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Hepatoprotective effect of *Ecballium Elaterium* fruit juice against paracetamol induced hepatotoxicity in male albino rats

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ABSTRACT

This study was designed to investigate the antioxidant and hepatoprotective activity of *Ecballium elaterium* "Fruit juice" extract against paracetamol induced hepatotoxicity in male albino rats. The hepatotoxicity was induced by acetaminophen (PCM) at dose of 400 mg/kg in male albino rats. It was administered orally once a *day, every 48 h* at the same time for twenty two days. The biochemical liver functional tests ALT, AST, ALP, total bilirubin, total protein, antioxidant enzymes (GR, GPx, CAT, SOD), and histopathological changes were examined. Our results showed that Levels of liver enzymes ALT, AST, ALP, G-GT and total bilirubin and MDA level were significantly enhanced by administration of acetaminophen and level of total protein while antioxidant enzymes "GR, GPx, CAT, SOD" were decreased. However, the pretreatment with The *E. elaterium* "fruit juice" at 1 ml/kg orally revealed attenuation of serum ALT, AST, ALP. The histopathological studies also supported the protective properties of *E. elaterium* "fruit juice". The area of necrosis and degeneration of hepatocytes were observed in the toxic group. The prophylactic and curative groups showed a marked protective effect with decreased necrotic zones and hepatocellular degeneration. The present results clearly demonstrate the marked antihepatotoxic effects of *E. elaterium* "fruit juice" extract through its antioxidant activity on acetaminophen induced hepatotoxicity in rats.

***Key Words*:** ALT, AST, ALP, MDA, SOD, antioxidant.

INTRODUCTION

Acetaminophen (APAP) (paracetamol) is a commonly used analgesic and antipyretic drug and is safe at therapeutic levels, but overdose can leads to potentially fatal hepatic necrosis in humans and experimental model animals (Proudfoot and Wright, 1970; Prescott, 1980; *Kim et al,*. 2009). APAP overdose is one of the most common/frequent causes of liver failure in western world (Lee, 2004). At overdose APAP is metabolized in the liver by cytochrome P450 (CYP) into reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) (Dahlin *et al.*, 1984; James *et al.*, 2003). NAPQI is known to deplete cellular glutathione (GSH), a natural antioxidant level and generate oxidative stress that turn into production of free radicals such as reactive oxygen (ROS) and reactive nitrogen species (NOS) (Mitchell *et al.*, 1973). This results in imbalance of cellular antioxidant defense mechanism in liver hepatocyte cells and thus finally leads to hepatotoxicity (Reid *et al.*, 2005). Due to known mode of its hepatotoxicity APAP is widely used as a model liver toxin for experimental validation of hepatoprotective drugs.

The production of various free radicals and successive oxidative stress leads to adverse effect on cellular level of an organ. Herbal antioxidants are widely used for the treatment and prevention of several diseases (Uttara *et al.*, 2009). Current therapeutic research is directed towards finding naturally occurring antioxidants particularly of plant origin. Many plant species reported to possess potential biomolecules to become a source of hepatoprotective drugs and search is still going on to find the best one. Tangjang *et al.* (2011) has reported the uses of different plant species as hepatoprotective among the traditional medicine practitioners of Arunachal Pradesh, India.

There are several herbal formulation claimed have possessed beneficial activity in treating hepatic disorder. In one of our field survey we found that a widely grown plant *Ecballium elaterium* which has claimed to possess hepatoprotective property. The plant contains many biologically active compounds, includingcontains phenolic compounds, flavonoids "phytomelin" and triterpenoid (Chaudhari *et al.*, 2009).

E. elaterium is a medicinal plant, whose fruit juice is used for the treatment of jaundice in folk medicine (Elayan *et al.*, 1989). In Turkey, the fresh fruit juice of this plant is directly applied into the nostrils for the treatment of sinusitis as herbal folk remedy (Nidaljaradat *et al.*, 2012). The juice of *E. elaterium* is fiber free but contains proteins, lipids, sugars, and minerals (Greige *et al.*, 2007). The antioxidant properties of cucurbitacin B glucosides and cucurbitacin E glucosides (cucurbitacinglucoside combination, CGC) and their direct free radical scavenging properties were established (Andrea, 1997).

