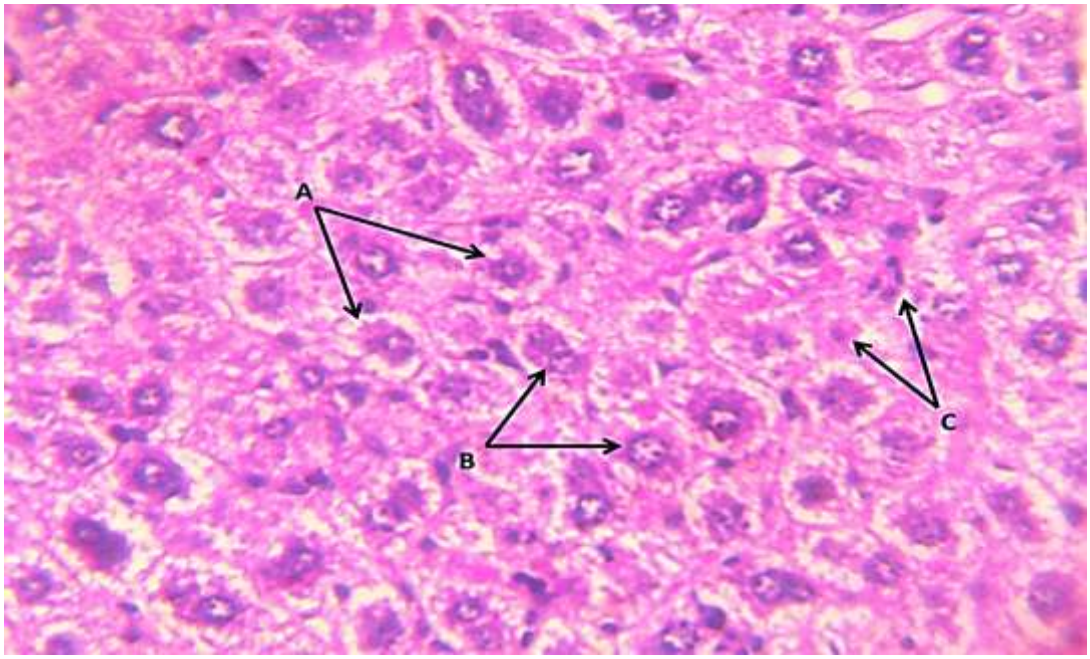


Fig.4.30 : Transverse section of liver after treated with high dose of ginger with warfarin which showed A- hepatocyte , B- hepatocyte nuclei, C- normal distribution of kupffer cells. **H&E** stain X40.

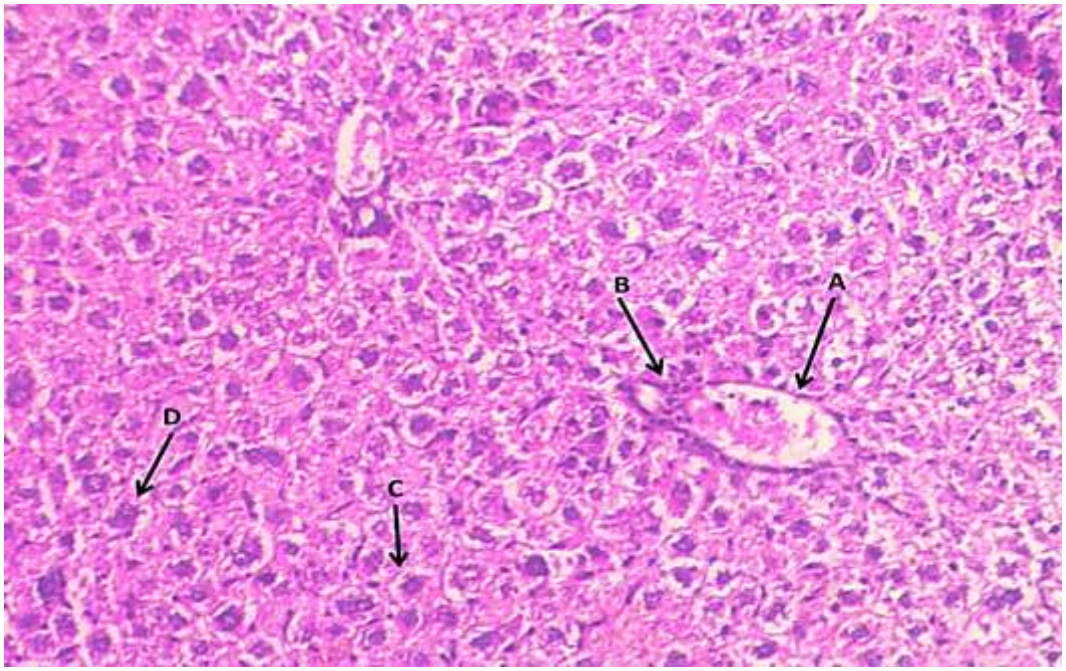


Fig.4.31: Transverse section of liver after treated with high dose of ginger with warfarin which showed A- portal area, B-cellular proliferation, C-normal hepatic tissue, D-normal distribution of hepatocyte. **H&E** stain X40.

