

Chemical constituents of *Eruca sativa* and treatment activity against paracetamol inducing hepatic injury in experimental rats

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Abstract

In the present study, gross chemical composition and some minerals content of *Eruca sativa* leaves and seeds were estimated. Also, hepatotreatment activity of *Eruca sativa* leaves, juice, oil and seeds against paracetamol induced liver damage in rats were investigated. Results of this study revealed that *Eruca sativa* (rocket salad) seeds had higher content of protein, fat and carbohydrate and minerals (Zn, Fe, Ca, K and Cu). *Eruca sativa* leaves had higher content of fibre, ash and moisture. Paracetamol induced liver injury rat groups treated with *Eruca sativa* leaves, juice, oil and seeds improved weight gain percent and food intake that appeared within normal. However, they decreased total serum protein and urea with significant increase in total bilirubin, haemoglobin (Hb) and packed cell volume (PCV) compared with control (+ve) group. Moreover, they lowered liver function enzymes, liver cholesterol, total lipids and MDA and elevated liver triglyceride, glycogen and liver antioxidant enzymes compared with control (+ve) group.

In conclusion, *Eruca sativa* could help the liver against paracetamol induced hepatic injury through its potent antioxidant activity in rats.

Keywords: *Eruca sativa*; Liver Injury; paracetamol; liver enzymes; antioxidant; rats.

Introduction

Liver plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage and also detoxification and synthesizes useful principles. Hepatotoxic agents cause structural abnormality in the liver which alters normal physiological functions. Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity (**Eisenberg et al., 1998 and Stickel and Schuppan 2007**).

Medicinal plants may serve as a vital source of potentially useful new compounds for the development of effective therapy to combat a variety of liver problems. *Eruca sativa* (syn. *E. vesicaria* subsp. *sativa* (Miller) Thell.) is an edible annual plant, often weedy, belongs to the Brassicaceae family of plants that originated in the Mediterranean region and Western Asia, and is now widely cultivated for its piquant leaves which are used in salads and as a cooked green. *Eruca sativa* is known as salad rocket because of their strong flavour and their content of putative health-promoting compounds. Among such compounds, glucosinolates and phenolics are well-known phytochemicals with an important role in determining the characteristic flavour of these species (**Lazzeri et al., 2004 and Pasini et al., 2011**).

In Asia, *Eruca sativa* has typically been cultivated for its seed oil for culinary purposes including pickling as well as industrial uses as a lubricant and as a source of erucic acid used in oil paints and as a lubricant. In traditional

medicine, *Eruca sativa* has been used as a stimulant and diuretic, and to treat stomach disorders. *Eruca sativa* (rocket) contains a wide range of compounds with nutraceutical and organoleptical properties (**Flanders and AbdulKarim 1985**). It has diversified medicinal and therapeutic properties including inhibition of tumorigenesis, anti-ulcer, and hepatoprotective activities (**Lynn et al., 2006 and Alqasoumi et al., 2009**). The rocket seeds and tender leaves are known in Arabian countries to increase sexual desire and are considered to be an aphrodisiac. *Eruca sativa* exert a beneficial antidiabetic effect in rats by reducing oxidative stress. It is also used as a carminative and to alleviate abdominal discomfort and improve digestion. The seed ethanolic extract possesses potent antioxidant and renal protective and diuretic activities (**Yanir et al., 1998, Sarwar et al., 2007 and Ansari 2014**).

This research aimed to investigate the chemical constituents of *Eruca sativa* and treatment activity against paracetamol induced hepatic injury in rats.

Materials and Methods

A – Materials:

Fresh leaves of *Eruca sativa* were obtained from grocery in Cairo while seeds and oil of *E. sativa* were purchased from the local herbal store. Specimens were identified in Taxonomy Lab. in the Department of Botany, Agriculture faculty, Cairo University. Paracetamol drug was obtained from Kahira Pharm & Chem. Ind. Co., Cairo- Egypt. Kits for biochemical analysis were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt. Forty eight adult male of white albino rats (Sprague dawley strain), weighing 185 ± 5 g. were obtained from of National Research Center, Cairo, Egypt. The rat basal diet was performed according to **Reeves et al., (1993)**.