MATERIALS AND METHODS

Chemicals

Paracetamol was purchased from Sigma Aldrich, commercial kits to estimate antioxidant enzymes were from Biodignostic company, liver function tests were carried out in Benghazi medical center.

Preparation of *Ecballium elaterium* "fruit juice"

The fruits of *Ecballium elaterium* were well crushed and then refined by filtration using Whatman filter paper No. 4. The refined crude juice was used as a plant material to study its effect in treatment of hepatotoxicity.

Animals

Twenty eight healthy adult male albino rats weighing between 110-120g were used for this study. The animals were kept in polypropylene cages and maintained at 24 ±

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3°C and a constant light-dark schedule (12 hours light and 12 hours dark cycle). The animals were allowed free access standard commercial rat chow (pellet form, in the sack, Benghazi Animal Feed Company, Benghazi, Libya) and water ad libitum.

Experimental design
The rats were adapted to laboratory condition for seven days before commencement of the experiment. Animals were divided into four groups of seven rats each and treated orally as below for 22 days (Roberts *et al.*, 1991)-
Group 1: Rats were fed on the standard diet and served as negative control group.
Group 2: Rats were administered with paracetamol 400 mg/kg body weight in distilled water orally every 48 h and served as positive control group.
Group 3: Rats were administered *Ecballium elaterium* "fruit juice" at a dose of 1 ml/kg body weight orally1 hour before oral administration of paracetamol.
Group 4: Rats were orally dosed daily with a single dose of 300 mg/kg body weight of vitamin C, 1 hour before oral administration of paracetamol.

Sample collection and biochemical assays
The blood samples obtained were collected into plain sample tubes and centrifuged at 1000 rev/min for 5 minutes to separate serum. Serum was carefully withdrawn and kept in eppendrof tubes for the determination of the biochemical parameters.

Assessment of serum marker enzymes
Serum level of ALT and AST, the potential biomarker of hepatic injury was estimated using a commercial kit (Medsource Ozone Biomedicals Pvt. Ltd) (Reitman and Frankel, 1957). Lactate Dehydrogenase (LDH) was determined using the method (Buchl *et al.*, 1978). G-Glutamyltransferase (GGT) by method (Shaw *et al.*, 1983).Serum total protein (T. Protein was determined using the method) (Doumas et al 1981). Serum albumin (ALB) was analyzed by method described by Doumas and Biggs (1972). Serum total bilirubin (T. BIL) was determined using the method (Lott J A 1987). Alkaline phosphatase (ALP) was determined using the method (Tietz *et al.*, 1983) were assayed using standard Diagnostic kits at Benghazi medical center.

Assessment of antioxidant enzymes
The activities of Glutathione reductase (GR) was determined using the method (Goldberg *et al.*, 1983), Glutathione peroxidase (GPx) was determined using the method (Paglia and valentine, 1967). Catalase (CAT) was determined using the method (Aebi, 1984), Superoxide dismutase (SOD) was determined using the method (Nishikimi *et al.*, 1972). Malondialdehyde (MDA) was determined using the method (Ohkawa *et al.*, 1979). . Where assayed in research laboratory of biochemistry in Benghazi medical center.

Histological assessment
At the end of experiments, animals in all groups were scarified dislocation for histopathological studies, Liver samples were fixed in 10% neutral buffered formalin overnight, washed well in running tap water, dehydrated, cleared in xylene and embedded in paraffin. Liver sections were cut into 5µm thickness, processed in alcohol grades and stained in haematoxylin and eosin (H&E) (Luna, 1968) for histopathological examinations. Sections were photographed using canon digital image recorder.

