

## Protective and Anti-oxidant Activity of the *Euryops arabicus* against Paracetamol Induced Hepatorenal Toxicity in Rats

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### Abstract

Paracetamol overdose is a predominant cause of hepatorenal toxicity in both humans and experimental animals. The extract of *Euryops arabicus* was evaluated for its protective and anti-oxidant effect against paracetamol-induced hepatic and renal injuries in rats. The extract of *Euryops arabicus* was administered at 100 mg/kg and 200 mg/kg followed by paracetamol at 1g/kg for seven days. The animals were sacrificed after 24 hrs of paracetamol challenge. Indices of liver such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), serum albumin (SA) and renal indices blood urea (BU) and serum creatinine (SC) were measured. Liver and kidney homogenates were analyzed for oxidative stress biomarkers namely Thiobarbituric acid reactive substances TBARS. Finally, histopathological examinations of both previous organs were examined. The significantly disturbed liver and kidney functions by paracetamol toxicity were restored to almost normal values by administration of *Euryops arabicus*. Also, the elevated TBARS in groups received paracetamol alone decreased in groups given paracetamol and *Euryops arabicus*. Histological effect of paracetamol on liver and kidney was also markedly abolished by co-administration of *Euryops arabicus*. These results suggest that *Euryops arabicus* may protect from paracetamol-induced liver and kidney toxicity.

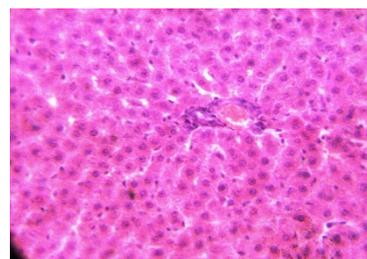
**Keywords:** *Euryops arabicus*; paracetamol; Thiobarbituric acid reactive substances; hepatorenal histopathology; protective and anti-oxidant

### Introduction

Asteraceae is a large family of herbaceous plants contains about 1100 genera and 25,000 species, widely distributed in the tropical and subtropical regions. It is economically important as a source of food, such as lettuce and artichokes, cooking oils, sweetening agents, and tea infusions [1]. Among 100 species of the genus *Euryops*, only *Euryops arabicus* (*E. arabicus*) is known in Saudi Arabia [2].

*E. arabicus* (Jabur) is dome shaped shrub ranged from three to five feet tall with interesting two inches long narrow lobed leathery leaves which are clustered at the tips of the branches. The fall appears in summer and the composite heads of spaced out yellow ray flowers and orange-yellow disk flowers are appeared. It is attractive plant that it has been described as a dwarf pine tree because of its elongated and narrow leaves. It is native to the Arabian Peninsula south to Somalia where the heated leaves and stems were once used in the treatment of wounds. The first description had been done by Ernst Gottlieb von Steudel, a German physician in 1852. The name for the genus comes from the Greek words 'eury' (or 'eurys') meaning "large" or "broad" and 'ops' (or 'opos') meaning "resemblance", "sight" or "the eye" probably in reference to the large eye-like flowers [1-3]. Metabolites from *E. arabicus* were studied for their anti-oxidant, antimicrobial and antiproliferative effects. For instances; flavonoids,

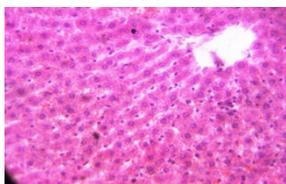
secofuroermophilanes, furoermophilanes and eremophilanolides were identified from the genus *Euryops* [3-6].



**Figure 1:** Group I rat liver served as normal negative control and they received normal saline 1 mL/kg bw, orally, normal architecture (H and E x 200).

Drug-induced liver injury is a potential complication of virtually every prescribed medication, because the liver occupies the main role in the metabolic disposition of all drugs and foreign substances. Most of the hepatotoxic chemicals damage liver cells mainly by lipid peroxidation and other oxidative damages and this applies also to paracetamol which is a widely used analgesic and antipyretic which is safe when used at therapeutic levels [4]. Paracetamol is representing the drug of choice in children. However, paracetamol hepatotoxicity is the leading cause of drug induced liver failure and an acute or

cumulative overdose can cause severe liver injury with the potential to progress to liver failure [5].