B- Methods:

1-Chemical study:

The fresh *Eruca sativa* leaves were washed with tap water, part of them was ground in a blender and filtered by using funnel and filter paper to obtain juice while other part was dried with hot air oven ($40-60^{\circ}\text{C}$) and grinded to powder. *Eruca sativa* seeds were ground into powder form by using electrical grinder to fine particles (**A.O.A.C., 2005**). Proximate analysis of *Eruca sativa* leaves and seeds powder samples consists of moisture, total fat, protein, ash, fibre and carbohydrate content were estimated according to **Duke and Atchley (1984)**. Some minerals as Zn, Fe, Ca, K and Cu were also determined according to **Pupsa et al., (1994)**.

2- Biological design

After a week for adaptation period, rats were housed as six groups (8 rats each) in wire cages under the normal laboratory conditions. First group was kept as normal group (- ve control) and the others were given a single dose of 2 g/kg of paracetamol by stomach tube for each rat to induce liver injury (**Rafael et al., 1999**). Groups of rats with baracetamol induced liver injury were divided into control +ve group and four treated groups with *Eruca sativa* leaves powder (5%instead of diet cellulose), juice (300mg/kg orally), oil (3ml/kg orally) and seeds powder (5%instead of diet cellulose). Daily food intake, weekly body weight gain and feed efficiency ratio (FER) were determined according to **Chapman et al., (1950)**. At the end of experimental period (42days), rats were anesthetized and blood sample were collected from hepatic portal vein in clean centrifuge tubes. Liver was removed and blotted on filter paper.

4- Biochemical analysis:

The packed cell volume (PCV) was determined by the microhaematocrit method and the haemoglobin (Hb) concentration by the cyanmethaemoglobin method according to **Mc Inory (1954) and Vankampen and Ziglstra (1961)**, respectively.

The blood was left to coagulate then centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum total bilirubin, total protein and urea were determined according to *Jendrassik (1938) Weichselbaum (1946), and Patton and Crouch (1977)*, respectively. Serum aspartate and alanine aminotransferase (AST, ALT) activity, alkaline phosphatase (ALP) and gamma glutamyl transferase (γ GT) enzymes activity were estimated according to *Reitman and Frankel (1957), Kind and King (1954) and Meister et al., (1981)*, respectively. Liver cholesterol, total lipids, triglyceride and glycogen were determined according to *Richmond (1973), Folch et al., (1957), Scheletter and Nussel (1975) and Rerup and Lundquist (1967)*, respectively. Liver glutathione S-transferase (GST), superoxide dismutase (SOD), glutathione peroxidase (GPX), and malondialdehyde (MDA) were estimated according to *Ellman (1958), Beuchamp and Fridovich (1971), Weiss et al.,(1980), and Uchiyama and Mihara (1978)*, respectively.

Statistical analysis

Data are expressed as mean \pm SD. Statistical analysis was done by using analysis of variance (ANOVA) followed by student's t-test and P values of 5% and less were considered to be significant according to *Artimage and Berry (1987)*.

Results

Table (1)

Gross chemical composition of *Eruca sativa* seeds and leaves powder (%)

	Protein	Fat	Fibre	Ash	Moisture	carbohydrate
Seeds	25.77	26.11	3.12	9.11	10.77	25.12
Leaves	15.96	1.06	15.66	10.71	25.67	24.95

The chemical composition of *E. sativa* leaves and seeds was illustrated in table 1. *Eruca sativa* (rocket salad) seeds had higher content of crude protein, fat and carbohydrate (25.77, 26.11, and 25.12, respectively) than rocket salad leaves. However, higher content of fibre, ash and moisture as the value being 15.66, 10.71 and 25.67% was found in *Eruca sativa* leaves.

Table (2)

Some mineral content of *Eruca sativa* seeds and leaves powder (μ g/g)

	Zn	Fe	Ca	K	Cu
Seeds	50.66	58.56	1805.66	703.65	30.66
Leaves	1.25	35.71	655.66	319.96	19.77

Table 2 showed that seeds of *Eruca sativa* had higher content of minerals as the values of Zn, Fe, K and Cu were 50.66, 58.56, 703.65 and 30.66 μ g/g, respectively than *Eruca sativa* leaves. However, *Eruca sativa* leaves had lower content of Ca as the value was 655.66 μ g/g.

