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Hepato-protective, antioxidant activities and acute toxicity of a stem bark extract of *Erythrina senegalensis* DC.

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ABSTRACT

This study aimed at evaluating the *in vitro* antioxidant and hepatoprotective activities of different stem bark extracts of *Erythrina senegalensis* prepared with ethanol, and the *in vivo* hepatoprotective activity and acute toxicity of the best extract. The 2, 4-diphenyl-1-picryl-hydrazil (DPPH) and microsomal lipid peroxidation (MLP) models, and the rat liver slices system were respectively used for the *in vitro* study. The Methylene chloride/methanol (1:1 v/v) (Emc) and 40% ethanolic (E40) extracts were more efficient in inhibiting MLP and in scavenging DPPH radical. However, E40 was most effective with regards to lactate dehydrogenase (LDH) leakage inhibition from rat liver slices intoxicated with carbon tetrachloride (CCl_4). The *in vivo* hepatoprotective activity was evaluated against CCl_4-induced hepatotoxicity in rats**.** The E40 extract (100 mg/Kg) significantly reduced the increase in ALT, AST and lipid peroxidation in liver homogenate, showing that the extract is as protective as silymarin at the same dose. Acute toxicity was evaluated in mice and E40 did not produce any behavioural changes or mortality even at an oral dose of 16 g/kg. The extract was found to contain antioxidant classes of compounds (flavonoids and polyphenols). In conclusion, the E40 extract of *E. senegalensis* could be an important source of hepatoprotective compounds.
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Key words: *Erythrina senegalensis*, stem bark extract, antioxidant, hepatoprotective, carbon tetrachloride.

INTRODUCTION

Erythrina senegalensis DC (*Fabaceae*), locally known by the Bamun people in Cameroon as *'Megham njû'* is a thorny shrub or small tree, with a corky stem bark and bright red flowers, found in sudanese savannah regions (Malgras, 1992). The plant material (leaves, stem and roots) is considered to be a traditional source of medicine against various diseases among which are amenorrhoea, urinary bilharziasis, sterility, hepatitis, icteria, liver cirrhosis and the liver disorders (Magassouba et al., 2007; Togola et al., 2005, Moundipa et al., 2002, Malgras, 1992). In addition to these traditional uses, positive biological activities such as antiplasmodial, analgesic, anti-inflammatory, antimicrobial and 15-Lipoxygenase inhibitory activities have also been reported for this plant (Togola, 2008; Saidu et al., 2000). From phytochemical studies, alkaloids and glycosides (Saidu et al., 2000), erybraedin F, erybraedin A, C, D, eryvarin K, phaseollin, shinpterocarpin and the flavone

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carpachromene (Togola, 2008) have been identified as some constituents of the plant.

In screening studies, the methylene chloride-methanol (v/v 1:1) stem bark extract of *E. senegalensis* was shown to have antihepatotoxic potential activity (Njayou et al., 2004). This background prompted us to evaluate the *in vitro* antioxidant and hepatoprotective activities of different stem bark extracts of the plant prepared with ethanol, the *in vivo* hepatoprotective activity and acute toxicity of the best extract.

MATERIALS AND METHODS

Chemicals

All chemicals used in these experiments were of analytical grade and purchased from Sigma Chemical (Dorset, UK) and Prolabo (Paris, France).

Plant material

The stem bark of *E. senegalensis* DC was collected from Foumban (West-Cameroon), in August 2002. The plant was identified at the Cameroon National Herbarium (CNH), Yaoundé, with voucher specimen number 35259 YA.

Preparation of plant extracts

The plant stem bark was air dried and crushed into a powder. Each 100 g of the powder was macerated in a mixture of methylene chloride/methanol (1:1 v/v) (Emc) for 24 h with constant stirring or extracted under reflux for 2 h using distilled water (Ew), 40% ethanol (E40), 70% ethanol (E70) or 92% ethanol (E92) for *in vitro* experiments. Solvents were then removed using a rotary evaporator and oven dried at 40°C to obtain dried extracts. The respective yields were: 4%, 5.74%, 3.32%, 2.62% and 1.88% w/w for the Emc, Ew, E40, E70 and E92 extracts. For the *in vivo* experiments, 1 kg of plant material was extracted under reflux as described earlier.

