

Impact of Water and Alcoholic Plant Extracts of *Hypericum perforatum* on Histological and Some Physiological Features in the Liver of Albino Rats

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ABSTRACT

Hypericum perforatum, which is known as St. John's wort (SJW), is a leafy herb that grows in the open areas of the temperate regions throughout the world. Recent research suggests the effectiveness of this plant in treating some diseases, such as cancer, bacterial and viral diseases, and some inflammation-related disorders, and as a neuroprotective agent and an antioxidant. The current study aimed to investigate the Impact of water and alcoholic plant extracts of *H. perforatum* on histological and some physiological features in the liver of albino rats. 21 male rats were used in the current study. They were divided into groups; each group contains 7 members of animals. The animals were raised a month ago in the animal house before conducting the study to ensure their suitability to the environment of the animal house. Before the start of the study, an appropriate amount of animal blood was obtained through the caudate vein of rats, in order to evaluate the variables of the physiological study before starting treatment with plant extracts. One of the groups (Group 1) was used as a standard control group, receiving only standard water and feed. The other group (Group 2) was treated with St. John's water extract at a concentration of 300 mg/kg/body weight, while the last group (Group 3) was treated with St. John's alcoholic plant extract at a concentration of 300 mg/kg/body weight. Aqueous and alcoholic plant extract were prepared for *H. perforatum*. Histological slides were prepared from the liver of each group of the study as well as, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were evaluated for the rates in each group. Histological examination shows the presence of necrosis in various stages and the presence of Foamy Cells and the occurrence of infiltration of a number of inflammatory cells within the tissue as well as the occurrence of congestion in the central vein and hemorrhage spread throughout the rest of the tissue. We also note the occurrence of necrosis and desquamation of the central vein lining and infiltration of some inflammatory cells in some areas. The effect of treatments on the aminotransferases (AST, ALT) enzymes and the ALP, where appeared as a significant increase of 0.05 in their concentration when comparing the concentrations of each groups before and after treatment with plant extracts. We can conclude that *H. perforatum* aqueous and alcoholic plant extract can causes mild damages on liver histological features that can be reflected on livers physiological states generally and on the ALT, AST and ALP enzymes specifically.

Keywords- *Hypericum perforatum*, plant extracts, water extracts, alcoholic extracts, histological, physiological features, liver, albino rats.

I. INTRODUCTION

Hypericum perforatum, which is known as St. John's wort (SJW), is a leafy herb that grows in the open areas of the temperate regions throughout the world. This species has been used as an herbal therapy to treat different several external and internal illnesses since the time of the ancient Greeks. As a result of that it had used as a popular treatment for depression, anxiety, burns, and cuts. The current data suggests that this herb is effective in treating other malfunctions, such as inflammation-related disorders, cancer, and viral and bacterial diseases, and as a neuroprotective and antioxidant agent.[1]

Hypericin can be considered the main constituent of SJW that can act as an antidepressant, and can stimulate blood flow in the capillaries[2]. Other studies on brain mitochondria of the rat found that hypericin have a strongly inhibitory effect on the monoamine oxidase (MAO) enzymes type A and B[3][4] which in turn is involved in the amine neurotransmitters degradation. Inhibiting the degradation of the neurotransmitters increases the levels of the neurotransmitter in the synapse. On the other hand, some studies suggested that the inhibitory ability of hypericin's on MAO was lower than what was expected before. Moreover, the sufficient levels of hypericin that are necessary to inhibit the MAO significantly were far higher than the levels that can be found in the tissue of the human brain at normal doses[5][6]. Other studies showed that hypericin have a high affinity for the sigma receptors that in turn regulate the dopamine levels. It also serve as an antagonist some receptors such as, the gamma aminobutyric acid (GABA), benzodiazepine, inositol triphosphate, and adenosine receptors, which in turn regulate the resulted action potentials from the neurotransmitters activity[6][7]. In spite of the antidepressant properties of the hypericin, it cannot

completely represent SJW antidepressant activity by itself[8].