Table (3)

The Mean values \pm SD of weight gain, weight gain %, food intake and FER of the experimental groups

		Weight gain	Weight gain %	Food intake	FER
Normal		85.77 \pm 8.20a	46.11 \pm 5.10a	20.71 \pm 1.21a	0.098 \pm 0.001a
Control +ve		20.77 \pm 1.17d***	11.10 \pm 0.88b**	17.77 \pm 1.40b**	0.027 \pm 0.003d***
Eruca sativa	Leaves	60.77 \pm 2.55bc**	32.32 \pm 3.01a	19.55 \pm 1.37a	0.074 \pm 0.004c**
	Juice	65.96 \pm 4.66b**	35.27 \pm 2.96a	19.91 \pm 1.40a	0.078 \pm 0.002c**
	Oil	72.66 \pm 6.80ab	38.64 \pm 3.16a	20.11 \pm 1.67a	0.086 \pm 0.005b*
	Seeds	70.11 \pm 6.8ab	37.09 \pm 3.11a	19.54 \pm 1.31a	0.085 \pm 0.006b*

Significant difference with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Values with the same letters in column indicate non- significant difference (P>0.05)

Data in table 3 represent the paracetamol induced liver injury in rats the control +ve group showed significant decrease in weight gain (P<0.001), weight gain % (P<0.01), food intake (P<0.01) and FER (P<0.001) compared with normal control (- ve) group. Rat groups with liver injury when treated with Eruca sativa leaves powder and juice showed significant decrease in weight gain (P<0.01) and FER (P<0.01) while rat groups treated with Eruca sativa oil and seeds powder showed less significant decrease (P<0.05) compared with normal control group. Treatment rat groups with Eruca sativa leaves, juice, oil and seeds could improve weight gain % and food intake that appeared close to normal values and also increase weight gain and FER compared with control (+ve) group. The most favourable results appeared in rat groups treated with Eruca sativa oil and seeds.

Table (4)

The Mean values \pm SD of total protein, total bilirubin, urea, Hb and PCV of the experimental groups

		Total protein (g/dl)	Total bilirubin (g/dl)	Urea (μ /mg)	Hb (g/dL)	PCV (%)
Normal		0.55 \pm 0.08d	7.33 \pm 0.44a	19.55 \pm 1.70c	13.67 \pm 1.18a	35.71 \pm 3.41a
Control (+ve)		2.11 \pm 0.10a***	5.11 \pm 0.52b***	44.71 \pm 4.03a**	9.54 \pm 1.03b***	27.71 \pm 2.66c***
Eruca sativa	Leaves	0.66 \pm 0.02c*	7.14 \pm 0.80a	26.71 \pm 2.66b*	12.66 \pm 1.11a	32.33 \pm 3.16ab
	Juice	0.61 \pm 0.04cd	6.49 \pm 0.66a	21.71 \pm 2.15c	13.21 \pm 1.16a	43.71 \pm 3.61a
	Oil	0.75 \pm 0.01bc*	6.45 \pm 0.87a	23.14 \pm 2.19bc	12.33 \pm 1.14a	31.21 \pm 3.24a
	Seeds	0.88 \pm 0.02b**	6.77 \pm 0.88a	25.11 \pm 3.20b*	12.11 \pm 1.15a	33.60 \pm 2.96a

Significant difference with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Values with the same letters in column indicate non- significant difference (P>0.05) and vice versa

The activities of total serum protein and urea were significantly increased at p<0.001&0.01, respectively and significant decrease in total bilirubin, Hb and PCV at p<0.001 in control (+ve) group compared with normal (control -ve) group. Liver injury rat groups treated with Eruca sativa leaves and seeds powder showed significant increase in total

protein ($p < 0.05$ & 0.01) and urea ($p < 0.05$) and non significant decrease total bilirubin, Hb and PCV at $p > 0.05$ while rat groups treated with *Eruca sativa* oil showed significant increase in total protein ($p < 0.05$) and non significant decrease total bilirubin, Hb and PCV and also non significant increase in urea at $p > 0.05$ compared with normal control group. Rat groups treated with *Eruca sativa* juice showed that the values of the above mentioned parameters were within normal. Treatment rat groups with *Eruca sativa* leaves, juice, oil and seeds could decrease total protein and urea and significant increase in total bilirubin, Hb and PCV compared with control (+ve) group. The best results appeared in rat groups treated with *Eruca sativa* juice as shown in table 4.

