

## Effect of The Aqueous Extract of Rocket( *Eruca sativa L.*) Leaves on the Histological Structure of Some Organs in Male Mice

Susan.I.Bajilan  
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### Abstract

In this study we have assessed the effect of the hot aqueous extract of (*Eruca sativa*) leaves on the histological structure of (Kidney, liver and spleen) in male albino mice. Two experimental groups were used in this study(250 and 500mg/kg body weight).The extract was given orally for 30 days. There was a significant increase in weighs of the liver for the two treated groups as compared with control and only the 500mg/kg group shows a significant increase in weighs of kidney. The histological study for the treated groups shows hypertrophy of the hepatic cells with the accumulation of glycoprotein granules. Sections of the spleen revealed expansion of the white pulp and red pulp areas in addition to the presence of megakaryocytes and haemosiderosis in some areas. While sections of the kidney did not show a remarkable changes. A detection for some active ingredients found in the plant extract reveals the presence of (saponins ,alkaloids, flavenoids, tannins and glycosides).

### Introduction:-

Medicinal plants play an important role in man's life because they are widely used for the treatment and prevention of various diseases and often contain highly active pharmacological compounds. *Datura stramonium* plant is effective against mammary gland adenocarcinoma [1]. Another plant is *Seidlitzia rosemarinus* in which its extract has an antimicrobial effect against some pathogenic microorganisms causing vaginal infections [2]. There is a chemical study of *Ocimum basilicum* plant involving evaluation the activity of this plant extract on some pathogenic microorganisms [3]. *Eruca sativa L.* which is commonly known as Rocket is used in this study. It belongs to the Brassica plant family(Cruciferae),and is immensely used as vegetable and spice ,it originated in Mediterranean region and now is found around the world [4].The plant also has a wide spread medicinal use. Traditionally, it is used as astringent ,diuretic ,digestive ,emollient ,tonic ,depurative, laxative ,rubefacient and stimulant is well documented [5].Rocket is also called girgir in Arabic was used as garden crop and spice and as medicinal plant, it was used as aphrodisiac ,for eye infection and for digestive and kidney problems [6].The main medicinal and therapeutic properties of this plant include antigentoxic effect against human hepatoma

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(HePG2) cells which is attributed to the presence of erucin and erucolin compounds in the plant extract [7].It has also antihyperlipidemic, antihyperglycemic ,antiephrolethiatic and hepatoprotective activity [8].Rocket and other Cruciferous vegetables contain a group of anticancer compounds known as glucosinolates, these compounds exert antioxidant activity ,and are potent stimulator of natural detoxifying enzymes in the body ,such compound exert anti-secretary ,anti-ulcer and cytoprotective properties in the ethanolic extract of the plant in rats [9].The plant also has antifungal activity due to the presence of antioxidant constituents: glucosinolate, flavonoids ,carotenoids in addition to the volatile fractions [10]. It was reported that the ethanolic extract of *Eruca sativa* plant has androgenic activity or stimulate testicular steroid production which enhance the preputial gland as well as increase spermatogenesis in the testis of male mice [11].Besides, the ethanolic extract of the seeds yields organosulfer compounds including isothiocyanate in which has anticarcinogenic ,antiinflammatory and antiproliferative activity [12].This extract also possesses potential antioxidant and renal protective activity and preclude oxidative damage inflicted to the kidney [5].The studies also showed that *Eruca sativa* helps regulate cholesterol levels, regulate blood pressure ,improve wound healing and support weight loss [13].The nutrition compounds and trace elements in both leaves and seeds of this plant were analyzed , the results referred to the higher amounts of crude fibers ,total minerals, Mg and Fe in the leaves as compared to the seeds [8].Many medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another [14].Therefore, we have designed this experiment to find out the effect of the hot aqueous extract of *Eruca sativa* L. leaves on some organs(liver ,spleen ,and kidney)in male mice. The weighs of these organs were taken and the histological changes of these organs have been taken by using routine stains (hematoxylin and eosin).

### Materials and Methods:- Preparation of the hot aqueous extract of *Eruca sativa* leaves:-

The hot aqueous extract of *Eruca sativa* leaves was prepared by weighing 50 gm of dry powder of the leaves and put it in a conical flask then 500 ml of hot distilled water was added on the powder which makes the ratio(1/10) (W/V).After that the mixture was shacked by using a magnetic stirrer for (24hr),the mixture was filtered by using 4 layers of quaze then centrifuged by a centrifuge(2000rpm for 10 min)the supernatant then was filtered again using filter paper wattman No.4.The filtrated mixture was concentrated by using oven on( 40°C) for 72hr,to obtain crude extract .This extract was stored in a dark sterile screw bottle in( 4°C) until use[15] .

