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GINGER ROOT EXTRACT ROLE IN TREATED THE WARFARIN SIDE EFFECTS ON THE BLOOD VESSELS WALL

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Abstract

Warfarin drug have prominent effects on the blood vessels. One of the important effect was calcifications of blood vessel wall, so the present study focused on the investigation on the role of watery ginger root extract in processing the histological changes in the blood vessels after treated with warfarin for 30 days. The results after treated time with warfarin showed weak tunica intima in the wall of blood vessels, interaction or trading between tunica intima and tunica media without internal elastic lamina in the small artery, irregular shape of fibers and appeared as small piece in tunica adventitia, while the results in the group after treated with warfarin and ginger showed the tunica intima was normally, prominent thin internal elastic lamina under the tunica media which separated between tunica intima and tunica media and normal tunica adventitia that composed of loose connective tissue without any histological changes. Consequently the ginger can be used as improvement material for treatment side effects of warfarin.

Keywords: warfarin, ginger, blood vessel.

Introduction:

The herbs are used in every parts 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). 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 which have essential action as an antioxidant factors (Hashim *et al*., 2010).

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).

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, magnesium 45.02, phosphorus 32.56, calcium 15.76, manganese 0.70, copper 0.58, iron 0.54 and 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).

Vascular calcification plays a crucial role in the pathophysiology of coronary artery disease, ischemic stroke and peripheral artery disease, commonly known risk factors for vascular calcification include hypertension, diabetes, aging, chronic kidney disease, cigarette smoking, and systemic inflammation, vitamin K antagonists, such as warfarin, are associated with vascular calcification, even though other risk factors are regulated (Weijs *et al*., 2011).

A significant number of 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 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).

Methods and Materials

Experimental animal:

Sixty adult male white mice, the average weight was 28-30gm, the animals housed at 25-28ºC and humidity about 40 to 45% with feeding by using standard pellets and water. Mice accommodated to the laboratory conditions for 2 weeks before starting the experiment.

Experimental design: The mice were divided into three groups, each group composed of 20 mice. Group A: 20 animals were served as control group. Group B: 20 animals treated with 0.1 ml of warfarin solution for 30 days. Group C: mice received orally 0.1 of warfarin and 0.3 ml of watery ginger root extract for 30 days.

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

preparation of watery extract of Zingiber officinale: According to (Hernandez *at el*., 1994), mode (10 gm) of plant powdered added to (200ml) of distilled water in a sterile glass beaker and left 24 hours with continued jolt ,then passed on the layers of sterile soft cloth for its candidacy and then separation of the filterate 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 refrigerator to until use.

Preparation of histological slide: The tissues samples fixed in formalin solution for 48 h. after that dehydrated the samples in graduated levels of ethanol, cleared in xylene, and embedded in paraffin wax for cutting, the tissue section have 5-µm in thssickness, placed on glass slides, and stained with hematoxylin and eosin stain for light microscopic examination (Luna, 1968).

Results and Discussion:

Effect of warfarin on blood vessels:

In our study, the tissue section showed the thickness of blood vessel wall in control group was (9.85±0.153µm) (Table1), while histological findings of blood vessels wall in mice after 30 days of oral administration with 0.1 ml warfarin noted the wall thickness was (7.94±0.284µm) (Table1), which have significant decreased compared with control group, the tunica intima of blood vessels after treated with warfarin showed abnormal tunica intima with prominent destruction of endothelial layer, the histological sections showed interaction or trading between tunica intima and tunica media without internal elastic

lamina in the small artery, the tissue section noted appeared scattered smooth muscle cells in the tunica media and interaction with tunica adventitia (Fig2). The incidence occur may be because toxicity of warfarin that lead to acute degeneration in in layers of blood vessels wall, This results coincided with (Lerner *et al*., 2009) which proved that warfarin increased prevalence of aortic valve calcium in patients using warfarin with atrial fibrillation.