Table (5)
The Mean values \pm SD of ALT, AST, ALP and γ GT of the experimental rat groups

		ALT(μ /ml)	AST(μ /ml)	ALP(μ /ml)	γ GT(μ /ml)
Normal		45.11 \pm 5.31c	50.51 \pm 5.57c	40.31 \pm 4.11c	7.31 \pm 1.43c
Control +ve		139.91 \pm 17.36a***	145.41 \pm 11.20a***	120.77 \pm 10.71a***	18.31 \pm 1.21a***
<i>Eruca sativa</i>	Leaves	56.77 \pm 6.24b*	63.77 \pm 6.31b*	61.91 \pm 6.21b*	10.22 \pm 1.96b*
	Juice	53.27 \pm 6.70bc	59.14 \pm 6.41bc	60.41 \pm 6.71b*	9.50 \pm 1.67bc
	Oil	54.35 \pm 5.66bc	60.14 \pm 6.61bc	50.21 \pm 5.41c	9.14 \pm 1.72bc
	Seeds	59.61 \pm 6.20b*	65.11 \pm 6.03b*	55.11 \pm 5.31bc	10.81 \pm 1.84b*

Significant difference with control (-ve) group * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$
Values with the same letters in column indicate non- significant difference ($P > 0.05$)

Table 5 showed that the activities of serum ALT, AST, ALP and γ GT enzymes were highly significant increased in paracetamol induced liver injury rat group (control +ve) at $p < 0.001$ and in liver injury rat group treated with *Eruca sativa* leaves powder at $p < 0.05$ compared to rats of normal group. Liver injury rat group treated with *Eruca sativa* juice showed significant increase in ALP at $p < 0.05$ and non significant increase in ALT, AST and γ GT enzymes however liver injury rat group treated with *Eruca sativa* seeds powder showed significant increase in ALT, AST and γ GT enzymes at $p < 0.05$ and non significant increase ALP compared to rats of normal group. Rat groups treated with *Eruca sativa* oil showed that liver function enzymes were within normal. Treatment rat groups with *Eruca sativa* leaves, juice, oil and seeds could lower these enzymes and improve liver function compared with control (+ve) group.

Table (6)
The Mean values \pm SD of liver cholesterol, total lipid, triglyceride and glycogen of the experimental rat groups

		Cholesterol(mg/g)	Total lipids(mg/g)	Triglyceride(mg/g)	Glycogen(mg/g)
Normal (-ve)		3.79 \pm 0.69bc	36.71 \pm 3.20bc	3.56 \pm 0.25a	5.99 \pm 0.56a
Control +ve		6.96 \pm 1.21a***	55.71 \pm 5.26a***	1.49 \pm 0.11c***	2.89 \pm 0.23c***
<i>Eruca sativa</i>	Leaves	4.61 \pm 0.54b	39.60 \pm 3.18b	3.07 \pm 0.24b*	4.51 \pm 0.56ab
	Juice	4.21 \pm 0.58b	35.60 \pm 4.01bc	3.17 \pm 0.23ab	4.60 \pm 0.44ab
	Oil	4.31 \pm 0.65b	40.21 \pm 3.99b	3.12 \pm 0.19ab	4.31 \pm 0.54ab
	Seeds	4.11 \pm 0.72b	41.21 \pm 4.52b	3.14 \pm 0.14ab	4.11 \pm 0.60ab

Significant difference with control (-ve) group * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Values with the same letters in column indicate non- significant difference (P>0.05)