Statistical analysis
Resulting data were represented as mean ± SD. Statistical data was analyzed by T-test, between control vs. all treated groups. A probability level of less than 5% ($p<0.05$) was considered significant.

RESULTS

Serum hepatic marker enzymes status
Oral administration of paracetamol significantly increased the activities of ALT, AST, LDH, G-GT, ALP and T. Bili.by 332.68%, 166.80%, 204.29%, 258.60%, 70% and 340%, respectively. Pretreatment of the rats with *E. elaterium* "fruit juice" at 1ml/kg ameliorated these increases by 61.53%, 51.28%, 56.46%, 62.21%, 35.68% and 40.88%,in ALT, AST, LDH, G-GT, ALP and T. Bili, respectively, when compared with positive group. The levels of total protein and albumin decreased significantly by 49.44%, 66.38% respectively. But the level of total protein and albumin increased by 61.67%, 135.78% after treatment with *E. elaterium* "fruit juice" at 1ml/kg when compared to the paracetamol treated group.

Antioxidant enzymes status
After the exposure of rats to paracetamol only a significant decrease in the activities of the antioxidant enzymes GR, GPx, CAT, and SOD, in comparison to the control group by 55.2%, 53.4%, 51.1%, and 60.7% respectively, but the MDA level shows significant increase by 153.5%. Pretreatment of the rats with *E. elaterium* "fruit juice" at 1ml/kg increase the activity of these enzymes GR, GPx, CAT, and SOD by 89.5%, 72.2%, 77.3%, and 111.5%, respectively, and significant decrease in MDA by 44.3% when compared to the paracetamol treated group.

Histopathology
Aphotomicrographs of liver sections showed (figure 1-4) effect of various treatments: (1) Liver section of rat showing normal architecture of hepatocytes (control); (2) Liver section of rat treated with 400mg/kg of paracetamol. (3) Liver section of rat treated with paracetamol and *Ecballium elaterium* "fruit juice" extract at 100 ml/kg. (4) Liver section of rat treated with paracetamol and vitamin C at 300 mg/kg.

DISCUSSION

Hepatotoxicity is the potential complication of paracetamol, which is widely, used in general medicine and an assessment of its relative toxicity is important. The primary toxicity of paracetamol is the result of drug metabolism in liver. At therapeutic doses, paracetamol is metabolized via glucuronidation and sulfation reactions result in the water-soluble metabolites that are excreted via the kidney. The result of the metabolic conversion of paracetamol by the microsomal P-450 enzyme system is that, a highly reactive intermediate, namely, N-Acetyl-P-Benzoquinone Imine (NAPQI) is produced. This metabolite is then reduced by Glutathione (GSH) (Jaeschkea *et al.*, 2002; Gardner *et al.*, 1998; Gardner *et al.*, 2002).

GSH depletion makes the hepatocytes susceptible to the toxic effects of NAPQI. Its depletion allows NAPQI to binds irreversibly and covalently to cell macromolecules. Primary cellular targets have been postulated to be mitochondrial proteins, with resulting loss of energy production (Doshi *et al.*, 2012).

This process disrupts homeostasis and initiates apoptosis "programmed cell death" leading to tissue necrosis and ultimately to organ dysfunction (Jaeschkea *et al.*, 2002). Because liver damage arising from necrosis or

Table 1: Effect of oral administration of paracetamol alone or with *Ecballium elaterium* "fruit juice" 1ml/kg or with vitamin C on serum liver functions.