The result appeared in tissue section of blood vessels prominent completely isolated the tunica intima from tunica media in most tissue sections of blood vessels, abnormal tunica media which characterized by dispersed most of the muscular layer, without normal arrangement as circular in shape, also noted some of inflammatory cells aggregated between the small pieces of destructed fibers in tunica adventitia, these findings were abnormal when compared with control group(Fig3). This histological results similar to (Siltari and Vapaatalo, 2018) which said warfarin therapy cause 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 section of blood vessels in the small artery noted weak tunica intima, so noted interaction tunica intima with tunica media, the tissue section showed wide space in the tunica adventitia and in the other site completely disappeared small part of tunica adventitia (Fig4). These results may be due to toxicity of warfarin that lead to degeneration of internal and external elastic lamina which lead to increased spaces between layers of blood vessels wall. 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 section of blood vessels in the small artery noted degeneration in tunica intima, tissue section showed many abnormal irregular spaces in the tunica media, the result of small artery wall didn't noted prominent external elastic lamina between tunica media and tunica adventitia in the wall of small artery in the treated groups compared with control group, The histological result noted the tunica adventitia in blood vessels wall after treated with warfarin noted wide cystic dilation in the tunica adventitia (Fig5). This histological changes may be due to distraction of internal and external elastic lamina which lead to increase the spaces between the histological layers that composed of the blood vessels wall. This result coincided with **(**Elantably *et al*., 2020) which noted

warfarin induced leukocytoclastic vacuities, lead to increase skin lesions, medication-induced leukocytoclastic vacuities can affect multiple organ systems and even cause death.

Effect of warfarin and ginger on blood vessels:

The histological finding appeared the blood vessels wall thickness have prominent width with diameter was (10.24±0.176µm) (Table1), which non-significant when compared with control group, but have significant increased compared with warfarin treated group, 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 histological result of blood vessels wall 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 histological result of the artery wall noted have normal tunica adventitia that composed of loose connective tissue without any histological changes(Fig6). This 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 tissue section didn't have prominent degeneration in the tunica intima compared with warfarin treated group, thin internal elastic lamina under the tunica media which separated between tunica intima and tunica media, and 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 (Fig7). 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 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 antioxidant beneficial effect on macrophage and protect smooth muscle cell in tunica media against the oxidative damage.

The tissue section of blood vessel wall showed the tunica adventitia was appeared as thin band completely separated from under tissue layers, (Fig8). 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 tissue field appeared many nodular lesions oval in shape which have high amount of cells. The nodular structure have prominent blood congested (Bassim and Duaa 2020).

The result appeared the value of potassium in control group was (8.21±0.273mg\dl). the potassium level in treated group with warfarin only was (10.00±0.101 mg\dl), While the level of potassium in the group that treated with both of watery ginger root extract with warfarin was (11.09±0.184 mg\dl). The potassium increased in the serum after treated with warfarin only, was may be as a result of to the warfarin lead to raise the rate of riches-in riches-out calcium-potassium which lead to release the potassium in blood and causes blood vessels calcification. While potassium level increased in treated group with both of ginger root extract and warfarin that because of the ginger root have high amount of potassium which lead to significant increase in potassium level. This results may be due to that ginger contained amount of potassium in 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.

The level of calcium in serum of control group was (9.03±0.066 mg\dl) (Table2), the level of calcium in treated group with warfarin only was (8.49±0.069 mg\dl), biochemical findings of calcium after treated with both of ginger root extract with warfarin was (10.04±0.134 mg\dl). The physiological results noted significant decrease in level of calcium in treated group with warfarin only, that may be as a result of to the role of warfarin in the calcification which lead to decrease the level of calcium in 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 blood.

While the level of calcium increased in the serum when treated with watery ginger root extract with warfarin may be because of the role of ginger root in reduced the effects of warfarin in calcification. These physiological findings were confirmed with histological result that noted the role of watery ginger root extract in treatment the warfarin side effects on calcification in the blood vessels wall histologically. The our physiological results referred to the watery ginger root extract consider the best in reduced of warfarin effects on the blood vessels and other organs for the patients that treated with warfarin for long time

Table (1): The diameter and parameter of blood vessels.

