In Vitro Antioxidant Activity and Hepatoprotective Potential Of Elaeis Guineensis Leaf Against Paracetamol Induced Damage in Mice

Soundararajan Vijayarathna, Subramanion L Jothy, Kwan Yuet Ping, Lachimanani Yoga Latha, Nadras Othman, and Sreenivasan Sasidharan

Abstract—Elaeis guineensis, a medicinal herb, is commonly used in folk medicine to treat various diseases. The aim of the present study is to evaluate the in vitro antioxidant and in vivo hepatoprotective activity of E. guineensis against experimentally induced liver injury through serum analysis. Serum activity was measured by monitoring the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin. The extract exhibits in vitro antioxidant activity with an IC50 value of 814 μg/mL in the DPPH radical scavenging activity and 37.48 μg/mL in xanthine oxidase inhibitory (XOI) activity. The results of the paracetamol-induced liver toxicity experiments indicated that mice treated with the E. guineensis leaf extract (200 mg/kg) showed a significant decrease in ALT, AST, and bilirubin levels, which were all elevated in the paracetamol treated group (p < 0.01). The hepatoprotective action is likely related to its potent in vitro antioxidant activity.

Index Terms—Elaeis guineensis, hepatoprotective, antioxidant, serum analysis.

I. INTRODUCTION

Although notable development in modern medicine, hepatic disease remains a global health problem, thus the search for new drugs is still ongoing. Hepatic cells participate in a variety of metabolic activities; therefore the development of liver protective agents is of paramount importance in the protection from liver damage. The literature has constantly shown that hepatoprotective effects are associated with plant extracts rich in antioxidants [1]. One of such plant known to have healing potential with various pharmacological activities is Elaeis guineensis Jacq (Arecaceae). E. guineensis has many therapeutic uses in traditional medicine practice. Every part of the plant can be used medicinally. Our previous study expressed that the E. guineensis leaf extract possessed good hepatoprotective activity [2]. In this study we further verified the hepatoprotective activity through serum analysis. Hence, the present study focused on evaluating the potential in vitro antioxidant activity and in vivo hepatoprotective effect of methanolic extract from E. guineensis leaves on paracetamol-induced liver injury in mice serum.

II. MATERIALS AND METHODS

A. Sample Collection

Fresh leaves of E. guineensis were collected from Semeling, Kedah in January 2010. The leaves were separated and cut into small pieces, which were first washed with tap water and then with distilled water. The leaves were then dried in an oven at 60°C for 7 days, after which the dried leaves were ground into fine powder using a grinder.

B. Extraction Procedure

Dried sample (approximately 100 g) was added to methanol (300 mL) and soaked for 4 days at room temperature (30 ± 2 °C). The suspension was stirred from time to time to allow the leaf powder to fully dissolve in the methanol. Removal of the sample from the solvents was done by filtration through cheesecloth followed by filter paper (Whatman No. 1); the filtrate was concentrated under vacuum to one-fifth its volume using a rotary evaporator at 60°C and then sterilized by filtration using a 0.22-mm membrane. The thick paste obtained was further dried in an oven at 40°C. The resultant extract was kept at 4°C for further analysis.

C. Antioxidant Activities

1) –Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

Free radical scavenging activity of E. guineensis was measured using DPPH assay was measured according to the method of Blois [3] and Bondet et al. [4]. Measurements were taken at in triplicate. DPPH radical’s concentrations were calculated and compared to commercial standards; Vitamin E and BHT.

2) Xanthine oxidase inhibitory (XOI) activity

The procedure is based on the principle described by Owen and Johns [5].

D. Animals

Wister albino mice of both sexes were used to study the hepatoprotective activity of the E. guineensis leaf extract. The Institution Animal Ethics Committee has approved the animal study for this project. The animals were kept at 27 ± 2°C, relative humidity 44–56% and light and dark cycles of 10 and 14 h respectively, for a week before and during the experiments. Animals were provided with standard diet (Lipton, India) and water ad libitum. The food was

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293
withdrawn 18–24 h before starting the experiment. All experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals [6].