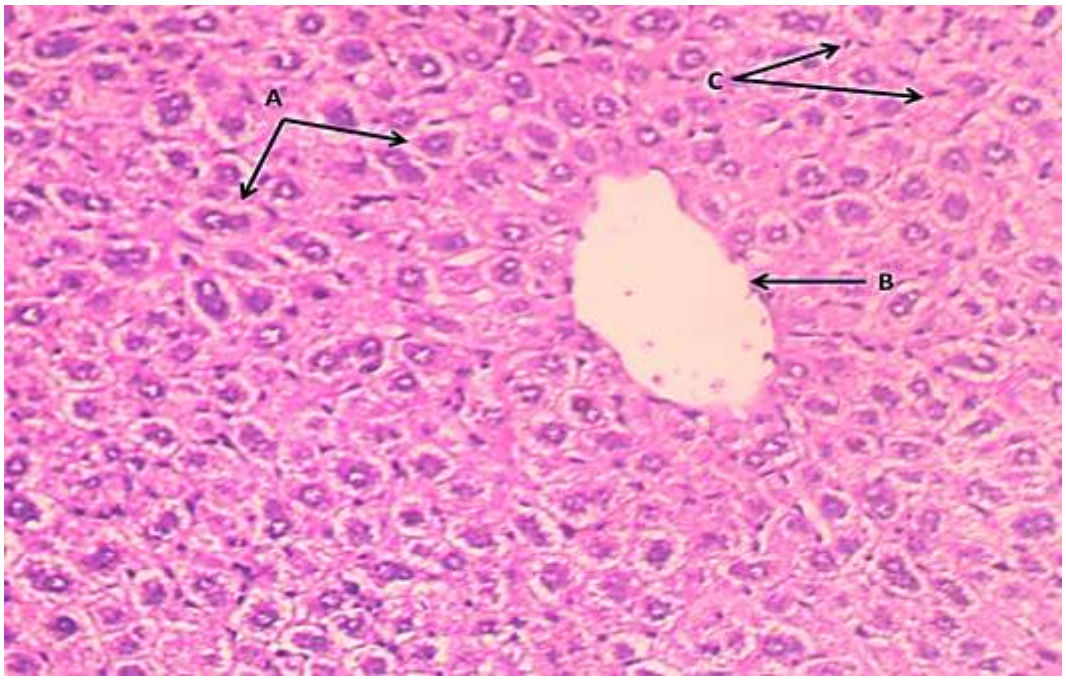


Fig.4.32 : Transverse section of liver after treated with high dose of ginger with warfarin which showed A- hepatocytes, B- central vein, C-normal of kupffer cells. **H&E** stain X40.

4.1.3 The Histological Results of blood vessels

4.1.3.1The histological results of the control group

The tissue section showed the thickness of the blood vessel wall was (9.85±0.153µm)(Table.4.5), the histological finding explained that the artery wall consists of the inner layer called tunica intima, which is a single layer of endothelial cells rest on the basement membrane. Tunica intima surrounded by a thin layer

composed of elastic fiber called internal elastic lamina, Tunica media was consist of smooth muscle cells as concentric muscle layers supported by elastic fibers. The outer layer is called tunica adventitia is composed of loose connective tissue and separated from under tunica media by external elastic lamina(Fig.4.33).

4.1.3.2 The histological results of blood vessels after treated with warfarin:

The histological findings of blood vessels wall noted the wall thickness was (7.94±0.284µm), which have significantly decreased compared with a control group, the tunica intima of blood vessels after treated with warfarin showed abnormal tunica intima with prominent calcification of the endothelial layer that lining the internal lumen of blood vessels, disappeared subendothelial layer under endothelia, the tissue section of arterial system noted clear exfoliated of internal endothelia. The tissue section of the blood vessels wall noted completely disappeared of the internal elastic lamina, the histological sections showed interaction or trading between tunica intima and tunica media without internal elastic lamina in the small artery (Fig.4.34,35). The incidence occurs may be because of toxicity of warfarin that lead to acute degeneration in layers of blood vessels wall, This results coincided with (Lerner *et al*., 2009) which proved that warfarin increased the prevalence of aortic valve calcium in patients using warfarin with atrial fibrillation.

The result appeared in the tissue section of blood vessels in the renal artery prominent completely isolated the tunica intima from tunica media in most tissue sections of blood vessels (Fig.4.36). The histological section of blood vessels in the small artery noted weak tunica intima in the wall of blood vessels, so noted interaction tunica intima with tunica media(Fig.4.37). This histological results similar to (Siltari and Vapaatalo, 2018) which said warfarin therapy causes vascular calcification characterized by an attack of inflammatory cells, accumulation of calcium and other minerals leading to a disturbance in the vascular endothelium and its regulatory role in arterial function.

The histological structures of blood vessels wall noted abnormal tunica media which characterized by dispersed most of the muscular layer, without normal arrangement as circular in shape, the smooth muscle cells have an elongated nucleus and dark in color, the tissue section showed isolated muscular layers from each other in the tunica media (Fig.4.36). these results may be due to the toxicity of warfarin that lead to degeneration of internal and external elastic lamina which leads to increased spaces between layers of blood vessels wall.

The tissue section noted appeared scattered smooth muscle cells in the tunica media and interaction with tunica adventitia(Fig.4.34,37), tissue section showed many abnormal irregular spaces in the tunica media, the result of renal artery wall didn't note prominent external elastic lamina between tunica media and tunica adventitia in the wall of a small artery in the treated groups compared with control group and noted prominent isolated between tunica media and tunica adventitia(Fig.4.35,38). This result coincided with (Liu *et al*., 2008) which said rats treated with warfarin therapy led to a rise in systolic blood pressure and aortic medial calcification, also noted that warfarin caused increase collagen but reduced elastin levels in the aorta.

The histological result of tunica adventitia in blood vessels walls of the treated group with warfarin noted dispersed tunica adventitia, the tissue section showed wide space in the tunica adventitia and in the other site completely disappeared small part of tunica adventitia, the tissue section of the renal artery wall noted irregular shape of fibers and appeared as small pieces which have abnormal distributed in the tunica adventitia and noted some of the inflammatory cells aggregated between the small pieces of destructed fibers in tunica adventitia, these findings were abnormal when compared with the control group(Fig.4.36). This result coincided with **(**Elantably *et al*., 2020) which noted warfarin induced leukocytoclastic vasculitis, lead to increase skin lesions, medication-induced leukocytoclastic vasculitis can affect multiple organ systems and even cause death.

The histological result noted the tunica adventitia in blood vessels wall after treated with warfarin noted wide space in the tunica adventitia, tissue section showed hemorrhage and bloody congestion in the tunica adventitia(Fig.4.37,38). These histological changes may be due to the distraction of internal and external elastic lamina which leads to an increase the spaces between the histological layers that composed of the blood vessels wall. This result constant with (Pautas *et al*.,2006) who said most of the bleeding associated with the use of warfarin occurs in the soft tissues and most extreme gastrointestinal hemorrhage.

4.1.3.3The histological results of blood vessels after treated with a low concentration of ginger with warfarin:

The histological results noted the wall thickness of blood vessels after treatment with both the low concentration of ginger and warfarin have a significant decrease in diameter with (8.50±0.335bμm)(Table4.5), compared with the control group, and non-significant with warfarin treated group. The histological result of blood vessel

wall after treatment with warfarin and watery ginger root extract showed the wall was similar to the blood vessels wall in the treated group with warfarin only.

The histological finding of tunica intima of the renal artery didn't have prominent structure, so have prominent damage in endothelial and sub endothelial layers that composed of tunica intima, the histological section of the small artery wall didn't show normal internal elastic lamina that separated between tunica intima and tunica media, the tissue section showed prominent exfoliated endothelial cells from internal layer that lining the internal surface of the renal artery, the tissue section showed no prominent separated between tunica intima and tunica media in the wall of the small artery(Fig.4.39). Tissue section (Fig.4.41) noted prominent destruction in the tunica intima and disappeared endothelial cells, the histological structures of tunica intima were abnormal in structure compared with control group, these result were similar of something to the histological findings in the wall of the small artery in the treated group with warfarin only. These results may be according to (Stary *et al* ., 1995) who noted ruptured plaques have increased the frequency of interruption of the internal elastic laminate, and the destruction of the elastic fibers has been associated with increased arterial stiffness and distension.