The monoamine neurotransmitters reuptake will be decreased due to the loss of the gradient. The hyperforin (another active part of the plant) was appear to act in a different mechanism from the other traditionally used antidepressants, which can opens the door for a new antidepressants class[9]. The results of other study demonstrated that the SJW extract therapeutic effective level is dependent directly on the hyperforin concentration[10].

The studies have been consensually indicated that *H. perforatum* administration has a positive effect on mild to moderate depression treatment and it has a good efficacy when the herb is administered to major depression cases. However, these good effects can be only applicable to SJW extracts that were already standardized such as WS 5570/2, ZE 117, and as LI 160. Most of the mentioned in the literature of the clinical trials suggest that *H. perforatum* is more tolerable than synthetic antidepressants as it causes fewer side effects and also shows similar adverse reactions as seen in placebo-controlled groups[11]. The current study aimed to investigate the Impact of water and alcoholic plant extracts of *H. perforatum* on histological and some physiological features in the liver of albino rats.

II. MATERIAL AND METHODS

The current study was conducted in the laboratories of Tikrit University - College of Science / Department of Biology - Iraq. 21 male rats were used in the current study. They were divided into groups; each group contains 7 members of animals. The animals were raised a week ago in the animal house before conducting the study to ensure their suitability to the environment of the animal house. Before the start of the study, an appropriate amount of animal blood was obtained through the caudate vein of rats, in order to evaluate the variables of the physiological study before starting treatment with plant extracts.

One of the groups (Group 1) was used as a standard control group, receiving only standard water and feed. The other group (Group 2) was treated with St. John's water extract at a concentration of 300 mg/kg/body weight, while the last group (Group 3) was treated with St. John's alcoholic plant extract at a concentration of 300 mg/kg/body weight.

The treatment period lasted for 3 weeks. After the end of the treatment period, the animals were anesthetized with an appropriate amount of chloroform and were compassionately slaughtered and blood collected to measure the study variables while the liver tissue was isolated and washed using an osmotic neutral solution (normal saline) and then transferred to formalin 10% for subsequent preparations for the histological examination.

Plant extract preparation

The extracts were prepared by Naik, *et al.*,(2015) method, which is summarized by placing 50 gm of the dried and milled plant part and then placed in a Soxhlet extractor using 400 ml for all different solvents at a concentration of 96% (ethanol and Distilled water) for a period of 72 hours at room temperature. Then the extract was evaporated by a rotary evaporator at a temperature of 45 ° C, ° for the ethanol extract, while the extract of distilled water was at 100 degrees[12]

Histological preparations:

According to[13], the liver tissue of experimental animals were prepared for histological examination. The liver tissue was taken and cut into parts, each piece less than 1 cm, then left in formalin 10% for 24 hours and then transferred to escalated concentrations of alcohols (30%, 50%, 70%, 80%, 90%, 96%, 100%) to remove water from the liver tissue, then it was transferred to xylol for less than two minutes, then transferred to paraffin wax in an oven at a temperature of 62 ° C for two hours, and then the tissue sample was poured into molds of paraffin wax. After that, the molds were trimmed with the tissue sample using the microtome, then the strips produced from the microtome were transferred to a water bath at a temperature of 37 ° C, after which they were loaded into glass slides and then transferred to xylol until the wax melted and then transferred to lower concentrations of alcohol to re-irrigate the tissue sample and then were stained using. The hematoxylin stain that stains the nuclei is then transferred to the eosin stain, which stains the rest of the cytoplasm capacity of the cell. The microscopy was examined using an Olympus light microscope connected to a camera, where the slides were examined and the resulting damage was confirmed.

Physiological parameters:

The concentrations of amine transporting enzymes (ALT, AST, ALP) were determined before and after treatment with plant extracts using a ready-made number from the French company, Bio-Labo, according to the manufacturer's directions.

Statistical analysis:

The ANOVA test in the famous statistical program SPSS version 21 was used to compare the results before and after the study and compare the results of the study groups.

III. THE RESULTS

Histological examination:

1- Group (1) standard control

Fig. (1) shows the liver tissue of the control group (Group 1). It shows the proper structure of the tissue, the regularity of the hepatic parenchyma and hepatic triads.

