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## Comparing the Histological Effect of Thymol from Multiple Sources with Blood Lipids Profile and Liver Enzymes in Albino Mice

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**Abstract---**The therapeutic value of the phenolic component and pure thymol was well known; this study comprised the extraction of crude phenol from two plants (*Thymus vulgaris* and *Artemisia annua*) which contain thymol with pure thymol and evaluate their effect on hematological and histological by using three different concentrations of each plant extract and pure thymol to tested them on lab mice. All the mice were allowed free access to water and feed for 21 days in laboratory conditions; orally, pure water was administered to the control mice (group I), while groups II, III, and IV were given orally with *T. vulgaris*, *A. annua*, combination of last two crude phenol plant extract 50:50 and pure thymol respectively. The levels of CHO, TRI, and HDL were significantly reduced in *A. annua* and *T. vulgaris* crude phenolic extract at 20 mg/ml as compared to the control treatment, while pure Thymol at 10 mg/ml gave the best result in reducing the levels of CHO, TRI, and HDL. In comparison to the control group, serum levels of liver enzymes in groups treated with in *A. annua*, *T. vulgaris* extract, and pure Thymol were substantially reduced in concentration: 20, 10 and 30 mg/ml, respectively. Also, there is a histopathological change in the liver and kidney of mice treated with plant extracts and pure thymol at a higher dose were indicated. In conclusion, thymol's hepatoprotective activity is dose-dependent, implying that it is more effective at moderate dosages and its effect becomes more severe at high concentrations. We recommend purifying the extract before using it in pharmaceutical treatments since it is more effective, and we can also highlight the importance of paying attention to medicinal plants because they are a major source of

active ingredients used in the development of new therapeutic medications.

**Keywords**---Albino mice, hematological parameter, histopathological changes, Thymol.

## Introduction

Traditional medicine is still widely regarded as the preferred main health-care system in many communities, with over 60% of the global population and around 80% of those in developing nations depend on medicinal plants directly for their specific treatment ([Shrestha et al., 2003](#)).

Toxicants are especially dangerous to the kidneys for two reasons: it filters a significant amount of toxins that can collect in the renal tubules as a large volume of blood flows through it, and it filters a significant amount of toxins that can accumulate in the renal tubules as a large volume of blood flows through it. The term "nephrotoxicity" refers to kidney toxicity. It can cause systemic toxicity, resulting in a diminished ability to eliminate body wastes, an inability to maintain body fluid and electrolyte balance, and a reduction in critical hormone synthesis ([Oduola et al., 2010](#)). Plant materials have been employed in Ayurveda to protect the liver from various poisons and dietary factors. As a result, herbal medications have grown in popularity in recent years due to their safety and capacity to cure such disorders. These drugs are also incredibly cost-effective when used for a long time ([Jannu et al., 2012](#)). Many medicinal plants found in various parts of India have been identified as hepatoprotective medications and are widely utilized to treat liver diseases. Hepatoprotective action can be found in a variety of plants and polyherbal preparations. Hepatoprotective action has been claimed for approximately 160 phytoconstituents and other phytochemicals ([Sharma et al., 2000](#)).

Thymol is a dietary monoterpene phenol that can be found in the oils of thyme and other plants. It has antibacterial, antifungal, anti-inflammatory, and radioprotective properties ([Buyukleyla, & Rencuzogullari, 2009](#)).

In recent years, there has been a significant increase in the use of and research into plant-based pharmaceuticals and food supplements. *Thymus vulgaris* extracts and oil are utilized in ethnopharmacology and have been extensively explored for their potential as a medicinal plant, as well as in flavorings, cosmetics, food manufacture, and therapy ([Horváthová et al., 2016](#)). Several publications have established the antibacterial, antifungicidal, antioxidant, anti-tumor, anti-inflammatory and immunomodulatory effects of carvacrol and thymol, which are the primary phenolic compounds contained in *T. vulgaris* preparations ([Habashy et al., 2018](#)).