Enzyme	Control group				Warfarin group				Warfarin and ginger group				Sig.
	N	Mean	St. Dev.	Std. Error	N	Mean	St. Dev.	Std. Error	N	Mean	St. Dev.	Std. Error	
Thickness o blood Vessels wall	50	9.8588	2.00898	.28411	50	7.9444	1.24635	.17626	50	10.2480	2.37170	.33541	.000
Potassium	20	8.2125	1.22238	.27333	20	10.0045	.45405	.10153	20	11.0935	.82730	.18499	.000
Calcium	20	9.0335	.29819	.06668	20	9.4925	.31124	.06960	20	10.0450	.60129	.13445	.000

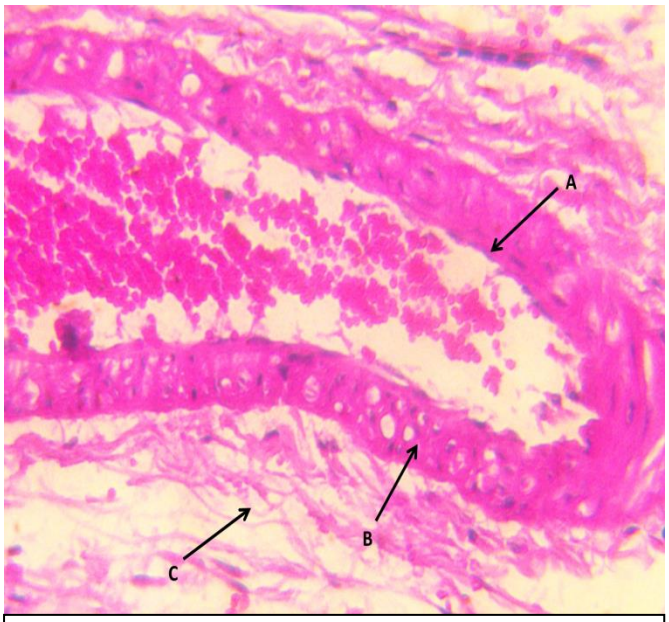

Fig.-1: Transverse section of blood vessels in control group which showed A- tunica intima, B-tunica media, C-tunica adventitia. H&E stain 40X.

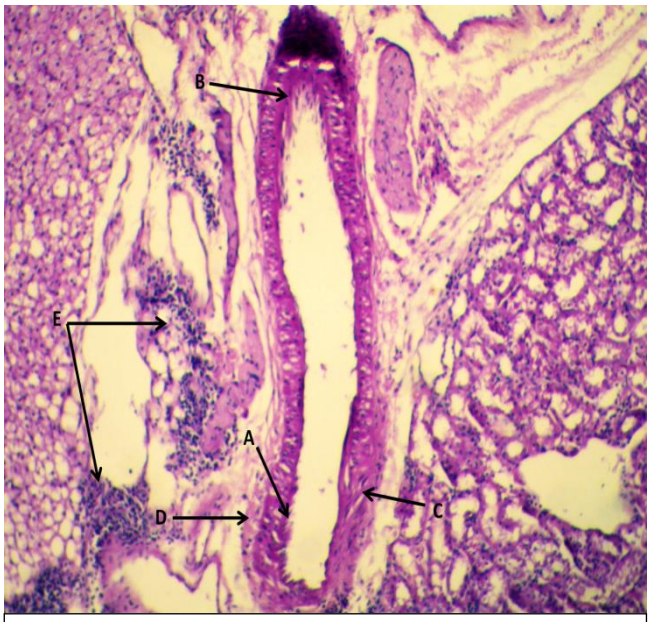

Fig.-2: Transverse section of blood vessels after treated with warfarin which showed A-abnormal tunica intima, B-exfoliated endothelial cells, C-tunica media, D-tunica adventitia, E-inflammatory cells. . **H&E** stain X40