### Detection for some active compounds found in the plant extract:-

In this study, a detection for some active compounds found in the plant extract

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was done by using chemical reagents .Benedict reagent [16] was used for the detection of glycosides. A diluted picric acid solution(50%)was used for the detection of alkaloids [17].Ferric chloride solution(1%) was used for the detection of tannins [18].The method of Jaffer *et al* (1983)was used to detect the presence of flavonoids. While saponin compounds were detected by shaking the tube of the extract and the presence of persistent foam indicates the presence of saponin.

## Animal and Dosing:-

Male mice approximately of the same age weighing (25-27)gm were fed on *ad libidum* .They were randomly divided into three experimental groups of six mice in each .Aqueous extract of *Eruca sativa* leaves was freshly prepared before administration ,two doses of (250and500mg/kg B.W) [9] were given orally by using a micropipette The mice were sacrificed after the end of the experimental period (30)days and the organs(Liver ,kidney and spleen)were removed ,their weights were taken then they were fixed in formaldehyde (10%) for sectioning and study the histological changes.

## Histological study:-

Organs(liver,spleen and kidney)which were fixed in formaline(10%) for a few days were processed for sectioning then embedded in paraffin blocks and sections about (5 $\mu$ )thick were cut using rotary microtome. The sections were stained with Heamatoxylin and Eosin using routine procedure [20].The slides were examined microscopically for observing the pathological changes.

## Statistical analysis:-

Values are given as(mean + S.E).Data were analyzed by using one way analysis of variance(ANOVA)Follwed by students test[21].

## Results:-

### Active ingredients found in the aqueous extract of *Eruca sativa* leaves.

Active ingredients	Glycosides	Saponins	Alkaloids	Tanins	Flavonoids
Results	++	+	+	+	+
	Red precipitate	persist foam when shaking the tube of the extract	White precipitate	Green color with ferric chloride solution (1%)	Dark yellow precipitate

+ Present , ++ present in quantity

### Effect of *Eruca sativa* leaves extract on weights of some organs:

#### Organ weights:-

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The results for some organs weights are presented in Table-1-.Significant differences (p<0.05) were found for mean liver weights between the two treated groups (250 and 500)mg/kg B.W. as compared with control. Significant differences (p<0.05) were also found between the two treated groups. There were also significant differences (p<0.05) in weights of spleen for the treated categories as compared with control and when we did a comparison in weights of this organ between the two treated groups, a significant differences (p<0.05)were found. Concerning weights of kidneys, there were significant differences(p<0.05) between the treated groups and control. Significant differences(p<0.05) were found between the treated groups in weights of this organ.

**Table1:-Mean ± S.E relative organs weights(mg) for the control group and the treated groups (250and500mg/kg) with the aqueous extract of *Eruca sativa* leaves.**

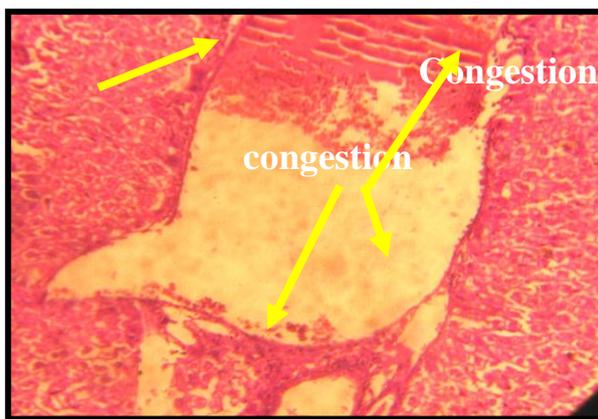
Organs	Treatments		
	control	250mg/kg	500mg/kg
Liver	1.63 ± 3.88 a	1.66 ± 8.81 ab	1.59 ± 0.16 ab
Spleen	0.22 ± 1.67 a	0.23 ± 5.99 a	0.11 ± 1.00 a
Kidney	0.40 ± 2.51 a	0.47 ± 2.03 b	0.42 ± 5.34 b

Similar letters in one raw mean there is no significant differences while different letters in one raw mean there is a significant differences(p<0.05).