Animals

Wistar albino rats (150-180 g) and Swiss albino mice (25-30 g) of both sexes were housed in plastic cages and used in this study. The animals were bred in the animal house of the Department of Biochemistry (University of Yaoundé I). They were placed on a semi- synthetic diet (LAVANET, Bockle, Cameroon) and given water *ad libitum*. These animals were handled according to ethical guidance of the Cameroon National Veterinary Laboratory.

Antioxidant activities screening

Free-Radical Scavenging Assay: The free-radical scavenging activities of the plant extracts were determined using the modified method of Brand-Williams et al. (1995). Into test tubes, 10 µl of different extracts or standard (Trolox and silymarin) were added to 1 ml of 0.3 mM DPPH in methanol, giving a final concentration of 200 µg/ml. The discoloration was monitored for 15 seconds by following the decrease in the optical density measured at 517 nm against a DPPH control containing only 10 µl of DMSO in place of the extract. Percentage scavenging activity was calculated using the expression:

Scavenging activity (%)= [(Absorbance of control – Absorbance of sample) / Absorbance of control] x 100.

***Microsomal lipid peroxidation inhibition assay*:** The livers of overnight-fasted 12-week-old male Wistar rats were used for the preparation of microsomes by calcium aggregation (Garle and Fry, 1989). Lipid peroxidation was initiated in the microsomal incubations using iron and ascorbate (Ulf et al., 1987). Five µl of each plant extract or standard (Trolox, silymarin) were added into tubes test to attain a final concentration of 200 µl. An appropriate volume of DMSO was added to the control test tube. After the incubations, the samples were assayed (Will, 1987) for thiobarbituric acid-reactive substances (TBA-RS). Lipid peroxidation was expressed as the change in absorbance of TBA-RS at 530 nm. The amount of TBA-RS which existed in the mixture before the peroxidation reaction was subtracted from the value obtained and the percentage of inhibition calculated using the equation:

Inhibition activity (%) = [(Absorbance of control – Absorbance of sample) / Absorbance of control] x 100.

***In vitro* screening of hepatoprotective activities**

The hepatoprotective effect of the extracts was studied by assessing the lactate dehydrogenase (LDH) leakage from the incubation of rat liver slices. The liver of a 12-week old male albino wistar rat killed by decapitation was sliced into pieces of about 0.5 x 0.5 x 0.5 mm on ice. The pieces were kept in Krebs Ringer Hepes (KRH) medium (Hepes 2.5 mM, NaCl 118 mM, KCl 2.85 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.15 mM, $MgSO_4$ 1.18 mM, Glucose 4 mM, beta-hydroxybutyrate 5 mM, pH 7.4) and incubated in the medium as described by Wormser and Zakinen (1990) with the following modifications. After washing the liver slices by renewing and oxygenating the medium every 10 min for 1 h at 37°C with constant shaking, test slice cultures in capped tubes were treated with plant extracts or silymarin at a final dose of 200 µg/ml. An appropriate volume of the toxin dilution solvent (DMSO) was put in the positive and negative controls. The liver slices were incubated for one more hour in the presence of plant extracts without renewing the medium. Thereafter, slice cultures were intoxicated with CCl_4 (40 mM) and incubated again for 2 h with oxygenation after every 10 min. Aliquots were immediately taken from the tubes after the intoxication and incubation and kept on ice for LDH activity assay (Benford and Hubbard, 1987). Each tube content was then homogenized and centrifuged (3000 rpm, 10 min, 4°C) and the supernatant kept for total enzymatic activity assay. LDH leakage percentage was then calculated according to the following formula:

LDH leakage (%) = [(LDH Activity in S_2 – LDH Activity in S_0) / LDH Activity in S_t] x 100;

S_0: Aliquot sampled from tubes immediately after slice culture intoxication with CCl_4.