The tissue section in the blood vessels wall (Fig.4.39,40) have abnormal muscular layers that composed the tunica media, which appeared as scattered smooth muscle cell have pale nuclei while other cells didn't have prominent nuclei, the histological structure of tunica media was similar to the tunica media in the treated group with warfarin only. So the result showed didn't found prominent external elastic lamina which separated between the tunica media and tunica adventitia, the tissue section showed abnormal thin tunica media and prominent discontinues of the external elastic lamina. These results may be according to (Price *et al*.,2006) who improved warfarin induce focal calcification of elastic lamellae in the media of major arteries and aortic heart valves in rats (Fig.4.41).

The tissue section in the wall of the small artery noted tunica adventitia have prominent histological changes with prominent dens fibrous tissue without clear external elastic lamina, the tunica adventitia was combined with under tunica media, these findings were similar to something treated with warfarin only (Fig.4.39,40). The degree of damage increases as a result of receiving warfarin therapy, this is results may be according to (Helin *et al*., 2014) who showed that long term anticoagulation therapy with warfarin can induce vascular calcification.

4.1.3.4 The histological results of blood vessels after treated with a high concentration of ginger with warfarin:

The histological finding appeared the blood vessels wall thickness have prominent width with diameter was (10.24±0.176µm) (Table4.5), which non-significant when compared with the control group, but have significant increased compared with warfarin treated group and with the group that treated with both low concentration of ginger and warfarin. The histological result of blood vessels wall after treated with both warfarin and watery ginger root extract showed the wall of small artery some the histological changes compared with previously treated groups, the tissue section of small artery wall showed the tunica intima was normally and have prominent endothelial layer belong the internal surface of the small artery, the tissue section didn't note prominent degeneration in the tunica intima compared with warfarin treated group, the histological section showed prominent thin internal elastic lamina under the tunica media which separated between tunica intima and tunica media, this result similar to something as histological findings in control group (Fig.4.42,44). These results agreement with (Hosseinzadeh *et al*., 2017) who showed ginger extract enhanced the expression of many antioxidant enzymes and decreased the production of lipid peroxidation.

The histological result of blood vessels walls noted the tunica media have prominent thick tunica media with prominent muscular layer arrangement with small circular space, the tunica media was separated from tunica intima and tunica adventitia, the tissue section of the small artery wall have prominent external elastic lamina that separated between the tunica media and tunica adventitia, this findings were similar to control group but were completely different of the treated group with warfarin only (Fig.4.42,43,44). These results may be according to (Fuhrman *et al*., 2000) who demonstrated that the anti-atherosclerotic activity of ginger is attributed to its antioxidant effect, which defends smooth muscle cells in the media against oxidative damage in atherosclerosis.

The histological result of the artery wall noted have normal tunica adventitia that composed of loose connective tissue without any histological changes, the tissue section of the blood vessel wall showed the tunica adventitia was appeared as a thin band completely separated from under tunica media by prominent external elastic lamina, the histological result of tunica adventitia didn't note any inflammatory cells aggregation (Fig.4.42,43). These results agreement with (Kamel and El-rab, 2017) which explained the aorta of the rabbit in the group treated with ginger have prominent tunica intima and intact of the smooth muscle cell in the tunica media, so, noted intact tunica adventitia that made of loose connective tissue. These results may be due to according to (Fuhrman *et al*., 2000) who proved ginger have an antioxidant

beneficial effect on macrophage and protect smooth muscle cell in tunica media against the oxidative damage.

Table.4.5: Diameter of blood vessels wall of thickness in mice. µm

Treatment \ diameter	blood vessels wall of thickness mean ±S.E
Control group	9.85±0.153[a]
Warfarin group	7.94±0.284[b]
Warfarin and low dose of ginger	8.50±0.335[b]
Warfarin and high dose of ginger	10.24±0.176[a]

*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.01).

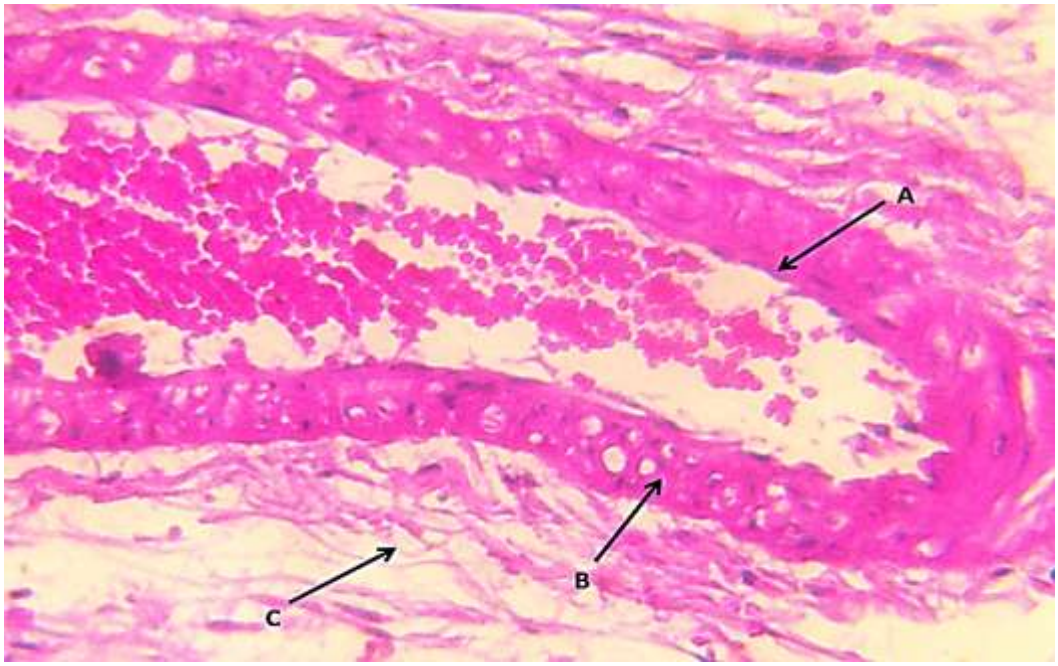


Fig.4.33 : Transverse section of blood vessels in control group which showed A- tunica intima, B-tunica media, C-tunica adventitia. H&E stain 40X.