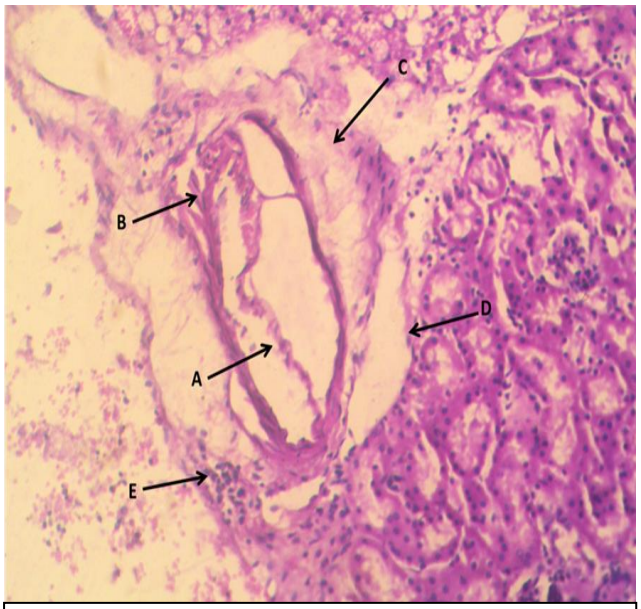

Fig.4.37 : Transverse section of blood vessels after treated with warfarin which showed A-isolated tunica intima, B- thin tunica media, C-tunica adventitia, D-cystic dilation, E-cellular proliferation. **H&E** stain X40

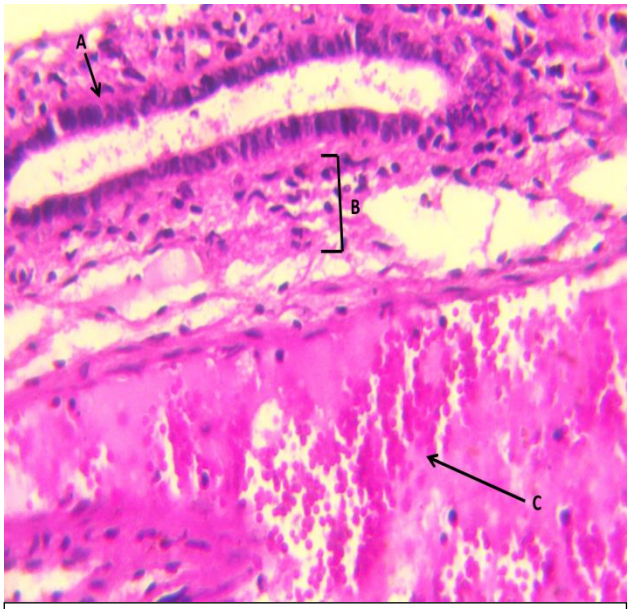

Fig.-4: Transverse section of blood vessels after treated with warfarin which showed A- degeneration in tunica intima, B-scattered smooth muscle cells introduction with tunica adventitia , C-hemorrhage. **H&E** stain X40

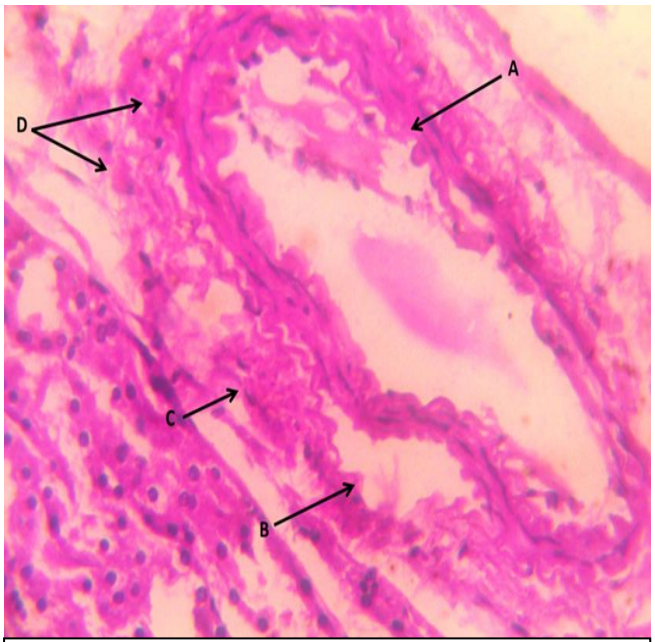

Fig.4-5: Transverse section of blood vessels after treated with warfarin which showed A- destruction in tunica intima, B-wide space between tunica media and tunica adventitia, C-hemorrhage, D-bloody congestion in tunica adventitia. **H&E** stain X40