S_2: Aliquot sampled from tubes at the end of 2 h incubation.

S_t: Supernatant obtained after homogenization and centrifugation of tube content.

***In vivo* hepatoprotective study**

Dose-response effect: Female Wistar albino rats were used in this experiment. The animals were randomly divided into 6 groups of 4 animals each and treated as follows: Group I animals served as normal controls and received corn oil (toxin vehicle) at 7 ml/kg b/w intraperitoneally (i.p) and normal saline at the appropriate volume orally. Group II animals, which constituted the hepatotoxin group, received 0.3 ml/kg b/w i.p CCl_4 suspended in corn oil and normal saline. Group III orally received the standard drug, silymarin in normal saline at 100 mg/kg b/w and hepatotoxin. Groups IV, V and VI respectively orally received the E40 extract (at 10, 100 and 200 mg/kg b/w) suspended in normal saline in a single administration. The drugs were given 30 min before CCl_4 injection and the animals were sacrificed by decapitation 24 h after its administration. Food was withdrawn 18 h before the sacrifice. Blood was collected and serum prepared. The liver tissue was excised, homogenized in ice-cold Tris-HCl 50 mM, KCl 150 mM pH 7.4 buffer and used for biochemical analysis.

Active extract dose-effect study: Male Wistar albino rats were used for this study. Four groups (I, II, III and IV) consisting of 5 animals each were constituted and treated as follows: Group I served as the control. Group II received hepatotoxin (CCl_4). Group III received silymarin at 100 mg/kg b/w and hepatotoxin and Group IV orally received the efficient dose (100 mg/kg b/w) of the extract and hepatotoxin. The animals of the test groups were given the extract or silymarin suspended in normal saline daily for 7 consecutive days. Simultaneously, control and hepatotoxin groups received normal saline instead of the drug. The rats were weighed every 2 days. On the last day, 30 min after drug administration, the animals of the hepatotoxin and test groups were intoxicated with CCl_4 as in the dose-response effect study, while the control group received the toxin vehicle alone and the experiment continued as above.

Assays: The colorimetric test of Reitman and Frankel (1957) as published by Rodier and Mallein (1983) was used for the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) assays in serum. The total hepatic protein (THP) was assessed by the method of Gornall et al. (1949) using

bovine serum albumin as a standard. Glutathione (GSH) (Ellman, 1959) and thiobarbituric acid reactive substances (TBA-RS) (Olusin, 2002) were assayed in liver tissue. The % of protection was calculated as:

Protection (%) = 100 x (ALT values of CCl_4 control – ALT values of sample)/(ALT values of CCl_4 control – ALT values of control).

General behaviour and acute toxicity study

The procedure followed was that of the World Health Organization (WHO, 1993). Different groups of 12 mice (6 males and 6 females) were fed with increasing doses of the efficient extract (2, 4, 8, 12 and 16 g/kg b/w) in distilled water after 16 h of starvation. One group of mice served as the control and received distilled water. The animals were observed for 1h continuously, then hourly for 4 h for any gross behavioural (locomotion, sensitivity, aggression and respiration) changes, and for a further 14 days with one daily observation. The animals were given food and water 2 h after drug administration. After every 2 days, the mice were weighed, and their diet and water consumption recorded.

Phytochemical study

Groups of phytochemical compounds (flavonoids, polyphenols, leucoanthocyanins, glycosides, tannins, triterpens and sterols, anthranoids) were tested (Harborne, 1984)

Statistical analysis

Data were expressed as mean ± SD. One-way analysis of variance (ANOVA) was used to estimate total variation in a set of data in case of normal distribution or Khruskall-Wallis otherwise. The Student-Newman Keuls t-test was used for group mean comparisons. $P < 0.05$ and $p < 0.001$ were regarded as significant.