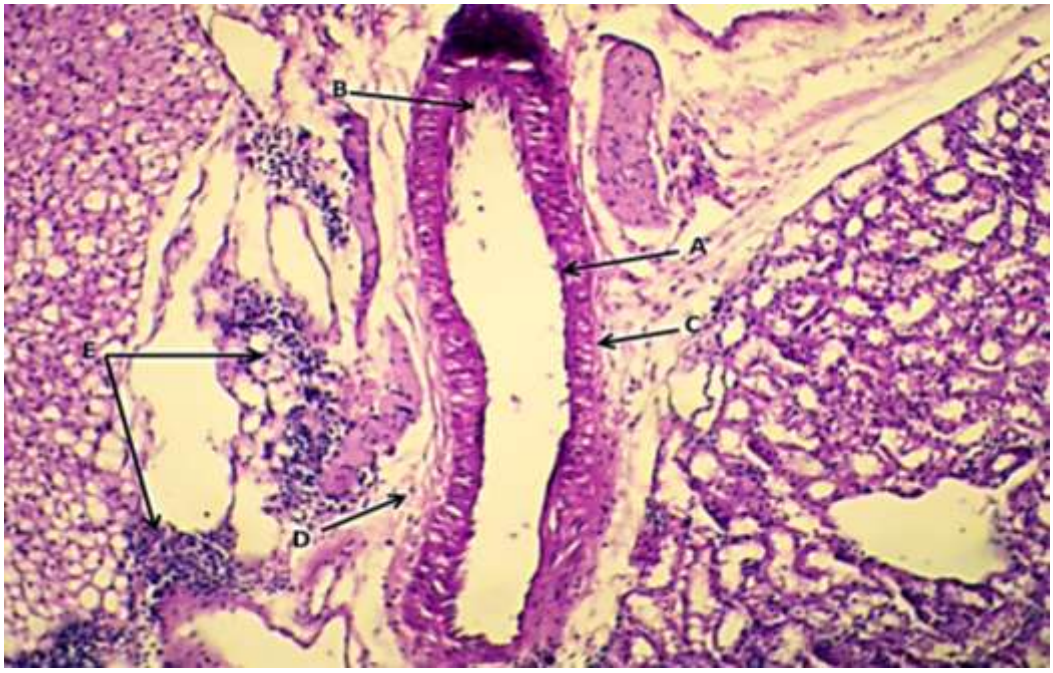


Fig.4.34 : Transverse section of blood vessels after treated with warfarin which showed A-abnormal tunica intima, B-calcification of endothelial layer, C-tunica media, D-tunica adventitia, E-inflammatory cells. **H&E** stain X40.

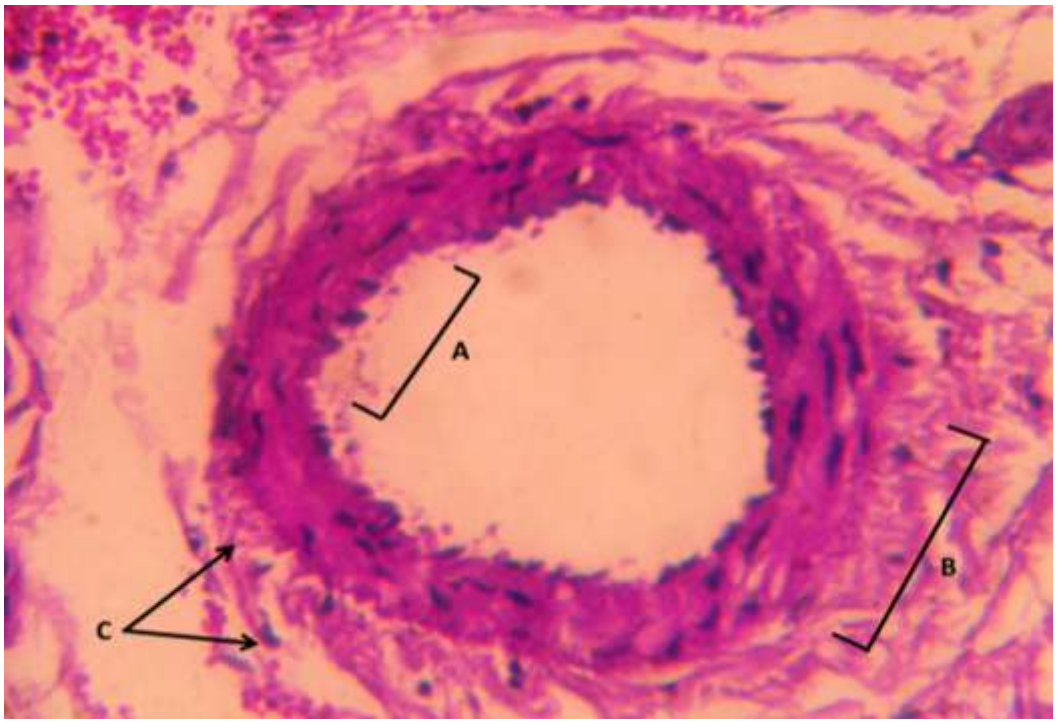


Fig.4.35 : Transverse section of blood vessels after treated with warfarin which showed A-abnormal endothelial cells, B-interaction tunica media with tunica adventitia , C- abnormal tunica adventitia. **H&E** stain X40.



Fig.4.36 : Transverse section of blood vessels after treated with warfarin which showed A-isolated tunica intima, B- thin tunica media, C-tunica adventitia, D-cystic dilation, E-cellular proliferation. **H&E** stain X40.