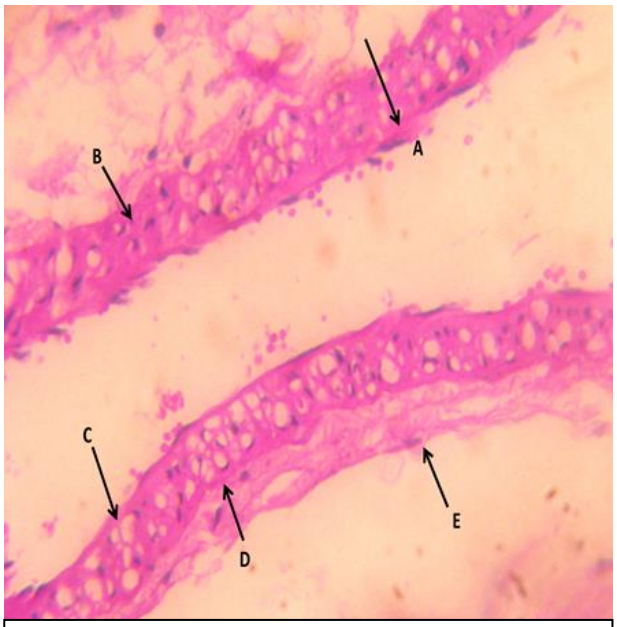

Fig.4-6: Transverse section of blood vessels after treated with ginger and warfarin which showed A-prominent tunica intima, B-tunica media, C-prominent internal elastic lamina, D-normal external elastic lamina, E-normal tunica adventitia. **H&E** stain X40.

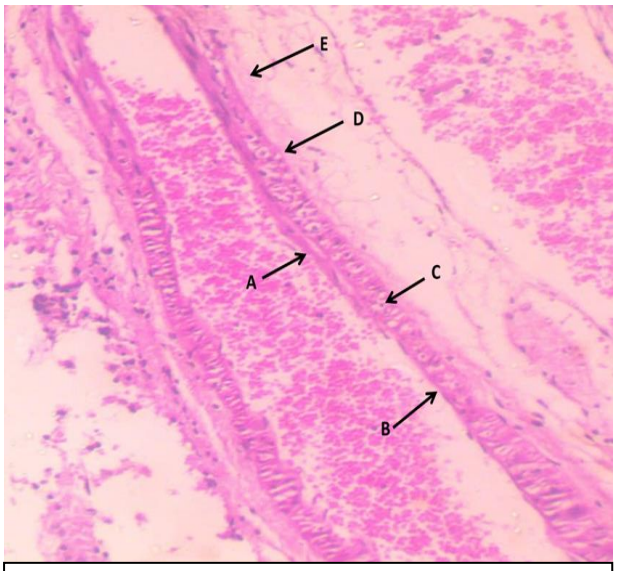

Fig.4-7: Transverse section of blood vessels after treated with ginger and warfarin which showed A-prominent tunica intima, B-prominent internal elastic lamina, C-tunica media, D-normal external elastic lamina E-tunica adventitia. **H&E** stain X40

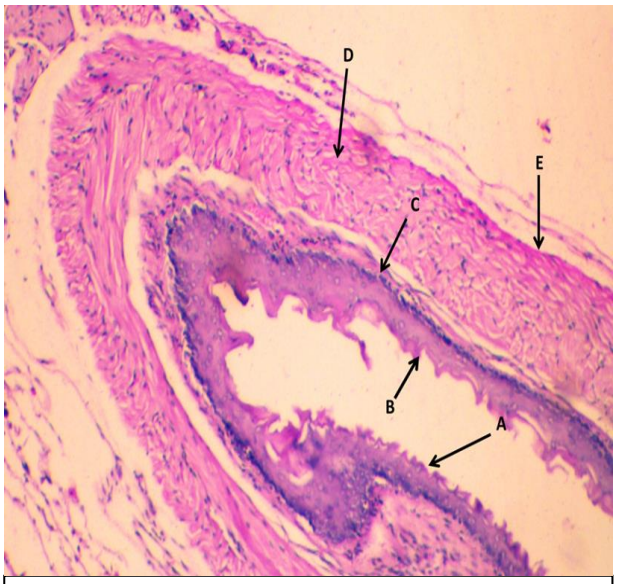

Fig.4-8 : Transverse section of blood vessels after treated with ginger and warfarin which showed A-prominent tunica intima, B-endothelial layer, C-prominent internal elastic lamina, D-tunica media, E-normal external elastic lamina. **H&E** stain X40

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