Parameter	Control	Positive	*E. elaterium*+ Paracetamol	Vitamin C + Paracetamol
ALT (u/l)	43.45±3.72	188.31±5.43 ^{a,***}	72.43±4.66 ^{b,***}	88.43±5.72 ^{b,***}
AST (u/l)	96.37±4.22	257.12±8.57 ^{a,***}	125.26±4.32 ^{b,***}	138.6±5.78 ^{b,***}
LDH (u/l)	40.54±1.82	123.36±4.52 ^{a,***}	53.71±3.22 ^{b,***}	48.87±5.01 ^{b,***}
G-GT (u/l)	3.72±1.61	13.34±2.31 ^{a,***}	5.04±0.57 ^{b,***}	5.85±0.97 ^{b,***}
ALP (u/l)	150±4.83	255±2.53 ^{a,***}	164±4.75 ^{b,***}	171±3.93 ^{b,***}
T. Bilirubin (mg/dl)	0.45±0.14	1.98±0.36 ^{a,***}	0.66±0.23 ^{b,***}	0.71±0.35 ^{b,***}
T. Protein (g/dl)	8.98±0.80	4.54±0.23 ^{a,***}	7.34±0.66 ^{b,***}	6.84±0.54 ^{b,***}
Albumin (g/dl)	9.31±0.53†	3.13±0.28 ^{a,***}	7.38±0.11 ^{b,**}	6.93±0.47 ^{b,**}

*Significant difference at $P < 0.05$; **highly sig. difference at $P < 0.01$; ***Very highly sig. difference at $P < 0.001$; ↓ Decrease; ↑ Increase; [a]compared with control group; [b]compared with paracetamol positive group.

Table 2: Effect of oral administration of paracetamol alone or with *Ecballium elaterium* "fruit juice" extract or with vitamin C on antioxidant enzymes activities.

Parameter	Control	Positive	*E. Elaterium*+ Paracetamol	Vitamin C + Paracetamol
GR	26.14±0.05	11.71±0.01 ^{a,***}	22.19±0.02^{b,***}	20.37±0.02^{b,***}
GPx	30.72±0.39	14.31 ± 0.03 ^{a,***}	24.65±1.21^{b,*}	21.23±2.14^{b,*}
CAT	46.45±3.99	22.71±3.32 ^{a,**}	40.26±5.01^{b,**}	38.11±4.77^{b,**}
SOD	13.53±1.93	5.31±1.57^{a,***}	11.23±2.21^{b,**}	9.81±2.31^{b,**}
MDA	19.35±3.66	49.05±2.31^{a,***}	27.30±3.09^{b,**}	22.11±4.05^{b,**}

*Significant difference at $P < 0.05$; ** highly sig. difference at $P < 0.01$; *** Very highly sig. difference at $P < 0.001$; ↓ Decrease; ↑ Increase; acompared with control group; bcompared with paracetamol positive group.

membrane damage normally releases the enzymes into circulation; therefore, measurement of these enzymes in serum gives an indication of the health status of the liver. An increase in AST and ALT, a hepatospecific enzymes that is principally found in the cytoplasm in the rats following administration of a hepatotoxin is attributed to the increased release of enzymes from the damaged liver. It is known that an increase in the enzymatic activity of ALT and AST in the serum directly reflects a major permeability or cell rupture (Walubo *et al.*, 2004; Kim *et al.*, 2009; Jin *et al.*, 2012).

Serum LDH, a cytoplasmic marker enzyme and G-GT, a membrane bound enzyme are the well-known indicators of cell and tissue damage by toxic substances and their levels are also substantially increased in paracetamol-intoxicated rats. LDH is an intracellular enzyme, the increase of which in serum is an indicator of cell damage (Gujral *et al.*, 2002). Serum ALP and bilirubin levels on other hand are related to the function of hepatic cell. (Kannan *et al.*, 2013) The decline in total protein and albumin content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases (Fakurazi *et al.*, 2012).

The decreased level of GSH has been associated with an enhanced level of lipid peroxidation in Paracetamol intoxicated groups of rats. Hepatic Oxidative Stress parameters where determined by elevated in the MAD level, while the activities of glutathione reductase (GSH), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) were decreased which consider as sensitive enzymatic index in liver injury caused by ROS and oxidative stress. All these were noted in paracetamol treated group. It is thought to be that fundamental antioxidant enzymes are closely related to the directly elimination of reactive oxygen species. Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Evan *et al.*, 2009).