The genus *Artemisia* belongs to the Asteraceae (Compositae) plant family, which has around 300 distinct species. Sweet worm wood (*Artemisia annua*) is a plant that is also known as *A. annua*. Several isolated compounds from this species have been demonstrated to have antimalarial, antibacterial, anti-inflammatory,

plant growth regulating, and anticancer properties (Abad et al., 2012). Coumarins, flavonoids, and other terpenoids found in *A. annua* L. have been found to have significant pharmacological activity, including anticancer and antibacterial activities, contributing to the herb's medical effects (Bhakuni et al., 2001).

Besides these documented effects of thymol-containing plant extracts, thymol has been linked to a variety of physiological actions, including antibacterial, antioxidant, and anti-hepatotoxic properties. Despite the fact that thymol has a variety of physiological actions, its link to obesity or metabolic disorders has never been investigated (Alam et al., 1999; Shapiro & Guggenheim, 1995). The aim of this was to evaluate the effect of *T. vulgaris*, *A. annua* phenolic extract which contain thymol, and pure thymol on lipid profiles and liver enzymes in albino mice, as well as observe histological changes in the liver and kidneys.

## Material and Methods

### Extraction

The leaves of *T. vulgaris* and *A. annua* were obtained from a local herbarium market in Baghdad city. It was maintained at 4°C for further investigation after the plants were air dried and powdered. The finely ground sample (50 g) of plants leaves was extracted with ethanol using Soxhlet apparatus for 5 hr. a rotary evaporator was being used to filter and dehydrate the mixture (Ng et al., 2014). The final dried materials were kept in labelled sterile bottles. Pure Thymol was obtained from the drug testing laboratories of the Iraqi Ministry of Health, national center for drug control and research.

### Preparation of phenolic plant extracts in various concentrations

Plant extracts and pure thymol were created by dissolving a certain weight of each plant extract in Distilled Water to achieve the desired concentration. Various concentrations of plant phenolic extracts and pure thymol (10, 20 and 30) mg/ml concentration (Saravanan & Pari 2015), were made by using the following equation:

$$\text{Concentration mg/ml} = \text{weight/volume} * 1000$$

### Experimental animals

Forty-five adult white albino mice male weighing between (20 – 25 g) were kept in animal house of Biotechnology Research Center/Al-Nahrain University under laboratory condition of 25-28 °C, and supplied food and water for the period of research. Albino mice were divided into five groups (I, II, III, IV and V) all groups were administered orally at dose 10, 20 and 30 mg\ml conc. of plant extract and pure thymol (Scarpa & Guerri 1982).

### Experimental design

All the mice were allowed free access to water and feed for 21 days to acclimatize them to laboratory conditions; orally, pure water was administered to the control

mice (group I), while groups II, III, and IV were given orally with *T. vulgaris*, *A. annua*, combination of last two plant extract 50:50 and pure thymol respectively (Windholz, 1976). At the end of the 21 days of oral administration of ethanol plant extract and pure thymol, blood samples were taken directly from punch heart and collected in clean and dried EDTA. Tube was used for the analysis of hematological parameters like liver enzymes and lipid profile.

### **Histopathology**

All animals that treated with 30 mg/ml plant extract and pure thymol were submitting pathological exams of some major internal organs. The animals' organs, such as the liver and kidney, were removed for histology after weighed and kept in 10% neutral buffered formalin before being cut and stained with hematoxylin and eosin for histological analysis (AL-Jborrey et al. 2018). Glutamate Pyruvate Transaminase (GPT), Glutamic Oxaloacetic Transaminase (GOT), Alkaline Phosphatase (ALk), and Total Serum Bilirubin (TSB) were determined by spectrophotometric methods using specified kits in Al-Munim medical lab, analyses of pathological, Iraq.