RESULTS

***In vitro* antioxidant activity**

DPPH-radical scavenging and microsomal lipid peroxidation inhibition (MLP) rates of the extracts were examined. All the extracts differently scavenged the DPPH free radical and inhibited lipid peroxidation. As shown in Table 1, Emc and E40 extracts as compared to positive control (Trolox and silymarin) were most efficient with percentages of 58.36 ± 1.34 and 46.47 ± 2.43 in DPPH scavenging assay and, 94.25 ± 1.71 and 60.47 ± 2.48 in MLP inhibition test.

***In vitro* hepatoprotective activity**

All the extracts inhibited the enzyme leakage from intoxicated liver slices. Emc and E40 extracts, as compared to the CCl_4 control, showed a significant protective activity ($p< 0.001$) indicated by their respective low LDH leakage percentages (30.24 ± 2.6 and 21.12 ± 3.48) in the studied model of hepatitis. Percentage leakage values were not significantly different from that of silymarin. Data are presented in Table 1.

Hepatoprotective activity

A significant increase ($P < 0.05$) in the activities of serum ALT and AST and the TBA-RS content in the liver of rats treated with CCl_4 only was observed when compared with the control (Table 2). The change effected by the administration of CCl_4 only was significantly reversed ($P < 0.05$) by the simultaneous administration of CCl_4 with the E40 extract. In dose-response effect, 100 mg/kg body weight of the extract was found to be efficient and the dose exhibited 80.60% of hepatoprotection against 82% of silymarin.

General behaviour and acute toxicity

A single oral dose up to 16g/kg of the 40% hydroethanolic extract of *E. senegalensis* was devoid of any lethal effect in mice and no apparent behavioural changes were observed. The weight of the females was reduced to a maximum growth percentage of 15.4 as compared to 28.71% in the control group (Table 3). The diet consumption of the females also significantly decreased ($p< 0.001$) at the 2, 4 and 16 g/kg doses as compared to the controls (Table 4).

Phytochemical study

Many compounds, including flavonoids, polyphenols, reducing sugars, anthocyanins and tanins were tested positive in the E40 extract.

Table 1: Scavenging and inhibiting effects on DPPH radicals, MLP and LDH leakage from CCl_4 intoxicated rat liver slices by extracts from *E. senegalensis*.

	Scavenging/inhibition/leakage percentages		
Extracts	**DPPH**	**MLP**	**LDH**
Control			10.16 ± 1.44
CCl4 control			55.40 ± 1.06*
Trolox	100.00 ± 0.00	93.15 ± 2.15	
Silymarine	66.00 ± 1.66	89.00 ± 1.30	32.50 ± 1.50 **
Ew	40.50 ± 1.60	32.60 ± 1.60	33.28 ± 2.81 **
Emc	58.36 ± 1.34	94.25 ± 1.71	21.12 ± 1.78 **
E40	46.47 ± 2.43	60.47 ± 2.66	30.24 ± 2.60 **
E70	32.36 ± 2.32	42.70 ± 1.11	39.26 ± 1.80
E92	20.30 ± 2.10	20.00 ± 1.82	41.20 ± 1.50

MLP= microsomal lipid peroxidation; DPPH: 2, 4-diphenyl-1-picrylhydrazyl radical, LDH: Lactate dehydrogenase, Ew: water extract of *E senegalensis*; Emc: methylenechloride/methanol (1:1 v/v) extract of *E senegalensis*; E40: 40% ethanol extract of *E senegalensis*; E70: 70% ethanol extract of *E senegalensis* and E90: 92% ethanol extract of *E. senegalensis*. Values are mean ± SD of 2 or 3 experiments in triplicates. Values significantly different at *p <0.05 and **p <0.001 as compared to the control and CCl_4 Control respectively.

Table 2: Effect of different doses of *E. senegalensis* 40% hydroethanolic extract on ALT (UI/l), TBA-RS (nmol/mg tissue), GSH (µmol/mg tissue) and HTP (mg/g tissue) in CCl_4 intoxicated rats.