Administration of *E. elaterium* "fruit juice" (1ml/kg) on paracetamol induced hepatotoxicity resulted in decreased levels of AST, ALT, ALP, LDH, G-GT, bilirubin and elevated total protein and albumin. This effect clearly indicated that "fruit juice" of *E. elaterium* may offer protection by stabilizing the cell membrane in hepatic damage induced by paracetamol. Furthermore it has been reported that *E. elaterium* "fruit juice" decreases the liver marker enzymes during carbon tetrachloride induced hepatotoxicity (Al-Howiriny, 2008; Jaeschkea *et al.*, 2003).

Paracetamol is known to produce oxidative damage in the liver by enhancing peroxidation of membrane lipids, a deleterious process solely carried out by free radicals. Where hepatic peroxides are reported to be increased during oxidative stress (Jaeschkea *et al.*, 2002).The free radicals attack the cell membrane, thus leading to destabilization and disintegration of the cell membrane as a result of lipid peroxidation (Jaeschkea *et al.*, 2003). The depression in GSH contents along with GST activity makes the cells more susceptible to by toxic electrophilic compounds. In this study, the observed decrease in GPx an oxidative type of injury with acetaminophen-induced hepatotoxicity. The decrease in GPx is potentially ascribable to inactivation by the increase in reactive oxygen species (ROS) or lipid peroxides when oxidative damage is extreme (Lauterburg *et al.*, 1983; Smilkstein *et al.*, 1988).

Glutathione reductase (GR) is the enzyme responsible for the reduction of oxidized glutathione (GSSG) to GSH (Mitchell *et al.*, 1973). The level of reduced glutathione and the activities of glutathione-dependent enzymes were reduced significantly in rats administered with acetaminophenin the present investigation. The depression in GR contents along with GPx activity makes the cells more susceptible to toxic compounds. Administration of PCM caused a reduction of GR and GPx levels in plasma.

Catalase (CAT) is one of the most important intracellular antioxidant enzymes in the detoxification of the oxidant hydrogen peroxide. Catalase, widely distributed

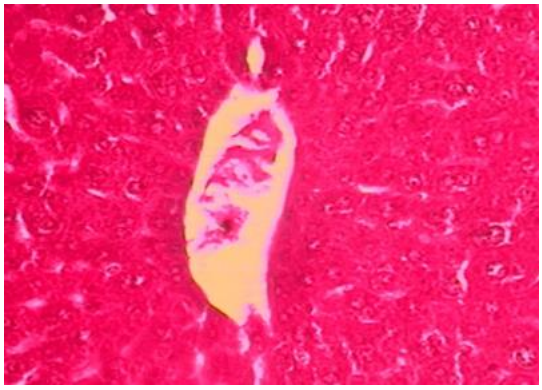

Figure 1: Liver cell of normal rat.

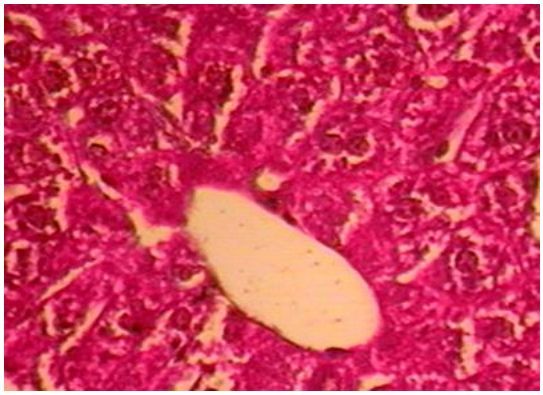

Figure 2: Liver cell of positive rat treated by 400mg/kg of paracetamol.