### **HPLC**

In an LC-20AD instrument, plant extracts and pure Thymol were quantified using a reversed-phase HPLC-UV technique (Shimadzu, Kyoto). UV detection was carried out at a wavelength of 278 nm in a 40°C oven. Without the use of safe guards, two columns were evaluated: Symmetry C18 (250x4.6 mm i.d., total) (A:B) Sulfuric acid (0.5 mL of 2.5M) in 500 mL of acetonitrile and Sulfuric acid (0.5 mL of 2.5M) in water (500ml). To get the best peak resolution, the flow rates were evaluated according to (Al-Mothafar & Al-Shahwany).

### **Statistical Analysis**

Complete Randomized Design was used in the experiment (CRD), the role of several plant extracts on albino mice was studied using statistical analysis. When  $P \leq 0.05$  was utilized to see if there was a significant difference between the two means, the least significant difference (LSD) was used (Mason et al., 2003).

### **Results and Discussion**

#### **Analysis of Thymol by HPLC**

The results showed that the plant extracts of *T. vulgaris* and *A. annua* content high amount of thymol substance by using the HPLC method comparing with thymol pure. In addition HPLC peaks of these compounds for each plant showed in the figures (1&2).

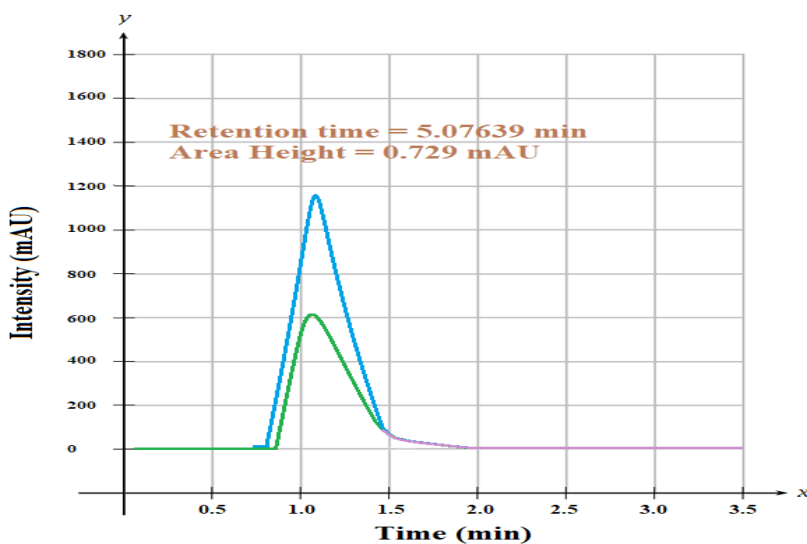


Figure 1. HPLC of standard (green) and *Artemisia* (blue)

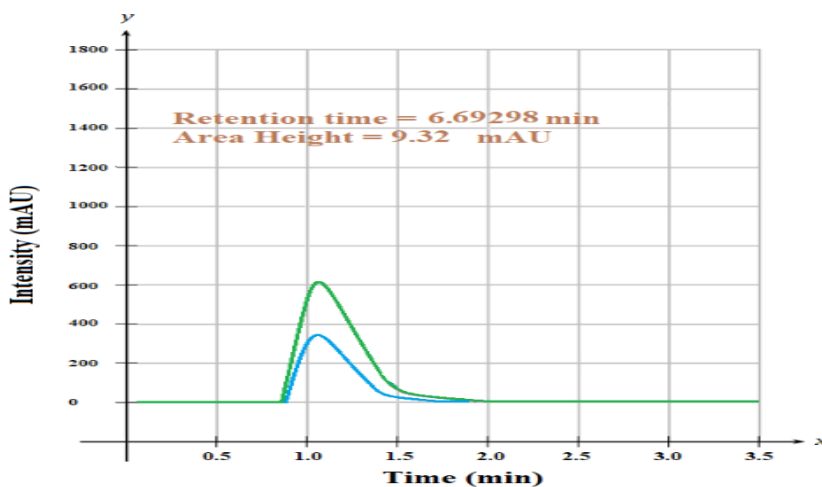


Figure 2. HPLC of standard (green) and *Thymus* (blue)

#### Hematological parameter

The result in tables 1&2 summarize the hematological profiles of the treated and control groups, including lipid profiles and liver enzymes.