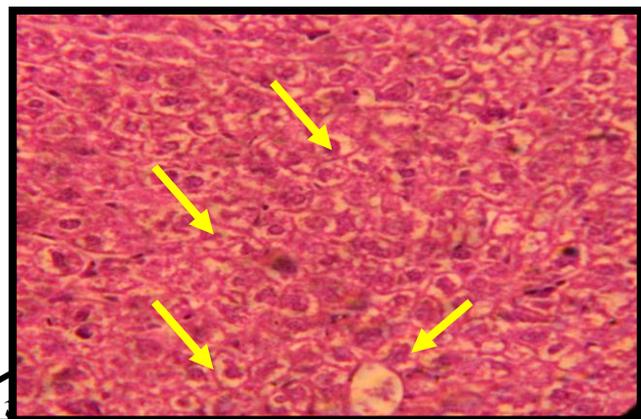
## Histological Study:-

### 1-The liver:

The group which was treated with 250mg/kg of the plant extract showed swollen of hepatocytes or it is known as hypertrophy .There was also congestion and dilation of sinusoids with cytoplasmic vaculation. In some areas hepatocytic vaculation appeared so severe that the vacuoles occupied the entire cell. Haemosiderosis slightly appeared in many sections of the organ .The same changes were found in the second group (500mg/kg) but in more severity. Figures:(1)&(2).



**Figure (2):** Liver section for the group treated with 500 mg/kg of *Eruca sativa* leaves extract showing congestion in some blood vessels (arrows) 400X.



**Figure (1):** liver section for the group treated with 500 mg/ Kg of *Eruca sativa* leaves extract showing vaculation of hypertrophied hepatocytes (arrows) 400X.

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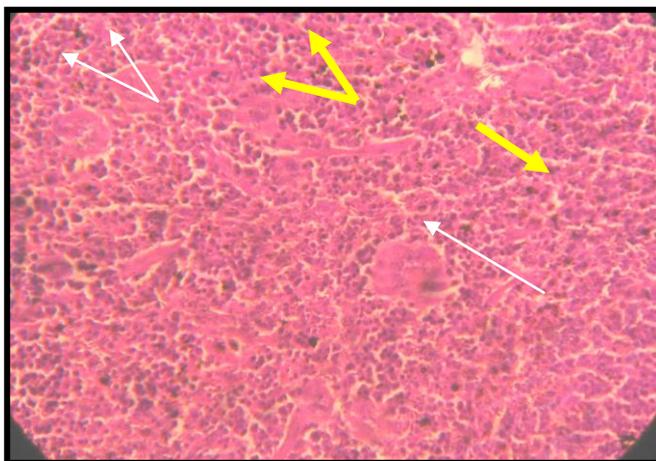
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## 2- The spleen:-

Sections of spleen for the first group(250mg/kg)show an increase in the white pulp and red pulp regions. Megakaryocytes have been seen in some areas of the section in addition to Haemosedirosis. The same changes were seen in the second group but they were more sever(500mg/kg).Figure (3).

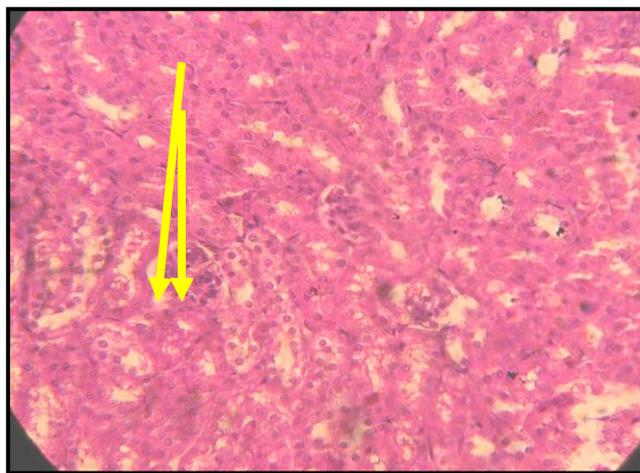
## 3-The kidney:-

Sections of the kidney for the group treated with 250mg/kg of the plant extract revealed mild degeneration in the renal tubules especially proximal convoluted tubules. The same changes were seen in the other treated group(500mg/kg).Figure (4).