**Figure 2:** Group II rat liver served as normal positive control and they received 1% carboxy methyl cellulose 1 mL/kg bw orally, normal architecture (H and E x 200).

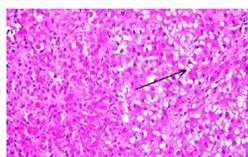
Paracetamol induced toxicity in rats is one of the widely used experimental models to evaluate the hepatoprotective nature of herbal extracts [6]. A computer survey including different databases, especially Scifinder, indicated no publication had been reported about in-vivo anti-oxidant activity of *E. arabicus*.

The main goal of the current study is to evaluate whether the extract of *E. arabicus* has in-vivo antioxidant and protective effect against hepatorenal toxicity induced by paracetamol. The in-vitro antioxidant effect was studied employing the volatile oil which obtained from the aerial part of *E. arabicus* and showed moderate activity [7]. Interesting is the chemical composition of *E. arabicus*, is mainly polyphenolic compounds. Thus the current study is interesting in the in-vivo study its antioxidant effect. A combination of biochemical and histological parameters were used to investigate the protective potential of *E. arabicus* extract on paracetamol induced hepatorenal toxicity.

## Materials and Methods

### Chemicals

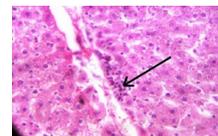
All the materials used for this experiment were of analytical grade. Paracetamol (EPICO Company), thiobarbituric acid and potassium phosphate buffer used for determination of Thiobarbituric acid reactive substances (TBARS) level, carboxy methyl cellulose (CMC) were purchased from Sigma and Merck Chemical Companies. Diagnostic kits for the estimation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum albumin (SA), total bilirubin (TB), blood urea (BU), and serum creatinine (SC) were manufactured by Ranbaxy Diagnostics Ltd. (UK).



**Figure 3:** Group III rat liver received paracetamol at dose of 1 g/kg bw p.o. (paracetamol) showing extensive areas of hepatocellular necrosis and inflammatory cell infiltration. Most of the centrilobular hepatocytes were swollen with marked cytoplasmic vacuolation and pyknotic nuclei with obliterated intervening hepatic sinusoids (H and E x 100).

### Plant material

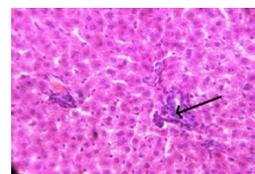
The aerial parts *E. arabicus* (Family Asteraceae) were collected from Al-Taeif, 200 Km south Jeddah, Saudi Arabia. The powdered air-dried *E. arabicus* was extracted and prepared according to Walied et al. [7]. The extract was dissolved in 1% CMC just before administration.



**Figure 4:** Group IV rat liver received paracetamol and 100 mg/kg bw, p.o. of *E. arabicus* leaves extract. H and E staining of liver showing scattered inflammatory cells (H and E x 200).

### Animals

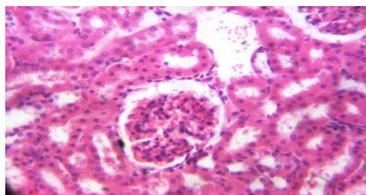
Healthy adult male albino rats weighting about 150-170 grams were obtained from the animal house in Faculty of Science Minia University. All animals were allowed free access to distilled water and laboratory chow ad libitum. To avoid stress of isolation or overcrowdings, 6 rats were housed per cage. They were left freely wandering in their cage for two weeks with 12 hour dark: light cycle for acclimatization before starting the experiment. They were fasted 12h before administration of paracetamol. All protocols used in this study were approved by the Committee of Minia University.



**Figure 5:** Group V rat liver received paracetamol and 200 mg/kg bw, p.o. of *E. arabicus* leaves extract. H and E staining of liver showing few scattered inflammatory cells (H and E x 200).