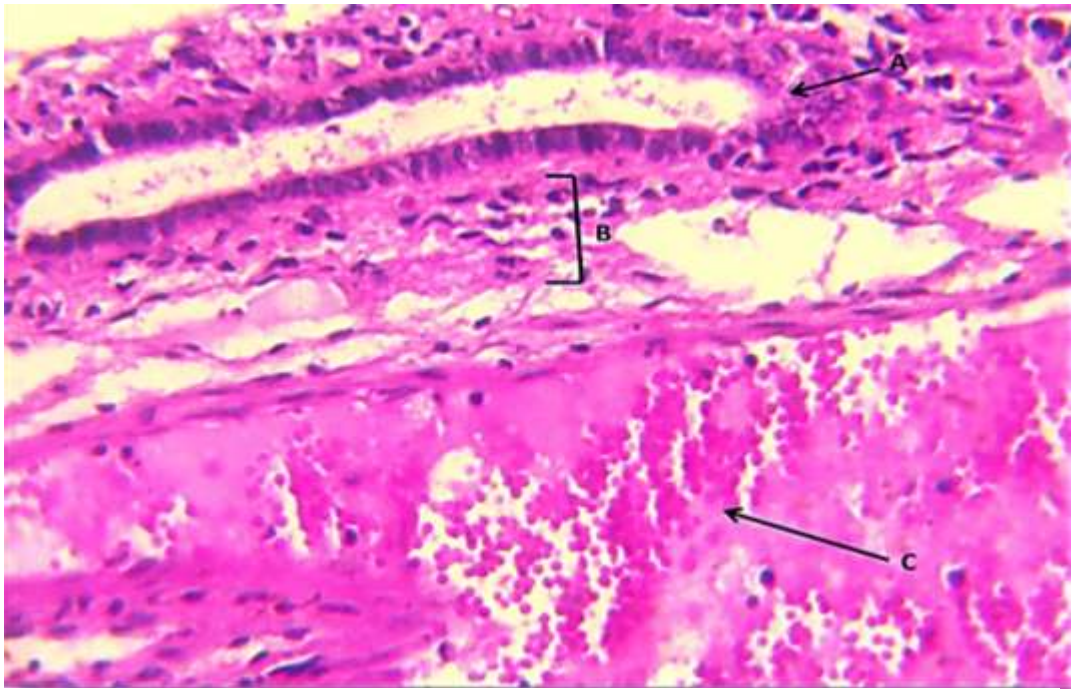


Fig.4.37 : Transverse section of blood vessels after treated with warfarin which showed A- degeneration in tunica intima, B-scattered smooth muscle cells introduction with tunica adventitia , C-hemorrhage. **H&E** stain X40.

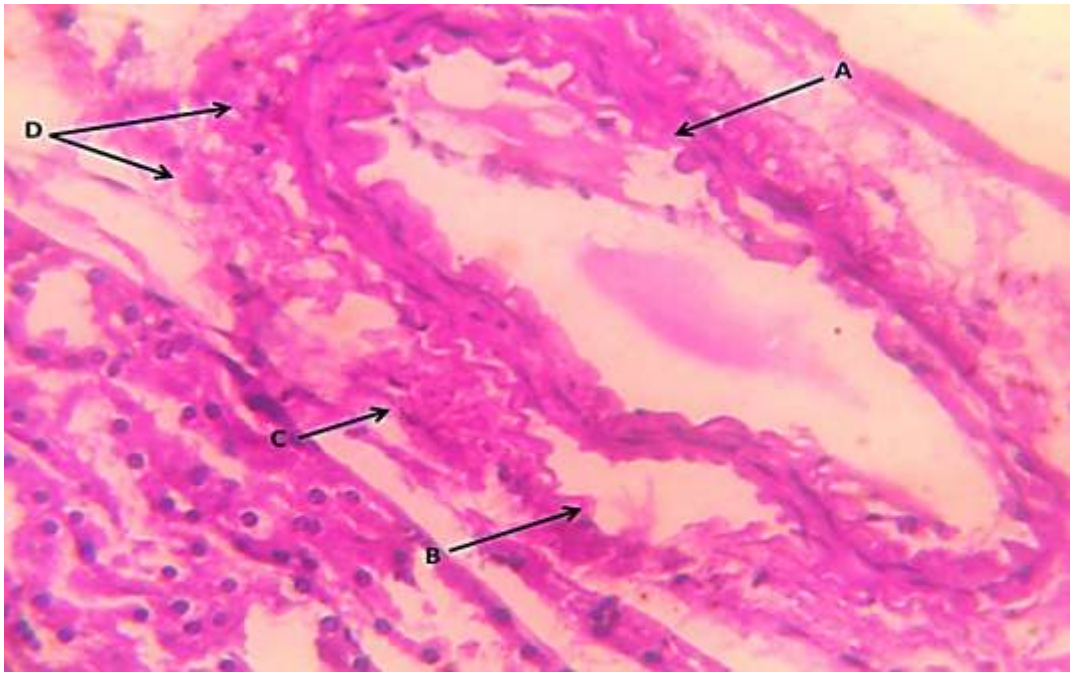


Fig.4.38 : Transverse section of blood vessels after treated with warfarin which showed A- destruction in tunica intima, B-wide space between tunica media and tunica adventitia, C-hemorrhage, D-bloody congestion in tunica adventitia. **H&E** stain X40.

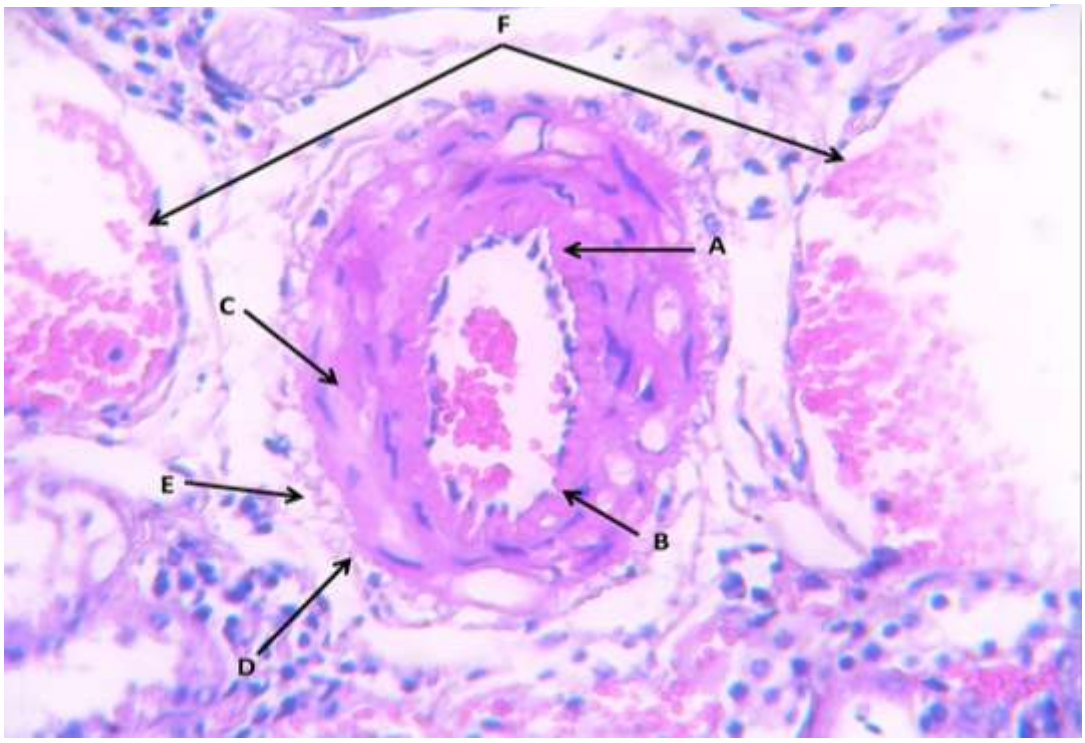


Fig.4. 39 : Transverse section of blood vessels after treated with low dose of ginger with warfarin which showed A- tunica intima, B-no founded internal elastic lamina, C-tunica media, D-no clear external elastic lamina, E-abnormal tunica adventitia, F- veins . **H&E** stain X40.