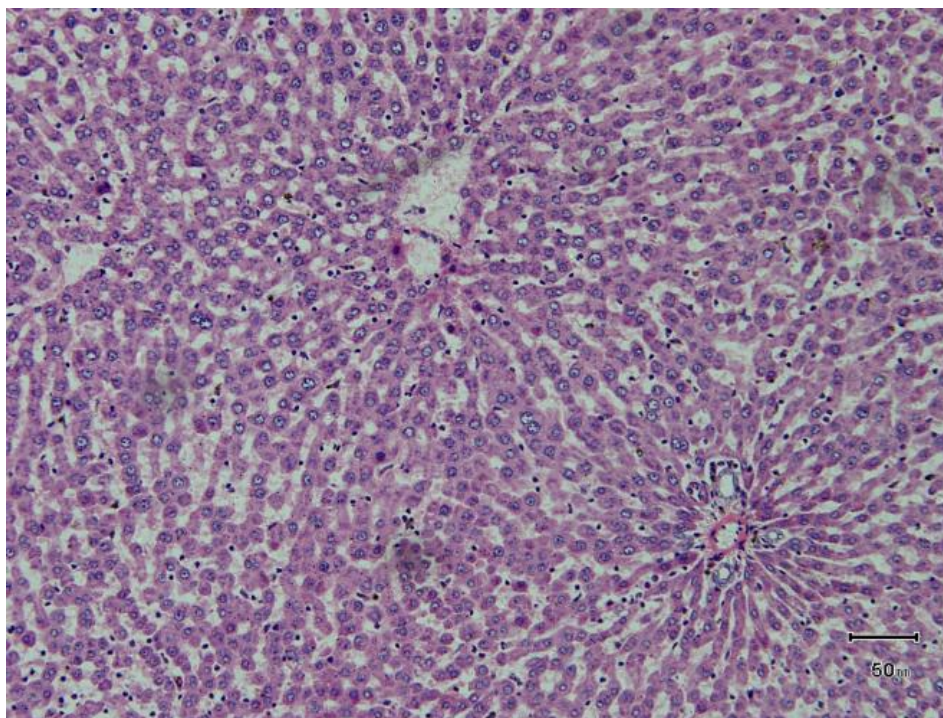


Fig. 1: Shows the liver tissue of the control group (Group 1) 200x H & E stain.

2- Group 2: 300 mg of aqueous extract

Fig. 2 shows the presence of necrosis in various stages and the presence of Foamy Cells and the

occurrence of infiltration of a number of inflammatory cells within the tissue.