### Acute toxicity of the extract

The acute toxicity of the extract was performed as per OECD-425 guidelines. Five rats of similar weight were chosen. One animal was fasted overnight with access to drinking water. They were given 2000 mg/kg of the extract and observed for 24h for mortality. The animal survived and then four additional animals were tested sequentially so that a total of five animals were tested. All the animals were observed closely for 24 h and daily for 14 days, no mortality was observed. Hence, we selected 200 mg/kg (1/10th of 2000 mg/kg) as maximum safety dose with descending dose levels with 2 fold interval i.e., 100 mg/kg and 200 mg/kg body weight of the test animal.



**Figure 6:** Group I rat renal tissue served as normal negative control and they received normal saline 1 mL/kg bw, orally normal architecture (H and E x 200).

Rats were divided into five groups of 6 animals each and were given orally the following treatment for seven days.

**Group I:** served as normal negative control and they received normal saline 1 ml/kg body weight, orally (bw, p.o).

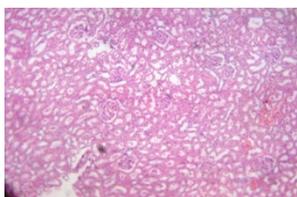
**Group II:** served as normal positive control and they received 1% carboxy methyl cellulose 1 mL/kg bw, p.o.

**Group III:** received paracetamol at dose of 1 g/kg bw, p.o.

**Group IV:** received 100 mg/kg *E. arabicus* leaves extract followed by 1g /kg paracetamol bw, p.o.

**Group V:** received 200 mg/kg *E. arabicus* leaves extract followed by 1g /kg paracetamol bw, p.o.

After 24 h of the last treatment, blood was collected from retro-orbital plexus, allowed to clot for 1 h at room temperature and serum was separated by centrifugation at 2500 rpm for 15 min. The serum was then collected and analyzed for various biochemical parameters.



**Figure 7:** Group II rat renal tissue served as normal positive control and they received 1% carboxy methyl cellulose 1 mL/kg bw orally (H and E x 200).

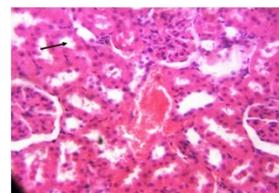
### Serum biochemical assays

The following tests were done to assess the hepatotoxicity of paracetamol AST, ALT, TB, and SA. Kidney functions were looked for by measuring BU and SC. Tests were used according to the standard procedures using commercially available diagnostic kits.

### Oxidative stress

Twenty four hour after the last treatment; rat were decapitated. The liver and kidney tissues were dissected from the surrounding fat and connective tissue. Left lobe of liver and left kidney specimens were longitudinally sectioned, kept at -20°C and subsequently homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The ratio of tissue weight to homogenization buffer was 1:10. The tissue specimens'

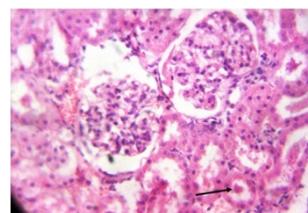
homogenates were centrifuged at 5000 r.p.m for 10 minutes at 4°C. The resulting supernatant was used for determination of TBARS level according to the method of Ohkawa et al. [8].



**Figure 8:** Group III rat renal tissue received paracetamol. H and E staining of kidney showing many tubules with cloudy swelling manifested as enlarged tubules with conical shaped cells, abundant eosinophilic cytoplasm, satellite shaped lumen and luminal cast (H and E x 200).

### Histological studies

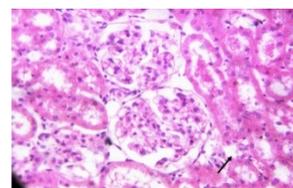
The right lobe of the liver and right kidney was fixed in 10% buffered formalin and was processed for paraffin sectioning. Sections of about 5 ml thickness were stained with haematoxylin and eosin to be examined and photographed [9].



**Figure 9:** Group IV rat renal tissue, received paracetamol and 100 mg/kg bw, p.o. of *E. arabicus* leaves extract. . H and E staining of kidney showing few tubules with cloudy swelling, less luminal cast (H and E x 200).

### Statistical analysis

All data were represented as mean  $\pm$  standard deviation (M  $\pm$  SD). For comparison between groups one-way analysis of variance was used. P value was considered significant if it was less than 0.05.