Paracetamol administration could increase the levels of liver cholesterol and total lipids and decrease triglyceride and glycogen at p<0.001 compared with the control (-ve) rat group. Liver injury rat group which treated with *Eruca sativa* leaves powder showed non significant increase in the levels of liver cholesterol, total lipids and glycogen and significant decrease in triglyceride at p<0.05 compared to rats of normal (-ve) group. However, Liver injury rat groups treated *Eruca sativa*, juice, oil and seeds showed normal values of these parameters. Treatment rat groups with *Eruca sativa* leave, juice, oil and seeds could lower the levels of liver cholesterol and total lipids and increase triglyceride and glycogen compared with control (+ve) group as illustrated in table 6.

Table (7)
Mean values ± SD of liver GST, SOD, GPX and MDA of the experimental rat groups (mmol/l)

		GST(μ/mg)	SOD(μ/mg)	GPX(μ/mg)	MDA(mmol/l)
Normal		1.35±0.03a	55.16±5.10a	49.41±4.27a	8.66±1.17b
Control +ve		0.65±0.002d***	22.24±2.11c***	20.55±2.66c***	17.85±2.41a***
<i>Eruca sativa</i>	Leaves	0.89±0.004bc**	41.21±4.17b*	35.16±3.66b*	9.11±1.03b
	Juice	1.01±0.05ab	49.61±4.22ab	40.71±5.11ab	8.21±1.15b
	Oil	0.91±0.02b*	47.21±5.14ab	39.61±4.71ab	9.11±1.14b
	Seeds	0.95±0.03b*	43.21±3.15b*	37.61±4.50b*	9.66±1.10b

Significant difference with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Values with the same letters in column indicate non- significant difference (P>0.05)

On paracetamol administration (control + ve) showed lower values of antioxidant liver enzymes as GST, SOD and GPX but showed higher value of MDA at p<0.001 compared with normal (control – ve) group. Liver injury rat groups treated with *Eruca sativa* leaves and seeds showed significant decrease in the levels of liver antioxidant enzymes and non significant increase in the value of MDA compared to rats of normal group. However, Liver injury rat groups treated with *Eruca sativa* juice showed normal values of these parameters while rat group treated with *Eruca sativa* oil showed significant decrease in the levels of liver GST, non significant decrease in the value of SOD and GPX and non significant increase in the value of MDA compared to rats of normal (-ve) group. Treatment rat groups with *Eruca sativa* leaves, juice, oil and seeds could increase values of liver antioxidant enzymes and lower MDA value compared with control (+ve) group as found in table 7.

Discussion

Medicinal plant has nutrient compositions that are essential for the physiological functions of human body. Such nutrients and biochemical's like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life. Minerals are essential for human nutrition and must be obtained from diet (*Adnan et al., 2010*). The obtained results of gross chemical analysis and minerals content of *Eruca* seeds and leaves agreed with *Flanders and Abdulkarim (1985), Ellahi et al., (2007) and Muhammad et al., (2011)*. The improvements in nutritional and growth results represented in table 3 were related to the chemical composition of *Eruca* seeds and phytochemical analysis. Seeds contain various nutrients which are required for food or feed purposes. *Bukhashi et al., (2007)* analyzed the nutrition compounds and trace elements in both leaves and seeds of this plant. The contents of oil depend on many factors including maturity of the seeds and the degree of plant irrigation crude protein is a good source of feed supplement (*Flanders and Abdulkarim 1985 and Villatoro-Pulido et al., 2012*). Both essential and non essential fatty

acids are present in *Eruca* oil as Erucic acid was the main fraction found in the seed oil. Higher concentration of Erucic acid was present in both free and triglyceride form (**Khoobchandani et al., 2010**). Mono and polyunsaturated fatty acids like Oleic acids and linoleic acid were abundant. Presence of these fatty acids support edible uses of *Eruca* seeds indicating that oil contained valuable fatty acids required for edible purposes (**Flanders and Abdulkarim 1985 and Alam et al., 2007**).