Treatment	ALT	TBARS	GSH	HTP
Control (G I)	64.40 ± 22.00	0.05 ± 0.01	6.70 ± 0.72	157.94 ± 25.36
CCl_4 (G II)	295.83± 80.61[a]	0.31 ± 0.05[a]	5.97 ± 0.78	140.79 ± 17.98
Si (100mg/kg) (G III)	88.80± 35.08	0.16 ± 0.03[b]	6.65 ± 0.69	147.56 ± 33.07
E40 (10mg/kg) (G IV)	225.84± 101.08	0.23 ± 0.04	5.71 ± 0.18	115.61 ± 18.60
E40 (100mg/kg) (G V)	96.40 ± 34.57	0.18 ± 0.03[b]	6.01 ± 0.49	147.42 ± 20.13
E40 (200mg/kg) (G VI)	197.50 ± 56.98	0.26 ± 0.12	5.61 ± 0.87	117.22 ± 52.42

ALT: alanine aminotransaminase; TBA-RS: thiobarbituric acid reactive substances; GSH: glutathione; HTP: hepatic total protein; E40: 40% hydroalcoholic extract of *E. Senegalensis*; Si: silymarin; G: Group. Values are mean ± S.D, n=4 animals per group. [a]$p < 0.05$, as compared to control group; [b]$p < 0.05$ as compared to CCl_4 group.

Table 3: Effect of *E. senegalensis* 40% hydroethanolic extract and silymarin fed daily for 7 days on transaminases (UI/l), TBA-RS (nmol/mg tissue), GSH (µmol/mg tissue) and total proteins in CCl_4 intoxicated rats.

Treatment	ALT	AST	TBARS	GSH	HTP
Control (G I)	48.66 ± 10.92	94.13 ± 12.39	0.14 ± 0.03	6.42 ± 0.78	242.81 ± 17.05
CCl_4 (G II)	280.92 ± 43.52[a]	305.86 ± 16.04[a]	0.66 ± 0.02[a]	5.0 ± 0.19	223.47 ± 35.29
Si (100mg/kg) (G III)	90.26 ± 15.54	105.47 ± 3.63[b]	0.36 ± 0.01[b]	6.20 ± 0.85	244.19 ± 81.04
E40(100mg/kg)(G IV)	93.73 ± 27.44	110.87 ± 10.78[b]	0.39 ± 0.04[b]	5.84 ± 0.72	245.30 ± 58.39

Values are mean ± S.D, n=5 animals per group. ALT: alanine aminotransaminase; AST: aspartate aminotransaminase; TBA-RS: thiobarbituric acid reactive substances; GSH: glutathione; HTP: hepatic total protein; E40: 40% hydroethanolic extract of *E. Senegalensis*; Si: silymarin; G: group [a]$p< 0.05$, as compared to control group; [b]$p < 0.05$ as compared to CCl_4 group.

Table 4: Body weight gain percentages of mice for 14 days after single administration of 40% hydroethanolic *E. senegalensis* extract.