E. Paracetamol Dose Regimen

Paracetamol tablets were obtained from a nearby clinic. Each tablet contains 500 mg of paracetamol. The dose administered to the mice was set as 1 g/kg. The paracetamol was made into fine powder using a mortar and pestle. The powdered paracetamol was suspended in saline and was administered according to the body weight of mice.

F. Mice Groupings and Treatments

Eighteen mice (25–30 g) were randomly divided into three groups and each group consists of 6 mice (Table I). The first group received 1 mL/kg of saline (control). Group II was given paracetamol (1.0 g/kg) orally and group III received orally both 1.0 g/kg paracetamol and 200 mg/kg of E. guineensis leaf extract respectively. Extract was administered three hours after the administration of paracetamol. Paracetamol 1g/kg was given to mice to induce hepatotoxicity. The treatments were continued for seven days and on the eighth day of the experiment; all animals were anesthetized and dissected [8].

TABLE I: MICE GROUPING AND TREATMENT ADMINISTERED

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 mL/kg of saline of per body weight</td>
</tr>
<tr>
<td>Induced</td>
<td>lg/kg of paracetamol per body weight</td>
</tr>
<tr>
<td>Treatment</td>
<td>lg/kg of paracetamol + 200 mg/kg of extract per body weight</td>
</tr>
</tbody>
</table>

18 adult mice were divided into 3 groups (n = 6).

G. Biochemical Parameters

The mice of each group were anaesthetized with ether, and blood was collected directly from the heart. It was centrifuged at 2,000 g for 10 min at 4 °C to separate the serum and kept at 4°C to assay the activities of serum enzymes. Aspartate aminotransferase (AST) and alanine transferase (ALT) were determined by the method described by Reitman and Frankel, [9]. Serum bilirubin level was estimated according to Malloy and Evelyn [10].

III. RESULTS AND DISCUSSIONS

The antioxidant activities of all of the solutions were determined in terms of the proportion (%) of DPPH scavenged by 1 mg/mL (Fig.1). When the purple DPPH containing unpaired electron receives a hydrogen atom from antioxidants, it becomes a stable diamagnetic molecule, yellow [11]. The E. guineensis methanolic extract exhibited 41.00 ± 0.020% while the standard Vitamin E and butylated hydroxytoluene (BHT) were found with the scavenging activities of 50.14 ± 1.711% and 66.00 ± 0.010% respectively. The radical scavenging activity increases with the increasing amount of extracts. Thus, the radical scavenging activities of E. guineensis extract and the standard compounds, Vitamin E and BHT are passably comparable with the Vitamin E showing lower radical comparatively while BHT contrarily expressed higher radical activity than the extract. Free radicals have long been associated to liver damages and the involvement of paracetamols has been clarified in exerting those damages [12]. Since E. guineensis methanolic extract can scavenge free radicals better compared to Cassia fistula methanolic seeds extract [13], this elucidates that E. guineensis extract fairly comparable and even better than other antioxidant compounds as a good source of natural antioxidant capable of inhibiting free radicals to limit the occurrence of damages to liver.

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**TABLE II: EFFECT OF E. GUINEENSIS LEA EXTRACT ON LIVER MARKER ENZYMES AND SERUM BILIRUBIN CONTENT.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Paracetamol Treated</th>
<th>Extract Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/L)</td>
<td>36.54 ± 4.58</td>
<td>105 ± 10.89**</td>
<td>47.26 ± 7.24**</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>33.42 ± 5.42</td>
<td>89.27 ± 9.27**</td>
<td>36.22 ± 4.88**</td>
</tr>
<tr>
<td>Bilirubin (mg/L)</td>
<td>1.47 ± 0.6</td>
<td>9.1 ± 2.8**</td>
<td>3.1 ± 2.4**</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M; * Statistically significant compared to paracetamol treated animals (p < 0.05); ** Statistically significant to control animals (p < 0.05).