**Figure 10:** Group V rat renal tissue received paracetamol and 200 mg/kg bw, p.o. of *E. arabicus* leaves extract. H and E staining of kidney showing very few tubules with cloudy swelling with no luminal cast (H and E x 200).

## Results

As shown in table 1 there is significant difference in hepatic and renal biochemistry parameters between the two control groups and paracetamol group indicating toxicity induced by paracetamol. There was significant elevation of AST, ALT, ALP, TB, BU, and SC in group that received paracetamol. Also, there was significant decrease in SA in paracetamol group compared with control ones.

In pretreatment groups with *E. Arabicus* there is significant lowering of the hepatic and renal injury indicators as there is a decrease of the increased level of AST, ALT, ALP, bilirubin, and increase albumin; also, decrease blood urea and serum creatinine.

There is also, much improvement in hepatic and renal function in group pretreated with 200 mg/kg *E. Arabicus* than in that treated with 100 mg/kg.

Table 2 demonstrates thiobarbituric acid reactive substances levels in liver and kidney tissues, there are statistically significant difference between TBARS level between different groups. It much increase in paracetamol group indicating oxidative damage produced by paracetamol but it decrease in rats pretreated with *E. Arabicus*, although it did not reach normal level. There are also little changes between 100 mg/kg pretreated group and 200 mg/kg one.

Groups/ parameters	Liver Function tests					Kidney function tests	
	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TB (mg/dl)	SA (gm/dl)	SC (mg/dl)	BU (mg/dl)
Group I (-ve control)	64.2 ± 1.23	39.8 ± 2.4	120.1 ± 4.3	0.4 ± 0.04	3.3 ± 0.60	0.2 ± 0.85	23.6 ± 3.12
Group II (+veControl)	61.4 ± 2.02	37.7 ± 2.34	115.3 ± 3.81	0.6 ± 0.05	4.1 ± 0.23	0.3 ± 0.39	28.3 ± 4.68
Group III (Paracetamol group)	160.3 ± 3.42***	89.7 ± 0.32***	389.8 ± 16.51***	0.9 ± 0.04***	1.9 ± 0.34***	1.3 ± 0.62***	55.7 ± 5.12***
Group IV (Paracetamol +100EA)	110.7 ± 2.3**	64.2 ± 2.81**	310.2 ± 17.17**	0.7 ± 0.03**	2.6 ± 0.43**	1.0 ± 0.23**	40.2 ± 5.90**
Group V (Paracetamol +200EA)	98.7 ± 1.24*	54.8 ± 1.91*	212.1 ± 14.50*	0.6 ± 0.04*	2.9 ± 0.67*	0.8 ± 0.42*	39.3 ± 4.70*

Data represent Mean ± SD of the each group. \*P <0.05, \*\*P < 0.01, \*\*\*P < 0.001

**Table 1:** Biochemistry levels of the groups.

Histopathological examination also provided supportive evidence for the results obtained from the enzyme analysis. Microscopically, liver slices from control animals stained with haematoxylin and eosin showed normal parenchymal architecture with cords of hepatocytes, portal tracts and terminal veins without noticeable alterations (Figures 1 and 2).

Liver section of paracetamol treated rats showing extensive areas of hepatocellular necrosis and inflammatory cell infiltration. Most of the centrilobular hepatocytes were swollen with marked cytoplasmic vacuolation and pyknotic nuclei with obliterated intervening hepatic sinusoids (Figure 3).

Liver section of rats treated with paracetamol and 100 mg/kg bw of *E. Arabicus* showed less inflammatory cells around central vein and absence of necrosis (Figure 4). Liver section of rats treated paracetamol and 200 mg/kg bw of *E. Arabicus* showed minimal inflammatory cellular infiltration, regeneration of hepatocytes around central vein was also observed and almost near normal liver architecture (Figure 5), indicating the hepatoprotective activity of *E. Arabicus*.

Regarding to the renal histology, group I and II show normal kidney architecture (Figures 6 and 7). In group III; rats received toxic dose of paracetamol, there are many tubules with cloudy swelling manifested as enlarged tubules with conical shaped cells, abundant eosinophilic cytoplasm, satellite shaped lumen and luminal cast (Figure 8). In group IV and V; there was much decrease in the inflammation and cast than paracetamol group with more improvement in group V comparing with group IV (Figures 9 and 10).