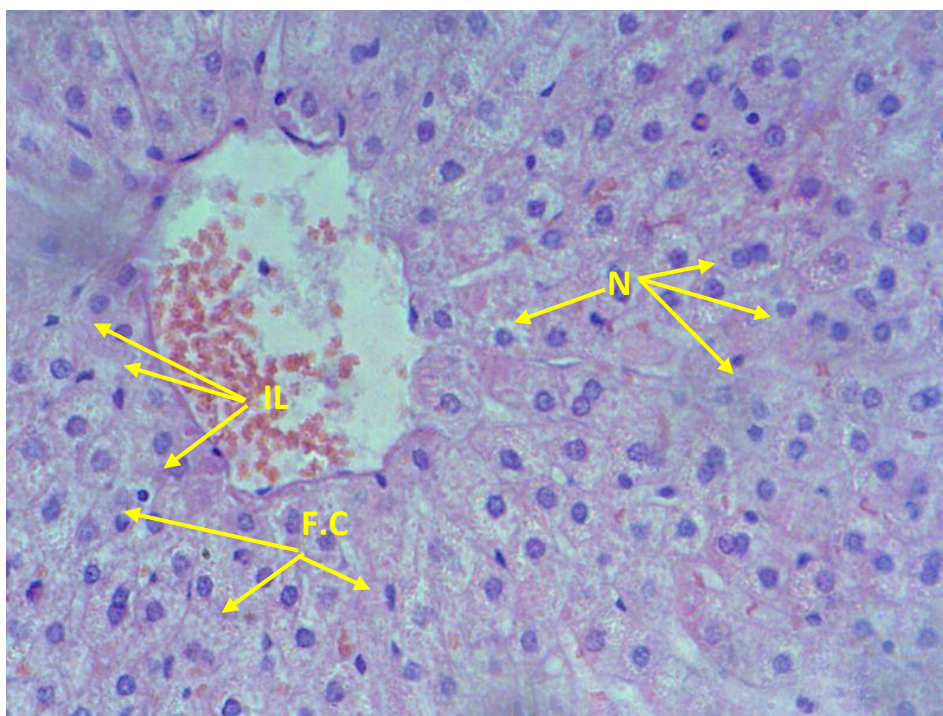


Fig. 2: Shows infiltration IL, Foamy cells F.C, and mild necrosis in different stages N. 400x H & E stain.

3- Group 3: 300 mg Alcoholic Extract

Fig. 3 shows the occurrence of congestion in the central vein and hemorrhage spread throughout the rest of

the tissue. We also note the occurrence of necrosis and desquamation of the central vein lining and infiltration of some inflammatory cells in some areas.

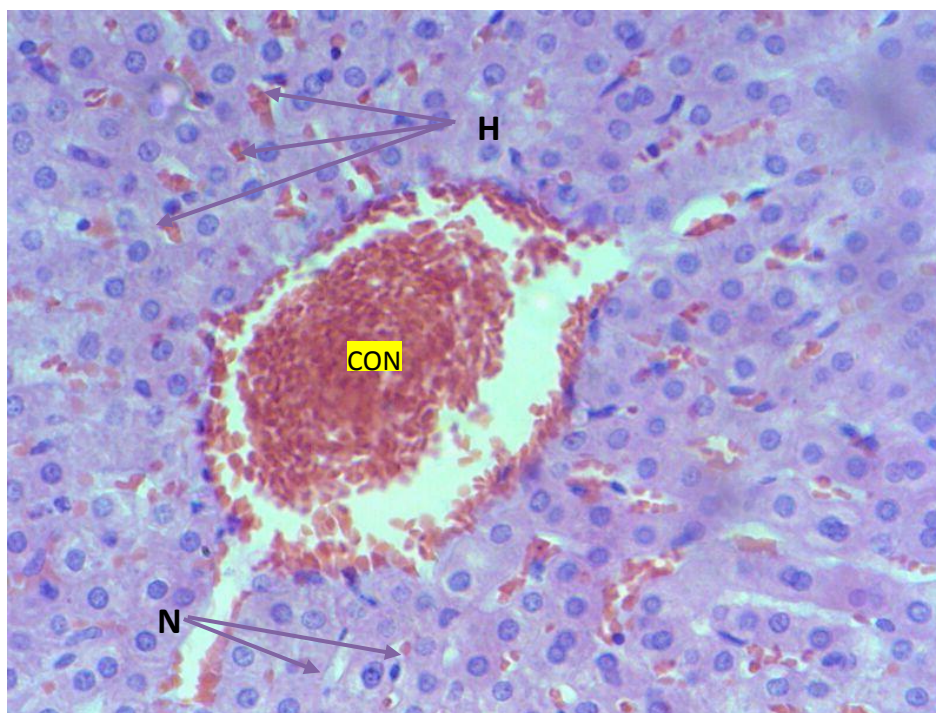


Fig. 3: Shows hemorrhage in the H tissue and congestion of the central vein CON with mild necrosis of N, desquamation of the central vein lining D.S, and infiltration of inflammatory cells IL. 400x H & E stain.

Table 1 shows the effect of treatments on the aminotransferases (AST, ALT) enzymes of amines and the alkaline phosphatase ALP, where a significant

increase of 0.05 was found in the concentration of AST, ALT and ALP when comparing the concentrations of groups before and after treatment with plant extracts.

Table 1: shows the effect of plant extracts on the AST, ALT and, ALP levels.