Doses (g/kg)		0	2	4	8	12	16
Days	**Sexe**						
1	**M**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	F	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3	**M**	3.72 ± 0.43	10.64 ± 0.85	9.00 ± 1.09	6.00 ± 0.82	6.34 ± 0.64	7.79 ± 0.98
	F	16.54 ± 0.89	6.05 ± 0.76	7.32 ± 1.08	7.38 ± 0.87	8.49 ± 0.87	15.41 ± 2.68
5	**M**	4.48 ± 0.56	10.64 ± 0.56	10.42 ± 2.82	8.00 ± 0.91	11.28 ± 1.89	12.04 ± 1.97
	F	21.76 ± 0.78	3.79 ± 0.67	7.32 ± 0.89	6.67 ± 1.07	4.62 ± 0.80	11.37 ± 0.34
7	**M**	8.20 ± 0.45	12.00 ± 1.09	9.71 ± 0.67	8.00 ± 0.78	10.57 ± 2.81	14.17 ± 2.51
	F	18.27 ± 0.03	6.77 ± 0.78	10.54 ± 2.08	3.69 ± 0.54	10.02 ± 0.98	12.20 ± 1.75
9	**M**	5.24 ± 0.37	16.00 ± 0.85	5.54 ± 0.79	9.32 ± 0.32	16.23 ± 0.85	12.04 ± 2.34
	F	23.49 ± 0.87	2.26 ± 0.35	6.49 ± 0.65	5.16 ± 0.78	0.78 ± 0.21	8.10 ± 0.71
11	**M**	6.72 ± 0.68	14.64 ± 2.61	10.42 ± 1.08	10.64 ± 2.09	17.62 ± 1.09	14.17 ± 0.85
	F	26.98 ± 0.89	6.05 ± 0.89	12.20 ± 2.54	6.67 ± 0.86	10.02 ± 0.76	13.80 ± 0.23
13	**M**	11.20 ± 0.96	20.00 ± 2.89	11.08 ± 0.97	10.64 ± 1.86	16.23 ± 0.89	21.28 ± 3.12
	F	28.71 ± 0.86	0.77 ± 0.20	8.93 ± 0.74	6.67 ± 0.86	7.71 ± 1.98	11.37 ± 0.76
15	**M**	17.15 ± 0.84	18.00 ± 1.08	15.96 ± 2.67	16.00 ± 3.61	21.85 ± 3.54	23.40 ± 2.32
	F	11.20 ± 0.67	0.00 ± 0.00	11.37 ± 1.08	10.36 ± 1.86	10.02 ± 0.89	7.32 ± 0.36

Values are mean ± SD (n= 6 animals); M: male; F: female

Table 5: Average diet and water consumption of mice every 2 days for 14 days after single administration of *E. senegalensis* 40% hydroethanolic extract.

Doses (g/kg)		**0**	**2**	**4**	**8**	**12**	**16**
Parameters	**Sex**						
Diet	M	46.66 ± 4.32	51.57 ± 5.71	48.33 ± 5.16	46 ± 9.2	52.28 ± 5.76	50.4± 7.09
consumption (g)	F	54.33 ± 5.46	34.14 ± 4.01[a]	40.42 ± 4.15[a]	42.14 ± 8.0	41.42 ± 4.15	37.14 ± 9.0[a]
Water	M	45.66 ± 7.0	45.5 ± 4.46	51.85 ± 2.8	44.0 ± 4.9	48.14 ± 5.63	50.50 ± 5.28
consumption (ml)	F	41.7 ± 4.95	39.0 ± 3.69	34.83 ± 5.15	40.1 ± 6.75	46.0 ± 4.7	39.42 ± 2.43

Values are mean ± SD (n= 6 animals); M: male; F: female; [a]p< 0.01, as compared to control group

DISCUSSION

Microsomal lipid peroxidation (Martinez et al., 2001; Czinnera et al., 2001) and DPPH radical scavenging (Gupta and Sharma 2010; Zheng et al., 2008) assays are often used for antioxidant activity screening of plant extracts and compounds. The liver slices system has also been used for the assessment of hepatotoxins and their antidotes (Wormser and Zakinen, 1990). The inhibition of MLP and the discoloration of DPPH reveal the antioxidant effect of an extract while the inhibition of LDH leakage from rat intoxicated liver slices indicates its hepatoprotective activity.

The Emc and E40 extracts were found to be efficient in scavenging DPPH radicals and inhibiting MLP (Table 1). Since the DPPH discoloration takes place by the transfer of H° atoms to the DPPH° radical, the structural differences between the chemical compounds present in each extract and bearing transferable hydrogen atoms may explain the different behaviour of the extracts regarding this activity as suggested (Brand-Williams et al., 1995) about the DPPH scavenging efficacy of some phenolic compounds. So, it may suggest that the above-mentioned extracts contain these types of compounds. The E40 extract strongly inhibited the LDH leakage in CCl_4-treated liver slices as compared to controls and this activity was not significantly different from that of silymarin (Table 1). These results show that the E40 extract has antioxidant and hepatoprotective properties. This may be due to the extract's high active compound content since pharmacological effect and active principle content of a medicinal plant depend on its extraction solvent (Mortier, 1990).