## Discussion

Paracetamol induced hepatic injury is considered as one of the most commonly used model for hepatoprotectivity drug screening through estimation of serum cytoplasmic enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum albumin (SA), total bilirubin (TB), blood urea (BU) and serum creatinine (SC) activities is a useful as quantitative markers of the extent, type of hepatocellular and renal affection by paracetamol [10,11]. In the current study, the significant elevation of the enzymes levels, particularly, serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin blood urea, and serum creatinine in the rats which treated with paracetamol, indicate the deterioration of the hepatic functions due to the toxic effects of the drug and consequently, they have been attributed to the damage of structural integrity of the liver, because these enzymes released into the circulation after autolytic breakdown or cellular necrosis [12].

The reduction in the level of hepatic markers by the *E. arabicus* is an indicator of stabilization of the plasma membrane as well as repair of hepatic tissue damage caused by paracetamol. This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [13].

There is statistically significant TBARS level difference as it increases in paracetamol group indicating oxidative damage produced by paracetamol but it decreases in rats pretreated with *E. Arabicus* although it did not reach normal level. There are also little changes between 100 mg/kg pretreated group and 200 mg/kg one. All these results are in agreement with published data [14-16], that indicated the

elevation in TBARS level in paracetamol group is an indicator of lipid peroxidation which is due to paracetamol induced tissue damage.

Groups/parameters	Hepatic TBARS	Renal TBARS
Group I (-ve control)	35.2 ± 5.20	22.9 ± 3.65
Group II (+veControl)	28.8 ± 6.78	21.2 ± 5.34
Group III (Paracetamol group)	93.1 ± 7.41***	37.2 ± 2.12***
Group IV (Paracetamol +100EA)	65.9 ± 7.97**	32.6 ± 1.3**
Group V (Paracetamol +200EA)	60.8 ± 8.11*	28.7 ± 4.3*

TBARS is measured in  $\mu\text{mol}$  of malondialdehyde/mg of tissue proteins. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001

**Table 2:** Thiobarbituric acid reactive substances in liver and kidney tissues.

Histological findings of tissue damage induced by paracetamol in the liver confirmed previous laboratory results that showing extensive areas of hepatocellular necrosis and inflammatory cell infiltration and this coincides with Roomi et al. [17] who found mild and significant lobular focal hepatitis in paracetamol studied group.

Regarding renal histological finding there are many tubules with cloudy swelling manifested as enlarged tubules with conical shaped cells, abundant eosinophilic cytoplasm, satellite shaped lumen and luminal cast. These results are in agreement with Madhukiran et al. [18] who reported that kidneys of paracetamol treated rats, the inflammatory cell infiltration in the interstitium and significant tubular damage were detected. In contrast, Ahmed et al. [19] who observed that the ingestion of paracetamol (500 mg/kg/day) did not produce papillary necrosis nor interstitial nephritis.

Flavonoids isolated from *E. arabicus* had been proved to increase the expression of the rate limiting enzyme in the synthesis of c-glutamylcysteine synthetase with a concomitant increase in the intracellular glutathione concentrations [20-21].

The reactive species mediated hepatotoxicity can be effectively managed upon administration of agents possessing anti-oxidant [22], free radical scavenger [23] and anti-lipid per oxidant activities [24]

A potential of hepatoprotective property underlying *E. arabicus* may be attributed to the polyphenolic principles, particularly, flavonoids. This finding could be explained by the comparing results with those plants that mainly used for treatment of liver disorders. For instance, *Curcuma longa* (turmeric), *Glycyrrhiza glabra* (licorice), and *Camellia sinensis* (Green tea), are reported to be hepatoprotective due to the powerful anti-oxidative properties [25-28]. In addition, the antioxidant properties of *Trichosanthes cucumerina* are attributed to flavonoids, carotenoids, lycopene, phenolics, and  $\beta$ -carotene [29].

## Conclusion

*E. arabicus* has the ability to protect the liver and kidneys from the damaging effects of paracetamol in acute toxic doses and stimulation of endogenous anti-oxidant defense system.

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