**Figure (3):** Spleen section of the group

treated with 500 mg/kg of *Eruca sativa* leaves extract showing an increase white pulp & red pulp areas with the presence of megakaryocytes (arrows) 400X .



**Figure (4):** Kidney section for the group treated with 500 mg/kg of *Eruca sativa* leaves extract showing mild degeneration of proximal renal tubules (400X)

## Discussion:-

In recent years, Rocket plant (*Eruca sativa*) has gained greater importance as a vegetable and spice around the world ,it is also considered to be an important chemoprotective plant. Many medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another [14].There are no studies concerning the effect of this plant alone on liver, spleen and kidney. Therefore, In this study, we tried to investigate the effect of high doses of (*Eruca sativa*) on these organs.

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Grossly there were an increase in weights of these organs for the two treated groups (250 and 500)mg/kg .This increase may be resulted from the presence of some trace elements (Cr,Cu,Fe,Mn and Zn) in the leaves of this plant [8].Copper (Cu) has been shown to be important for immune system function based on evidence that Cu metabolism affects the function of several classes of immune system cells particularly those involved in producing antibodies. Cu is also vital for the activity of an enzyme responsible for removing toxic free radicals from the body (Cu-Zn superoxide desmotase) as well as for the activity of phagocytes [22].The microscopic study of the liver showed some negative effects represented by cellular hypertrophy and vaculation of the cytoplasm of hepatocytes .These liver injuries can be attributed to effect on eiber cellular respiration or due to accumulation of lipid droplets or glycogen in the cytoplasm of hepatocytes. Arab, *et al.*(2009) showed that histological alteration on early stages of labor ischemia-reperfusion lead to hepatocytes vaculation [23].The detection for the active ingredients found in the leaves of the plant showed the presence of alkaloids.In general ,plant alkaloids are the leading plant toxins associated with human and animal hepatotoxicity[24].such toxic materials act by depletion of glutathione which initiate mitochondrial oxidation stress that lead to increase membrane permeability and cause the collapse of mitochondrial membrane potential which results in a diminished mitochondrial capacity to synthesize ATP[25,26]. However, other substances produce cytoplasmic vaculation of tissue act by accumulation of phospholipids and concentration membranous structures within secondary lysosomes of these cells [27]. Concerning the spleen the increase in the white pulp and red pulp areas can be attributed to the presence of saponin compounds.Researches on these compounds demonstrate that they have the unique ability to stimulate the cell mediated immune as well as enhance antibody production and have the advantage that only a low dose is needed for adjuvant activity [28].Besides, the presence of some minerals especially Cu which activate the functions of several classes of the immune system particularly those involved in antibodies production could cause this effect [8,22]. Megakaryocytes appeared in the sections of the spleen for both the treated groups and the presence of such cells in the spleen indicate an activation for the immune system, Kidneys showed mild degenerative changes in the renal tubules that means this extract has slight adverse effect on kidney. This result disagrees with the ability of this plant (*Eruca sativa*) to exert a protective activity effect on nephrotoxic agents [5].

### Recommendation

Study the effect of this plant on liver enzymes and make blood profile. Make a Biochemical study and assess the levels of some hormones

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### الخلاصة:-

تتضمن هذه الدراسة تقييم تأثير المستخلص المائي الحار في التركيب النسيجي لأعضاء الكبد والطحال والكلى في ذكور الفئران البيض.قسمت الحيوانات المختبرية إلى ثلاث مجاميع(مجموعة السيطرة ومجموعة جرعت ب250 ملغم/كغم من المستخلص المائي ومجموعة ثالثة جرعت ب500/ملغم/كغم).تم تجريع الحيوانات فمويا واستغرقت فترة التجربة30 يوما.أظهرت المقاطع النسيجية للمجاميع المعاملة وجود زيادة في حجوم خلايا الكبد مع تجمع حبيبات البروتين السكري داخل الخلايا ووجود احتقان في بعض الأوعية الدموية أما المقاطع النسيجية للطحال فقد أظهرت وجود توسع في منطقة اللب الأبيض واللب الأحمر مع ظهور خلايا ذات النوى الكبيرة Megakaryocytes في حين لم تظهر تغييرات ملحوظة في المقاطع النسيجية للكلى.وبالكشف عن المركبات الفعالة الموجودة في المستخلص المائي للنبات لوحظ وجود مركبات الصابونينات والقلويدات والفلافونات والتانينات والكلايكوسيدات.