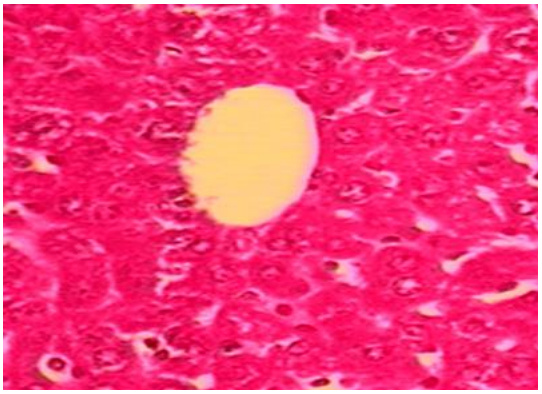

Figure 3: Liver cell treated by *Ecballium elaterium*" fruit juice" at 100 ml/kg.

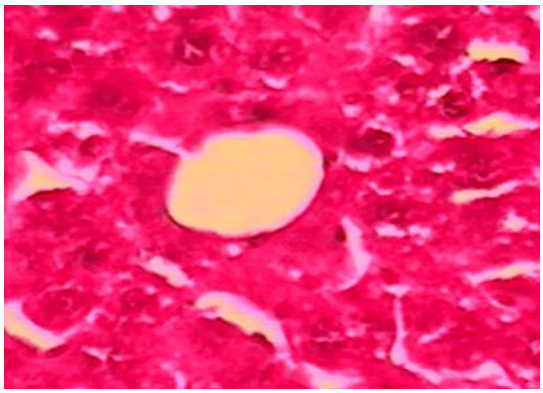

Figure 4: Liver cell treated by vitamin C at 300 mg/kg.

in all animal tissues, decomposes H_2O_2 by dismutating it to H_2O and O_2 (Bhakta *et al.*, 1999) and protects tissue from reactive hydroxyl radicals. The activity of these enzymes was inhibited due to high level of toxic metabolites which produced by administration of PCM that significantly reduced CAT activity, indicating oxidative stress. A decrease in SOD production can be attributed to an enhanced superoxide generation. The activity of SOD enzyme in plasma was also determined. The group treated with PCM showed significantly lower plasma SOD activity than the control group.

In the present study, an elevation in the levels of MDA in the plasma of animals treated with acetaminophen was observed. The increase in MDA level suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Administration of PCM induced oxidative stress with a significant increase in the levels of plasma MDA.

According to our results about the antioxidant enzymes "GR, GPx, SOD and CAT" in addition to the lipid peroxidation indicator "MDA", we may suggest that the therapeutic potential of *Ecballium elaterium* "fruit juice" is dependent on an antioxidant mechanism. These results concluded that *Ecballium elaterium* "fruit juice" inhibition of PCM-induced tissue damage is due to presence of various antioxidant bioactive compounds which mediates its antioxidant effect by scavenging free reactive oxygen species (ROS).

In the present study, the hepatic histoarchitecture of the paracetamol-treated rats resulted severe necrotic changes, inflammatory cell infiltration, fatty degeneration and vacuolization. It might be due to the formation of highly reactive radicals and subsequent lipid peroxidation induced by paracetamol. Administration of *Ecballium elaterium* "fruit juice" reduced the histological alterations provoked by paracetamol quite appreciable. It can be attributed to the antiradical/ antioxidant efficacy of *Ecballium elaterium* "fruit juice" which significantly reduced the oxidative stress leading to the reduction of histopathological alterations and restoration of normal physiological state of the liver.

CONCLUSION

In the present study, *Ecballium elaterium* "fruit juice" possessed strong hepatoprotective in a rat model of paracetamol-induced hepatotoxicity. The hepatoprotective activity of *Ecballium elaterium* "fruit juice" may be due to its free radical-scavenging and antioxidant activity, resulting from the presence of some phenolic compounds in the fruit juice of plant. In general, to use this plant as safe prophylactic agent, more studies should be carried out to know all the active components and their mechanism of actions weather synergistic or antagonist using different doses from this plant and another types of experimental animals for a long period in order to judgment if this plant could be used as safe agents or not in human therapy.

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