#### Effect of plant extract and pure Thymol on lipid profile:

The effect of plant extract in table 1 shows that there was a significant difference between plant phenolic extracts and pure Thymol, and that their concentration affects the levels of CHO, TRI, and HDL in mice blood serum. The levels of CHO, TRI, and HDL were significantly reduced in *A. annua* and *T. vulgaris* extract at 20 mg/ml conc. as compared to the control treatment, while pure Thymol at 10 mg/ml conc. gave the best result in reducing the levels of CHO, TRI, and HDL.

Table 1  
Effect of phenolic plant extract and pure thymol on CHOL, Tri and HDL levels of mice's blood serum

Treatments	Conc. mg/ml	Chol mg/dl	Tri mg/dl	HDL mg/dl
CONTROL		96.00	112.33	130.00
<i>Artemisia annua</i>	10	121.00	114.33	94.67
phenolic extract	20	78.67	102.67	110.00
	30	99.67	271.33	86.67
<i>Thymus vulgaris</i>	10	95.00	234.33	101.00
phenolic extract	20	95.67	99.67	87.00
	30	90.67	108.33	89.67
Combination	10	94.00	98.00	109.67
	20	88.00	116.00	90.00
	30	114.33	106.67	102.00
Pure Thymol	10	87.00	101.33	87.00
	20	88.00	105.33	89.33
	30	94.67	103.00	99.33
lsd5%		16.10**	48.41**	11.43**

Combination: mixture of *T. vulgaris* and *A. annua*, Chol: Cholesterol, Tri: Triglyceride, HDL: High-density lipoprotein, \*\*: Very significant

There is a significant different between treatments in cholesterol parameter, *A. annua* extract gave the best result 78.67 mg/dl in phenolic extracts concentration 20 mg/ml conc. and also gave the highest value about 121.00 mg/dl in phenolic extracts conc. 10 mg/ml. This study found that in *A. annua* leaves has been suggested as a mechanism to explain the potential cholesterol-lowering benefits by stimulating cholesterol binding to bile acids, and inhibition of micelle formation mixed with the effect of fermentation on short-chain fatty acid synthesis (Baghban-Kanani et al., 2018). While in Triglyceride parameter, the combination of *T. vulgaris* and *A. annua* gave the best value about 98.00 mg/dl in phenolic extracts 10 mg/ml conc., several investigations have shown that each individual plant extract in the blend has hypolipidimic action. The presence of flavonoids, polyphenols, tannins, mucilages, and other bioactive compounds with hypolipidimic and stimulatory activities was indicated by the phytochemical profile of this mixture (Williamson, 2001). In HDL the extract of *A. annua* gave a good result 86.67 mg/dl in phenolic extracts conc. 30 mg/ml, this reduction in HDL could have delayed the transit of free cholesterol and cholesteryl ester from tissues to the liver, where it is converted to bile acid (George & Pamplona, 2005).

#### Effect of phenolic plant extract and pure Thymol on liver enzymes:

In terms of liver enzymes, the serum levels of GPT, GOT, ALK, and TSB in the treated group were significantly lower than those in the control group (Table 2). In comparison to the control group, serum levels of liver enzymes in groups treated with in *A. annua*, *T. vulgaris* phenolic extract, and pure Thymol were substantially reduced in concentration: 20, 10 and 30 mg/ml conc., respectively.

