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, Lachimanan 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 billirubin. The extract exhibits *in vitro* antioxidant activity with an IC_{50} 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* leave 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.

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Soundararajan Vijayarathna MSc, Subramanion L Jothy MSc, Kwan Yuet Ping, Lachimanan Yoga Latha MSc, and Sreenivasan Sasidharan are with Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Pulau Pinang 11800, Malaysia (e-mail: srisasidharan@yahoo.com).

Nadras Othman is with School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia, 14300 Nibong Tebal, Penang, Malaysia.

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 ± 2ºC, 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

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 [7] 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 ADIISTRATED

Groups	Treatments
Control	1mL/kg of saline of per body weight
Induced	1g/kg of paracetamol per body weight
Treatment	1g/kg of paracetamol + 200 mg/kg of extract per body weight

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 ºC to separate the serum and kept at 4ºC 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.

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

Parameters	Control	Paracetamol Treated	Extract Treated
AST (IU/L)	36.54 ± 4.58	105 ± 10.89**	47.26 ± 7.24*
ALT (IU/L)	33.42 ± 5.42	89.27± 9.27**	36.22 ± 4.88**
Billirubin (mg/L)	1.47 ± 0.6	9.1 ± 2.8**	3.1 ± 2.4*

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 antioxidant activity of all of the solution was determined in terms of the proportion (%) of DPPH scavenged by 1mg/mL.

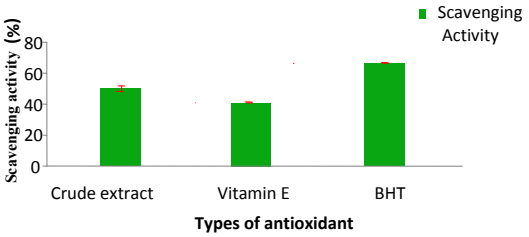

Fig. 1. Types of antioxidants and scavenging activity (P<0.05).

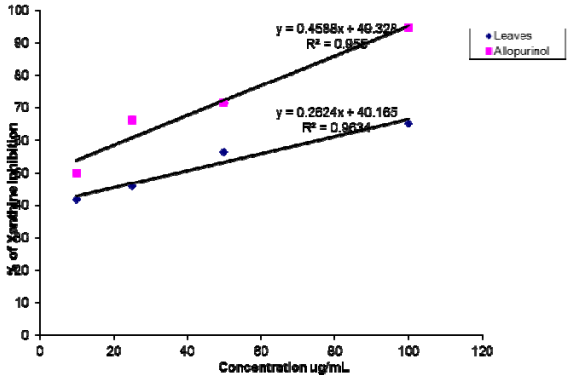

Fig. 2. Percentage of xanthine *oxidase* inhibition activity at different concentrations.

The result of XOI was illustrated in Fig.2 with relevance to linear regression graph, *E. guineensis* extract (y = 0.2624x + 40.165 and r^2 = 0.9634) and allopurinol (y = 0.4588x + 49.328 and r^2 = 0.955). Allopurinol is a known xanthine oxidase (XO) inhibitor and was used as a standard comparison. The IC_{50} value obtained for *E. guineensis* through the regression graph is 37.481 µg/mL while the allopurinol's 1.47 µg/mL. XOs 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 billirubin levels. Nevertheless, the paracetamol treated group, showed higher level AST, ALT and billirubin. This indicates that, paracetamol caused the liver injury at higher doses. The elevation of AST, ALT and billirubin were an indicative for the release of enzymes from disrupted cells. Alternatively, the *E. guineensis* leaf extract treated group showed a very interesting result. Based on the table, the results for *E. guineensis* leaf extract treated group is higher than the control group, however, it showed a much lower levels of AST, ALT and billirubin than the paracetamol treated group. *E. guineensis* leaf extract treatment significantly reduced the raised levels of AST, ALT and billirubin 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 obtained.

In the evaluation of liver damage, the determination of liver function tests enzyme levels such as AST and ALT is largely applied [18, 19]. In the current study, exposure to paracetamol resulted in a significant hepatic damage as elicited by the elevated level of serum marker enzymes, AST and ALT. These marker enzymes are cytoplasmic in origin and are released in to the circulation after cellular damage [20]. The rise in the enzyme AST is usually accompanied by an elevation in the levels of ALT, which plays a vital role in the conversion of amino acids to keto acids [21]. The decrease in the serum levels of these enzymes after the treatment of leave extract, further confirmed the hepatoprotective potential of *E. guineensis* leaf. The results of this study further verified the results of our *in vitro* 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 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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Vijayarathna Soundararajan was born in November 1984 and raised in Penang, Malaysia. She earned her Degree in Biotechnology in 2007 from Asian Institute for Medicine, Science and Technology (AIMST) and her masters in Molecular Medicine in 2012 from Universiti Sains Malaysia (USM). Vijayarathna joined USM in 2010 after two years of serving as LECTURER in the School for General and Foundation Studies at AIMST. Since coming to USM, she has published nearly 7 papers, 3 proceedings and contributed to a book chapter of which her latest articles are as following; The antimicrobial efficacy of *Elaeis guineensis*: Characterization, *in vitro* and *in vivo* studies. *Molecules*, 17(5), p. 4860-77. (2012). Cytotoxicity of methanol extracts of *Elaeis guineensis* on MCF-7 and Vero cell lines**.** *Asian Pacific Journal of Tropical Biomedicine*, p. 826-9.(2012) She is currently working her PhD project on Cytotoxicity and mechanism of actions of *Phaleria macrocarpa* (Boerl.) Scheff in USM. Ms. Vijayarathna had won the most "*Excellent Paper*" award for her oral presentation at 2012 3rd International Conference on Biotechnology and Food Science in Bangkok, Thailand and been nominated for Best Thesis Award by the Institute for Research in Molecular Medicine in USM for her MSc thesis.