Fig.4.40 : Transverse section of blood vessels after treated with low dose of ginger with warfarin which showed A- destruction tunica intima, B-exfoliated endothelial layer, C-abnormal tunica media, D-no clear external elastic lamina, E-tunica adventitia. **H&E** stain X40.

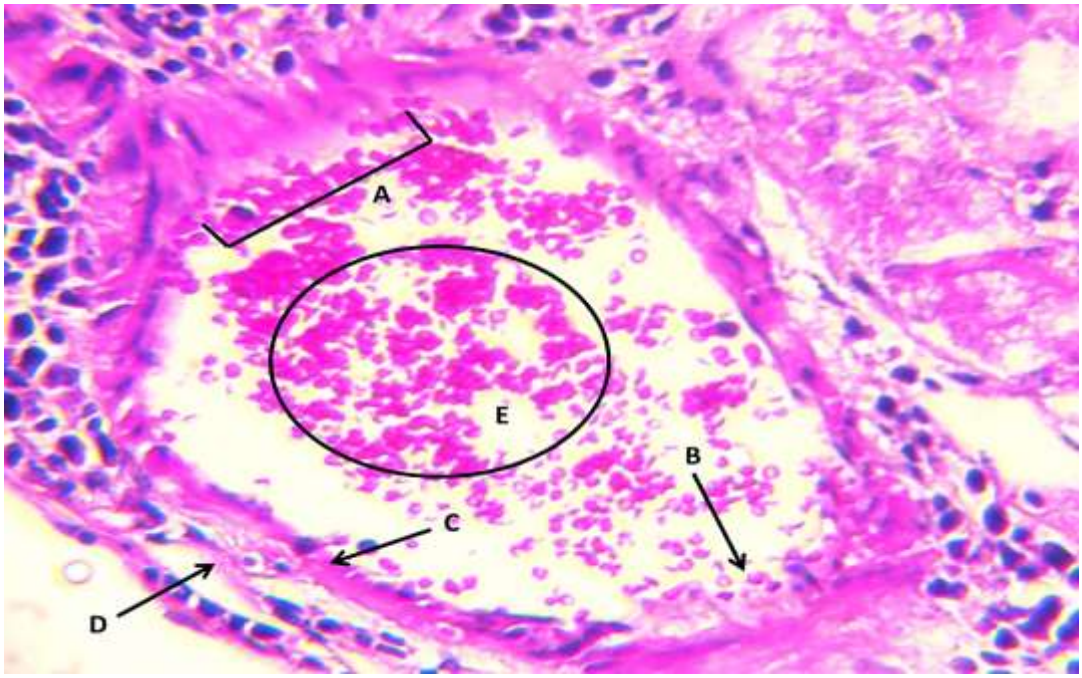


Fig.4.41 : Transverse section of blood vessels after treated with low dose of ginger with warfarin which showed A- disappeared endothelial cells, B-degeneration tunica intima, C-thin tunica media, D-discontinuous external elastic lamina, E-blood congestion. **H&E** stain X40.

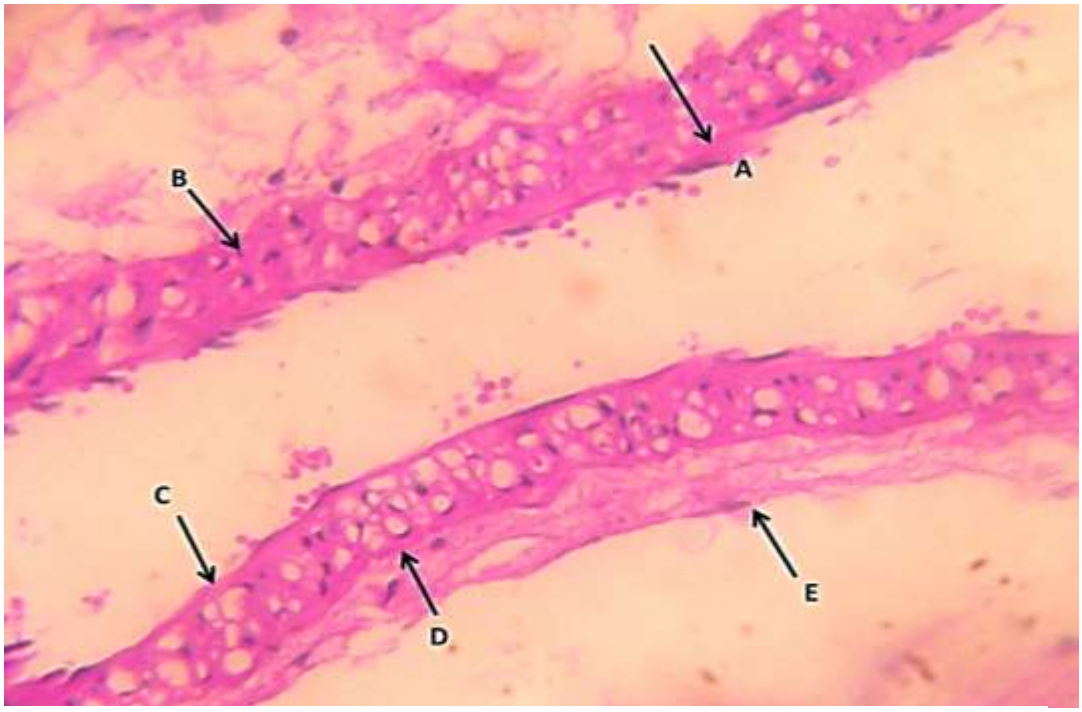


Fig.4.42 : Transverse section of blood vessels after treated with high dose of ginger with warfarin which showed A- prominent tunica intima, B-tunica media, C-prominent internal elastic lamina, D-normal external elastic lamina, E-normal tunica adventitia. **H&E** stain X40.