Table 2  
Effect of phenolic plant extract and pure Thymol on GPT, GOT, ALK and TSB on levels of mice blood serum

Treatments	Conc. mg/ml	GPT u/l	GOT u/l	ALK u/l	TSB mg/dl
CONTROL		40.33	260.33	193.00	3.00
<i>Artemisia annua</i> phenolic extract	10	13.33	268.33	199.67	3.67
	20	29.00	188.67	170.33	2.50
	30	30.00	213.67	361.67	3.13
<i>Thymus vulgaris</i> phenolic extract	10	30.33	208.67	227.00	2.80
	20	37.67	185.67	192.33	2.20
	30	40.67	242.00	265.67	2.83
Combination	10	60.00	261.67	212.00	2.70
	20	41.67	169.00	170.00	2.50
	30	57.33	251.33	400.00	2.60
Pure Thymol	10	32.33	163.33	148.67	2.87
	20	35.00	137.33	103.33	1.67
	30	36.67	279.67	481.00	1.47
lsd5%		7.198**	28.45**	34.20**	0.95**

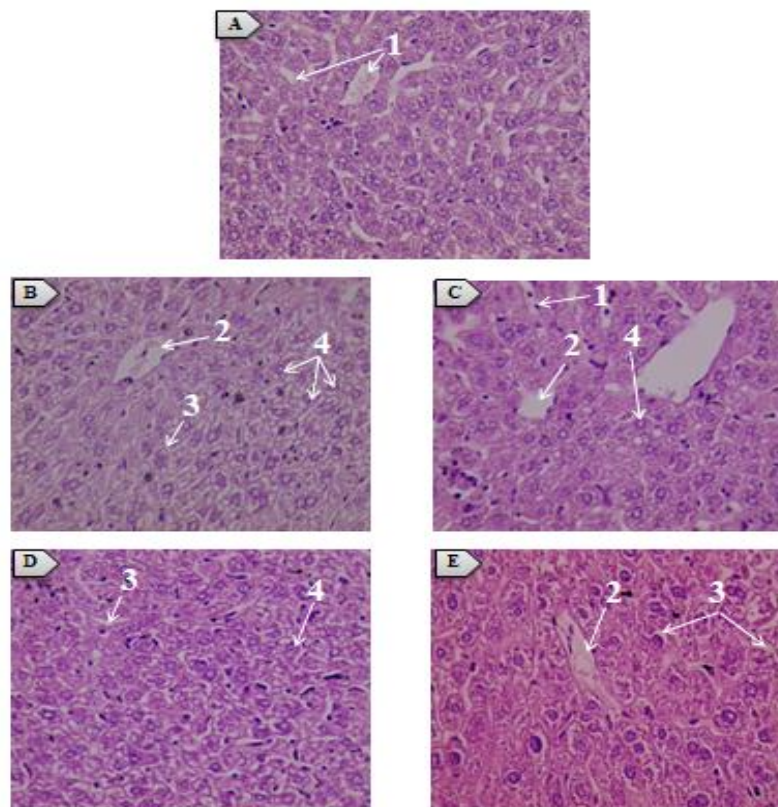
Combination: mixture of *T. vulgaris* and *A. annua*, GPT: Glutamate Pyruvate Transaminase, GOT: Glutamic Oxaloacetic Transaminase, ALK: Alkaline Phosphatase, TSB: Total Serum Bilirubin, \*\*: Very significant

The result of table 2 shown that *A. annua* extract gave the best value in GPT parameter 13.33 u/l, *Artemisia* plant contain many secondary metabolites (flavonoids, alkaloids, phenols, glycosides and terpenes) at various concentrations which have anti-diabetic and hepatoprotective potentials ([Ghaffari et al., 2012](#)). However, GPT increase with using the mixture of *T. vulgaris* and *A. annua* about 60.00 u/l. in GOT and ALK, the using of pure thymol in 20 mg/ml conc. gave the best result were 137.33 u/l and 103.33 u/l respectively and increasing the value in 30 mg/ml concentration, this may be Because of its powerful free radical scavenging and antioxidant action, thymol therapy restored the activities of liver marker enzymes ([Aboelwafa & Yousef, 2015](#)). Pure thymol using to decrease the TSB about 1.47 mg/dl in conc. 30 mg/ml, there has been limited studies on the influence of thymol on bilirubin markers in liver injury, studies on carvacrol have been published another important component of thyme essential oil phenol which, like thymol, have antioxidant effects ([Mastelic et al., 2008](#)).