The result of XOII was illustrated in Fig.2 with relevance to linear regression graph, E. guineensis extract (y = 0.2624x + 40.165 and r² = 0.9634) and allopurinol (y = 0.4588x + 49.328 and r² = 0.955). Allopurinol is a known xanthine oxidase (XO) inhibitor and was used as a standard comparison. The IC₅₀ value obtained for E. guineensis through the regression graph is 37.481 µg/mL while the allopurinol’s 1.47 µg/mL. XOIs are essential enzymes richly found in liver actively involved in nucleotide catabolism [14, 15]. When DNAs and RNAs are degraded into purines, XO will then breakdown them to xanthine and uric acid while forming superoxide anions as byproducts [16]. This
superoxide eventuates in oxidative damage on liver tissues [17]. Therefore when antioxidants inhibit XO, this will decrease the generation of the byproducts in purine catabolism ultimately limiting the exaggerative risk on liver tissues.

In this study, the E. guineensis extract stipulated in exhibiting comparatively good inhibition of XO and stands a chance as an alternative in reducing the XO production. The observed in vitro antioxidant activity was further confirmed by in vivo hepatoprotective study. The effect of E. guineensis leaf extract on liver marker enzymes and serum bilirubin content are given in Table II. Based on Table II, it can be seen that, the control group showed a normal range of AST, ALT and bilirubin levels. Nevertheless, the paracetamol treated group, showed higher level AST, ALT and bilirubin. This indicates that, paracetamol caused the liver injury at higher doses. The elevation of AST, ALT and bilirubin. This indicates that, paracetamol caused the liver injury at higher doses. The elevation of AST, ALT and bilirubin levels. Nevertheless, the paracetamol treated group, showed higher level AST, ALT and bilirubin. This indicates that, paracetamol caused the liver injury at higher doses. The elevation of AST, ALT and bilirubin levels. Nevertheless, the paracetamol treated group, showed higher level AST, ALT and bilirubin. This indicates that, paracetamol caused the liver injury at higher doses. The elevation of AST, ALT and bilirubin levels. Nevertheless, the paracetamol treated group, showed higher level AST, ALT and bilirubin. This indicates that, paracetamol caused the liver injury at higher doses. The elevation of AST, ALT and bilirubin levels. Nevertheless, the paracetamol treated group, showed higher level AST, ALT and bilirubin.

In the evaluation of liver damage, the determination of liver function tests enzyme levels such as AST and ALT is higher than the control group, however, it showed a much lower levels of AST, ALT and bilirubin than the paracetamol treated group. E. guineensis leaf extract treatment significantly reduced the raised levels of AST, ALT and bilirubin in hepatotoxic mice. The decrease in the serum levels of these enzymes might possibly be due to the presence of various antioxidant phytochemicals in the E. guineensis leaf extract that enhanced the regeneration ability of liver. E. guineensis leaves extract was used first time to study its hepatoprotective effect at serum level. One way ANOVA (Analysis of Variance) was done where p is less than 0.05 and n=6 to prove the significant of the result.

In the present study, E. guineensis leaf extract possessed good in vitro antioxidant activity and in vivo hepatoprotective activity in a mice model of paracetamol-induced at serum level. The hepatoprotective activity of E. guineensis leaf may be due to its various bioactive compounds in the extracts. Further studies are in progress to better understand the mechanism of action of E. guineensis that is responsible for the hepatoprotective and antioxidant activity.

IV. CONCLUSION

In the present study, E. guineensis leaf extract possessed good in vitro antioxidant activity and in vivo hepatoprotective activity in a mice model of paracetamol-induced at serum level. The hepatoprotective activity of E. guineensis leaf may be due to its various bioactive compounds in the extracts. Further studies are in progress to better understand the mechanism of action of E. guineensis that is responsible for the hepatoprotective and antioxidant activity.

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