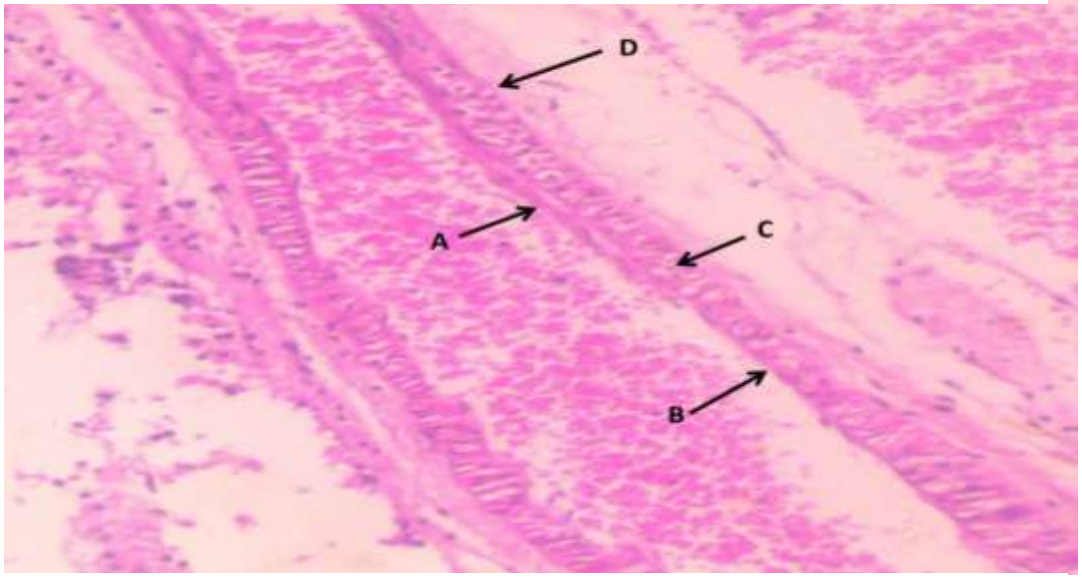


Fig.4.43: Transverse section of blood vessels after treated with high dose of ginger with warfarin which showed A- prominent tunica intima, B-prominent internal elastic lamina, C-tunica media, D-normal external elastic lamina E-tunica adventitia. **H&E** stain X40.



Fig.4.44 : Transverse section of blood vessels after treated with high dose of ginger with warfarin which showed A- prominent tunica intima, B-endothelial layer, C-prominent internal elastic lamina, D-tunica media, E-normal external elastic lamina. **H&E** stain X40.

4.2 The Biochemical results

4.2.1 The Biochemical results of Urea

(Table 4.6) explained the normal proportion of urea in the serum of a control group was (34.69±0.100 mg/dl), the value of urea in treated mice with warfarin only after 30 days was (135.17±0.136 mg/dl), the level of urea in the groups that treated with both warfarin and a low dose of watery ginger root extract was (60.67±0.370 mg/dl), while the urea concentration in the serum of group that treated with both warfarin and the high dose of watery ginger root extract was (28.68±1.942 mg/dl). The statistical study noted the level of urea in the treated group with warfarin have significant increased compared with the control and treated group with a high dose of ginger root extract, while no significant differences compared with group that treated with a low dose of ginger root extract.

Urea is the main end product of the synthesis of protein nitrogen, it is synthesized by the urea cycle in the liver from ammonium formed by amino acid deamination, urea is mostly excreted by the kidneys, blood urea nitrogen determination is the most commonly used diagnostic procedure for renal function, increases in blood urea nitrogen concentrations are seen in insufficient renal perfusion, shock, reduced blood flow (pre renal causes), chronic nephritis, nephron sclerosis, tubular necrosis, glomerular nephritis (renal causes) and urinary tract obstruction (post renal causes)(Rock et al., 1987).

The significant increase in urea level of experimental groups that treated with both warfarin and low dose of ginger root extract and warfarin only compared with the control group, which may be due to the warfarin toxicity that leads to these physiological deference's in the urea values which may be causes kidney dysfunction (injury in the kidney), this result constant with(Ishii, 2018) which noted blood urea increase as a result of receiving warfarin treatment.

Physiological changes after treated with both high dose of ginger and warfarin constant with (Mehrdad *et al*., 2007) who reported the mice receiving ginger extract that lead higher rate of urea excretion in the kidney, this because ginger have properties anti-free radicals abilities and exhibit antioxidants activity it may stimulate the liver action and urea synthesis.

Table.4.6: Levels of serum urea, in mice (mg/dl).

Parameter / Treatment	Urea mean ±S.E
Control group	34.69±0.100^{c}
Warfarin group	135.17±0.136^{a}
Warfarin and low dose of ginger	60.67±0.370^{b}
Warfarin and high dose of ginger	28.68±1.942^{d}

*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.01).

4.2.2 The Biochemical Results of Creatinine

Physiological results in (Table 4.6), noticed the normal Creatinine value in the serum of control group was(0.362±0.0076 mg/dl), the level of creatinine in treated group with warfarin only was (0.457±0074 mg/dl), the level of Creatinine in the groups that treated with both warfarin and a low dose of watery ginger root extract was(0.445±0.0060 mg/dl), while the creatinine value in the group that treated with both warfarin and a high dose of watery ginger root extract was (0.370±0.0080 mg/dl).

Creatinine is a break-down product of creatine phosphate in the muscle, and is usually produced at a fairly constant rate by the body, it is freely filtered by the glomeruli and under normal conditions, is not reabsorbed by the tubules. The increase in blood creatinine is found only with marked injury to the nephrons. (Fabiny and Ertinghausen, 1971).

The statistical study noted the level of creatinine in the treated group with warfarin only has significantly increased compared with both the control group and treated group with a high dose of ginger root extract. A high level of creatinine in the treated group with warfarin may be due to the toxicity of warfarin that leads to damage in renal corpuscle that effected on the infiltration rate, or the degeneration in tubules that leads to an increase in the reabsorption of creatinine from renal tubules. This physiological change after treated with warfarin only was similar to (Ozcan *et al*., 2012) which explained the increase in the concentration creatinine level in the rats with increasing the dose of warfarin. While the creatinine value no significant differences with the group that treated with both warfarin and a high dose of ginger root extract. This result coincide with (Ajith *et al*., 2007) who noted that the presence of flavonoids and polyphenols in the ginger extract may be responsible for the antioxidant nephron protective function and decreased levels of serum creatinine and urea.

Table.4.7: Levels of serum creatinine in mice (mg/dl).