### Histopathological analysis

Histopathological significant changes in the liver and kidney of mice treated with plant extracts and pure thymol at a higher dose were reported.



*In liver*

1:Sinusoidal dilatation, 2:central vein, 3:depletion of glycoprotein, 4:hepatocyte

Figure 3. Microscope sections in liver of mice: A: control, B: treated with 30 mg/ml of *A. annua* extract, C: treated with 30 mg/ml *T. vulgaris* extract, D: treated with 30 mg/ml Mixture of *T. vulgaris* and *A. annua* extract E: treated with 30 mg/ml of pure thymol (400X)

The liver sections in figure 1 picture (B) shows a mild depletion of glycoprotein inside hepatocyte cells in section when treated with *A. annua* extract, the obtained results indicated that an extract of *Artemisia* caused histological changes in the livers of rats. Pathological alterations in liver tissue, including inflammation and congestion, centrilobular necrosis, steatosis, and enlargement of hepatic cytoplasm, were shown in another investigation with extract of *A. dracuncululus*, which had similar results to this study. These effects may be caused by the presence of methylchavicol and other genotoxic substances in the extract, and this study found a direct link between extract dosage and three important variables: mutagenicity index, serum liver enzyme activity, and liver histology (Kalantari et al., 2013).

In figure 1 picture (C) the section showed a normal histological structure appearance of hepatic tissue when treated with 30 mg/ml of *T. vulgaris* phenolic extract protected hepatocytes against damage, indicating improved liver function

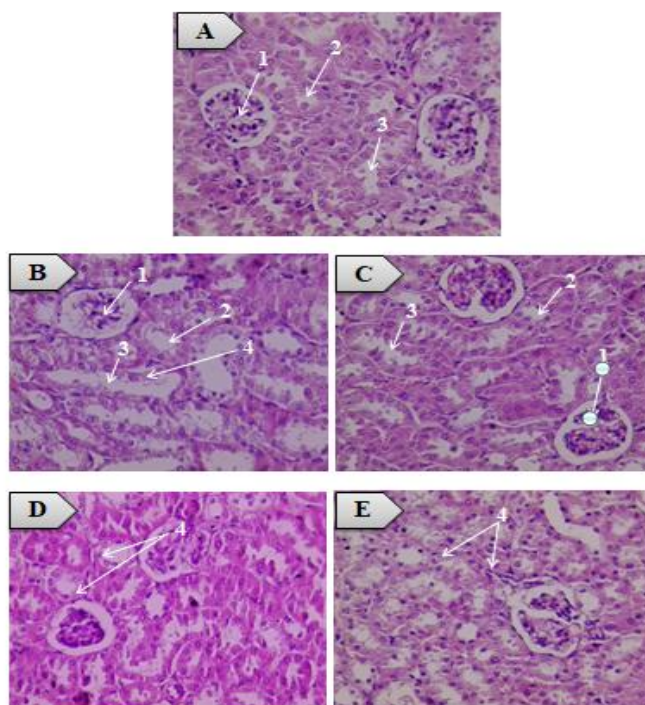


and a reduction in liver oxidative stress. Thyme's antioxidant and anti-inflammatory actions are mediated through activating antioxidant enzymes, altering lipid metabolism, and lowering lipid peroxidation (Raaof et al., 2013; El-Newary et al., 2017).

The liver section showing in Figure 1 picture (D) shown a normal histological structure appearance of hepatic tissue with very mild depletion of glycoprotein granules inside the hepatocyte cells. A mixture of several herbal extracts/fractions is likely to achieve the required effects in the treatment of severe liver illnesses. Medicinal herbs contain a number of phytochemicals with strong antioxidant properties, which have antihepatotoxic properties (Govind, 2011).