Parameter		Control	Group 1	Group 2
AST(U / L) ±S. D	Before treatment	32.6 ±1.2	34.5 ±2.3	31.2 ±3.2
	After treatment	31.9 1.5	35.2* ±2.8	30.8* ±1.4
ALT(U / L) ±S. D	Before treatment	22.2 ±3.9	26.3 2.1	24.5 3.2
	After treatment	21.5 2.5	27.8* 3.3	25.9* 2.8
ALP (U / L) ±S. D	Before treatment	55.2 ±2.9	54.4 ±5.3	64.3 ±4.7
	After treatment	58.1 3.7	59.2* 6.1	83.7* 5.7

Group 1: aqueous extract group, Group 2: alcoholic extract group, S. D: standard deviation * significant at P≥0.05.

IV. DISCUSSION

It is not surprising to notice significant tissue damage and lesions in the liver cells, being the organ in which many substances entering the body are metabolized and detoxified in one way or another. Jubb and his group[14] stated that the liver is the organ responsible for detoxification, through which the body gets rid of the largest. A possible number of toxic substances by destroying the unwanted substance, as well

as through the conjugated reactions to form other compounds that help the body to get rid of them and excrete them by the kidneys in the urine. In addition, it is known that about 75% of the blood supplied to the liver comes from the gastro-intestinal viscera and from the spleen by the portal vein[15]. This blood brings with it the absorbed materials in a concentrated form, where the liver enzymes work to remove the toxicity of some of the substances contained in the liver[16].

Al-Khatib and his group[17] also explained that the hemorrhage in the tissue is the result of increased pressure inside the vessels, so there is a breakdown in the vessel wall and the exit of red blood cells. The cause of the dissolution of blood cells is the interactions between toxic compounds and glutathione, forming compounds that lead to the dissolution of blood cells. The venous congestion leads to the expansion of swelling sinusoids and hepatic veins, which creates pressure on the hepatocytes, and the occurrence of necrosis is attributed to an imbalance in the distribution of enzymes in parts of the liver or the quality of cells in terms of function or blood supply since when the blood is affected, the peripheral areas are first hurt[18].

In a study conducted to verify the safety of plant administration, significant toxicity was observed in newborn animals whose mothers were treated with the plant extract at the highest dose. Lesions were evident in the liver as observed at a dose of 100 mg/kg per day, similar to that used to treat depression in humans. The 21-day-old mice also developed severe liver damage even at the lowest dose. The lesions observed were more severe than in the newborn, indicating a protective role of the placenta, or higher sensitivity of the target organs during this stage of development[19].

Barbara *et al.* concluded that chronic treatment with St. John's wort during pregnancy or lactation was responsible for the histological changes in the liver of mice. The effect is dose-dependent and also demonstrated with a dose similar to that used to treat depression[20].

It was reported by Meruelo *et al.*, [21] that treating animals with a plant of the genus *Hypericum* in the absence of light, hypericin was not activated, and no toxic effects occurred on animals. Conversely, in treated animals exposed to direct sunlight, the plant showed clear clinical signs of photosensitivity such as shade-seeking, tearing, insomnia, loss of appetite, skin lesions, erythema, edema with pruritus as well as macular lesions, congestion of all internal organs. Especially in the liver. These pathological signs may be due to the photosynthesis of fibrin accumulation in the body, the production of a large number of reactive oxygen species (ROS) leading to oxidative damage to the cell membrane, cell injury, and the release of histamine and other inflammatory chemical mediators[22][23]. Observed macular lesion, liver congestion, possibly due to the effects of elevated body temperature, as animal poisoning by *H. perforatum* resulted in central nervous system depression and noradrenaline accumulation in the hypothalamic temperature-regulating center [24].

In our study, the concentrations of ALT, AST, and ALP increased in groups treated with plant extracts. The increase in the activity of these enzymes represents an increase in oxidative factors due to the higher concentration of lipids in the blood. The results of this study are consistent with those of many previous studies. For example, Arhan *et al.* Increased blood lipids have been shown to reduce antioxidant defenses and increase

lipid peroxidation in the liver. In addition, oxidative stress eliminates the balance between peroxides and antioxidants in biological systems, followed by a rise in lipid oxidation and free radical production[25].

V. CONCLUSIONS

We can conclude that *Hypericum perforatum* aqueous and alcoholic plant extract can causes mild damages on liver histological features that can be reflected on livers physiological states generally and on the ALT, AST and ALP enzymes specifically.

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