The activities of liver function enzymes tended to decrease indicating improved liver function tests. Such reduction of liver enzyme activities and also reduction of total protein and urea concentration exhibit healthy effect of dietary rocket due to high content of sulphur and antioxidant status as reported by **Alam et al., (2007)**

In the present work, *Eruca sativa* has antihyperlipidemic, hepatoprotective and antihyperglycemic effects. These results were in agreement with **Bukhashi et al., (2007)**. Also, the obtained results showed that *Eruca sativa* produced potent antioxidant and renal protective activities. *Eruca sativa* seeds and leaves possess a potent free radical scavenging antioxidants and protect against oxidative damage by increasing and maintaining the levels of antioxidant molecules and antioxidant enzymes (**Bennett et al., 2006**). 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-secretory, anti-ulcer and cytoprotective properties in the ethanolic extract of the plant in rats (**Al-qasomi et al., 2009**). Several studies on phytochemical analysis of *Eruca sativa* leaves has shown the presence of many antioxidant compounds including glucosinolate, flavonoids (Quercetin, Kaempferol and isohamnetin), carotenoids and Vitamine C (**Jin et al., 2009**).

Regarding the present study it could be concluded that *Eruca sativa* produce hepatotreatment and diuretic effects in rats as result of paracetamol induced hepatic injury in rats. These effects could be due to antioxidant activity. Therefore, the study recommends that intake of *Eruca sativa* seeds, oil or leaves may be beneficial for patients who suffer from liver diseases and those on paracetamol therapy.

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التركيب الكيميائي والنشاط العلاجي للجرجير ضد الباراسيتامول المسبب لاصابة الكبد في فنران التجارب

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الملخص العربي

اجريت هذه الدراسة، لتقييم التركيب الكيميائي ومحتوى بعض المعادن لكل من اوراق وبذور الجرجير مع تقدير النشاط العلاجي للتسمم الكبدى الناتج عن تناول مركب الباراسيتامول المستخدم كمسكن للألم وخافض للحرارة وذلك لكل من اوراق أو عصير أو زيت أو بذور الجرجير علي الفنران.

حيث اظهرت نتائج هذه الدراسة المتحصل عليها أن بذور الجرجير تحتوى على كمية كبيرة من البروتين الخام و الدهون والكربوهيدرات والمعادن (الزنك، الحديد، الكالسيوم والبوتاسيوم والنحاس). كما تحتوى أوراق الجرجير ايضا على الألياف والرماد والرطوبة.

وان مجموعات الفنران التى تناولت دواء الباراسيتامول بجرعات عالية حدث تدمير فى خلايا الكبد التى اظهرت زيادة فى الانزيمات الكبدية بينما المجموعات التى تناولت دواء الباراسيتامول مع الجرجير سواء كان عصير أو زيت أو بذور اظهرت تحسن معنوى فى كل من الزيادة فى الوزن ونسبة كفاءة الطعام كما اظهرت النتائج ايضا تحسن معنوى فى مستوى سيرم الدم من البروتين الكلى واليوريا والبيليروبين و الهيموجلوبين وحجم كرات الدم وذلك بالمقارنة مع مجموعة الكنترول المريضة التى لم تناول الجرجير كما اظهرت النتائج تحسن معنوى فى كل من وظائف الكبد من خلال خفض الانزيمات الكبدية والكوليسترول في الكبد و الدهون الكلية والماتوالدهيد كما اظهرت النتائج ايضا ارتفاع معنوى لمستوى الدهون الثلاثية والجليكوجين في الكبد وكذلك الانزيمات المضادة للاكسدة وذلك فى المجموعات التى تناولت عصير او زيت او بذور بالمقارنة مع المريضة .

وتوصى هذه الدراسة باهمية استخدام الجرجير أو منتجاته ضمن الخطة الغذائية لمرضى الكبد وكذلك عند المرضى اللذين يستخدمون دواء الباراسيتامول بجرعات كبيرة ولفترات طويلة لتلاشى الآثار الجانبية لهذا الدواء على اصابة الكبد حيث اثبتت الدراسة فاعليتها فى حماية خلايا الكبد وكمضادات للاكسدة في الفنران

الكلمات المفتاحية :

الجرجير - إصابة الكبد- الباراسيتامول- انزيمات الكبد-مضادات الأكسدة- الفنران