The result in figure 1 picture (E) indicated a moderate depletion of glycoprotein granules inside the hepatocyte cell, also Thymol treatment considerably reduced liver injury caused by toxicity, according to the findings of the current study. Thymol's action could be mediated according to its anti-oxidative or anti-inflammatory properties, as well as up-regulation of PGE2 production. As a result, Thymol has the potential to be a powerful hepatoprotective in drug toxicity studies; nevertheless, the high dose of this molecule should be considered for further research (Geyikoglu et al., 2019).

#### *In kidney*



1: glomerular, 2: proximal tubule, 3: distal tubule, 4: degenerated change

Figure 4. Microscope sections of mice's kidney: A: control, B: treated with 30 mg/ml of *A. annua* extract, C: treated with 30 mg/ml *T. vulgaris* extract, D: treated with 30 mg/ml combination of *T. vulgaris* and *A. annua* extract E: treated with 30 mg/ml of pure thymol (400X)

This section of renal tissue demonstrating degenerative alterations in some renal tubules, (proximal and distal convoluted tubules) after treatment with 30 mg/ml of *A. annua* showing in figure 2 picture (B). This agrees with some research suggesting that this plant is toxic to the kidneys and may increase the risk of kidney failure (Brown, 2017), but disagrees with other research suggesting that the oil of *A. annua* administered alone has no adverse effects on kidney function, morphology, or tissue, but the oil has a mild nephroprotective effect in rats (Xia, et al., 2020).

The section in figure 2 picture (C) shows a normal histological structure of renal tissue with only minor depletion in a few renal tubules. This suggests that *T. vulgaris* had a negative impact on renal function and tissue, which is consistent with the findings of Abdallah et al. (2020), who found that thyme had no effect on renal tissue except for the heated oil, which caused damage to the tissue. According to several studies, thyme extract has a protective effect against some forms of renal oxidative stress and redox. Thyme extract's protective action is achieved by the modulation of serum and tissue antioxidants (Soliman et al., 2021).

The histological structure of the *T. vulgaris* and *A. annua* extract combination was normal, but there were very slight degenerative alterations in some renal tubules, as shown in figure 2 picture (D). Medicinal herbs have a number of phytochemicals with significant antioxidant characteristics, as well as antihepatotoxic properties (Govind, 2011), thus when they interact together they obtain best results.

In this study thymol with high concentration 30 mg /ml lead to majority of degenerative changes in renal tubules showing in figure 2 picture (E), A high dose of thymol disrupts the communication between CK isoenzymes, preventing attempts to regenerate ATP or increase the intracellular ATP use and consumption by facilitating the CK/PCr shuttle (Baldiissera et al., 2018). Other histopathological studies of kidney tissues revealed that thymol decreased extracellular mesangial matrix growth and glomerulosclerosis in diabetic mice, suggesting that thymol treatment provided significant protection against HFD-induced diabetic nephropathy (Saravanan, & Pari, 2016).

## Conclusion

Based on current research that has proved the occurrence of harmful effects on human and animal health with the long-term use of synthetic chemicals, so phytogetic feed additives (herbs, essential oils, extracts, powders, etc.) have been substituted for these synthetic compounds (Golestan, 2010; Popović, et al., 2016). The study also indicates that thymol's hepatoprotective activity is dose-dependent, implying that it is more effective at moderate dosages and its effect becomes more severe at high concentrations. Concerning the distribution of thymol, it may reach different tissues at different levels. Medicinal plants contain a variety of compounds that have substantial antioxidant and antihepatotoxic capabilities. The *T. vulgaris* and *A. annua* phenols combination had very mild degenerative modifications in some renal tubules, especially when they interacted, which produced the best results. Although thymol has a protective effect against

several liver enzymes, more research is needed to understand the role of thymol in serum and tissue antioxidant protection. We recommend purifying the extract before using it in pharmaceutical treatments since it is more effective, and we can also highlight the importance of paying attention to medicinal plants because they are a major source of active ingredients used in the development of new therapeutic medications.

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