Parameter / Treatment	Creatinine mean ±S.E
Control group	0.362±0.0076[b]
Warfarin group	0.457±0074[a]
Warfarin and low dose of ginger	0.445±0.0060[a]
Warfarin and high dose of ginger	0.370±0.0080[b]

*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.01).

4.2.3 The Biochemical Results of AST

The statistical results in (Table4.7), noted that the level of AST in the control group was (359.98±8.847 U/L), the physiological result showed the AST value in the treated group with warfarin only was (1121.85±51.831 U/L), the level of AST in the group that treated with both warfarin and a low dose of watery ginger root extract was(548.00±4.587 U/L), while the AST level in the group that treated with both warfarin and a high dose of watery ginger root extract was (411.30±14.807 U/L).

The enzyme aspartate aminotransferase (AST) is widely distributed in tissue, mainly hepatic, cardiac, muscle, and kidney, elevated serum levels are found in

diseases involving these tissues, liver diseases, such as cirrhosis, metastatic carcinoma, and viral hepatitis (Moss *et al*., 1987).

The statistical results noted the level of AST in the treated group with warfarin only have significantly increased compared with the control group and as well as group that treated with warfarin and a high dose of ginger root extract, so the AST value have significantly increased compared with the group that treated with both warfarin and a low dose of ginger root extract.

The high significant increase in the levels of AST enzymes after treatment with warfarin in mice, that may be occur due to the toxic effect of warfarin on hepatic tissue, and degeneration of hepatocytes that lead to the release the AST enzymes from the cytoplasm that lead to increase in this enzyme. The physiological results in treated group with both high doses of watery ginger root extract with warfarin constant with (Ismail and Attyah, 2012) who said ginger extract as used concomitantly with cisplatin protects the liver and heart from the toxicity of this cytotoxic drug due to the antioxidant properties of ginger.

Table.4.8: Levels of serum AST, in mice (U/L).

Parameter / Treatment	AST mean ±S.E
Control group	359.98±8.847^{c}
Warfarin group	1121.85±51.831^{a}
Warfarin and low dose of ginger	548.00±4.587^{b}
Warfarin and high dose of ginger	411.30±14.807^{c}

*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.01).

4.2.4 The Biochemical Results of ALT

(Table4.9) appeared that the level of ALT in the control group was (29.21±0.994 U/L)), the physiological result of the current study showed the ALT value in treated group with warfarin only was (436.45±6.385 U/L). While the level of AST in the groups that were treated with both low and high doses of watery ginger root extract with warfarin, individually was (72.14±6.657 U/L) (58.67±1.717 U/L) respectively.

The enzyme alanine aminotransferase (ALT) present in a variety of tissues, the main source of ALT is the liver, which has led to the measurement of ALT activity for the diagnosis of hepatic diseases, elevated serum ALT is found in hepatitis,

cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse (Moss *et al*., 1987).

The high significant increase in the levels of ALT after treatment with warfarin in experimental animals, that may be occur due to the acute degeneration in hepatic tissue, and the death of the hepatocytes because of the warfarin toxicity that lead to release the hepatic enzymes from broken hepatocytes. This physiological finding similar to (Hahn *et al*., 2015) which explained anticoagulant medications cause an increase in the level of liver enzymes**.**

The physiological results in the treated group with both high doses of watery ginger root extract with warfarin referred to the role of a high dose of ginger root extract in reduced the warfarin side effects on the level of ALT in the treated group compared with the control group.

Table.4.9: Levels of serum ALT, in mice (U/L).

Parameter / Treatment	ALT mean ±S.E
Control group	29.21±0.994[d]
Warfarin group	436.45±6.385[a]
Warfarin and low dose of ginger	72.14±6.657[b]
Warfarin and high dose of ginger	58.67±1.717[c]

*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.01).

4.2.5 The Biochemical Results of potassium

The current study in (Table 4.10), explained the potassium value in the control group was (8.21±0.273mm/L). the potassium level in the treated group with warfarin only was (10.00±0.101mm/L), While the level of potassium in the groups that treated with both low and high doses of watery ginger root extract with warfarin individually was (10.65±0.214mm/L) (11.09±0.184mm/L) respectively.

The potassium increased in the serum after treated with warfarin only may be as a result of the warfarin leads to raise the rate of riches-in riches-out calcium-potassium which lead to release the potassium in the blood and causes blood vessels calcification. While potassium level increased in treated groups with both low and high doses of ginger root extract and warfarin that because of the ginger root have a high amount of potassium which lead to significant increase in potassium level. These results may be due to that ginger contained an amount of potassium at the rate of (410.91±13.97)(Tanweer *et al*., 2014). this agreement with (Sun *et al*., 2017) who proved increased dietary potassium (2.1%) reduced vascular calcification and aortic stiffness.

Table.4.10: Levels of serum potassium, in mice (mm/L).

Parameter / Treatment	Potassium mean ±S.E
Control group	8.21±0.273[c]
Warfarin group	10.00±0.101[b]
Warfarin and low dose of ginger	10.65±0.214[a]
Warfarin and high dose of ginger	11.09±0.184[a]

*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.01).

4.2.6 The Biochemical Results of calcium

The level of calcium in the serum of control group was (9.03±0.066 mg/dl) (Table 4.11), the level of calcium in treated group with warfarin only was (9.49±0.069 mg/dl), biochemical findings of calcium after treated with both low and high doses of ginger root extract with warfarin individually were (9.59±0.081 mg/dl), (10.04±0.134 mg/dl) respectively.

The physiological results noted a significant decrease in the level of calcium in the treated group with warfarin only, which may be a result of to the role of warfarin in the calcification which lead to a decrease in the level of calcium in the blood, This results constant with (Helin *et al*., 2014) show that long term with warfarin therapy can promote vascular calcification and lowered calcium levels in the blood.

While the level of calcium increased in the serum when treated with high dose of watery ginger root extract may be because of the role of ginger root in reduceding the effects of warfarin in calcification. These physiological findings were confirmed

with the histological results that noted the role of the watery ginger root extract in the treatment the warfarin side effects on calcification in the blood vessels wall histologically. The our physiological results referred to the high dose of watery ginger root extract consider the best dose in reduced of warfarin effects on the blood vessels and other organs for the patients that treated with warfarin for a long time.

Table.4.11: Levels of serum calcium, in mice (mg/dl).

Parameter / Treatment	Calcium mean ±S.E
Control group	9.03±0.066[c]
Warfarin group	9.49±0.069[b]
Warfarin and low dose of ginger	9.59±0.081[b]
Warfarin and high dose of ginger	10.04±0.134[a]

*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.01).