The hepatoprotective effect of the E40 extract was then evaluated on CCl_4-induced hepatotoxicity in rats. Protection against CCl_4-induced toxicity has been used as a test for potential hepatoprotective activity by several investigations (Pattanayak and Priyashree, 2008; Iniaghe et al., 2008). It is established that CCl_4 toxicity is mediated by the production of free radicals leading to cellular membrane lipid peroxidation and the leakage of cytosolic enzymes (Recknagel et al., 1989; Wolf, 1999). The ability of an extract to reduce the injurious effect or to preserve the normal hepatic physiological mechanism that has been disturbed by the hepatotoxin is the index of its hepatoprotective effect.

In this study, no matter the pre-treatment modality with the extract, E40 was found to significantly decrease the serum ALT (Tables 2 and 3), AST (Table 3) and liver TBA-RS (Tables 2 and 3) levels as compared to controls ($p < 0.05$). Only the 100 mg/kg dose was effective with regards to the dose-response effect study. The effect of this active extract dose against CCl_4 hepatotoxicity was similar to that of silymarin at the same dose in the two conditions of pre-treatment. This dose was low compared to those used in literature (Pattanayak and Priyashree, 2008; Gilani and Janbaz, 1995), indicating the high hepatoprotective action of the E40 extract. This activity may be due to the presence in the extract of phytoconstituents that scavenge free radicals produced in the course of the metabolism of CCl_4 (Husain et al., 1987).

Flavonoids and polyphenols have been detected in the E40 extract and reported to be antioxidants (Kessler et al., 2003; Middleton et al., 2000). Flavonoids are furthermore known to be hepatoprotective (Kang et al.,

2006, Khalid et al., 2002, Middleton et al., 2000). Thus, the observed *in vitro* and *in vivo* effects of the E40 extract may particularly be attributed to the presence of these classes of compounds. The possible mechanism may be that the antioxidant potentiality of polyphenols and flavonoids can scavenge free radicals and protect the cell membrane from destruction. Hence, the transaminases (ALT/AST) may not leak into the blood from the necrotic hepatocytes.

In the acute toxicity study, there was no significant variation between treated males and the control group in contrast to the female groups (Table 4). Since E40 did not produce any gross behavioural changes or mortality even at an oral dose of 16 g/kg, it may be considered safe. However, the extract may induce anorexia in female mice, leading to a decrease in food consumption and slow weight gain in the female groups (Table 5).

These results show that E40 extract protects against CCl_4-induced liver damage. Our findings therefore provide scientific evidence for the traditional use of *E. senegalensis* in the healing of liver disorders and show a systematic mode of preparation of a more efficient extract of this plant.

In conclusion, the E40 extract of *E. senegalensis* could be an important source of hepatoprotective compounds. Since the CCl_4 toxin induces necrosis, steatosis and cirrhosis (Fleurentin and Joyeux, 1990), the action of the E40 extract may be of clinical importance and may contribute to the prevention of these pathologies.

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REFERENCES

Benford DJ, Hubbard SA. 1987. Preparation and culture of mammalian cells. In *Biochemical Toxicology, a Practical Approach Series*, Snell K, Mullock B (eds). IRL Press: Oxford, Washington; 73-75.

Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol.*, **28**: 25-30.

Czinnera E, Hagymasib K, Blazovicsb A, Szkea E, Lemberkovicsa E. 2001. The *in vitro* effect of *Helichrysi flos* on microsomal lipid peroxidation. *J. Ethnopharmacol.*, **77**(1): 31-35.

Ellman GL. 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys., **82**: 70-77.

Fleurentin J, Joyeux M. 1990. Les tests *in vivo* et *in vitro* dans l'évaluation des propriétés antihépatotoxiques des substances d'origine naturelle. In *Ethnopharmacologie: Sources, Méthodes, Objectifs*, Fleurentin J, Cabaillon P, Mazarj G, Santos JD, Younos C (eds). Actes du 1er colloque Européen d'Ethnopharmacologie, Metz (France) – ORSTOM; 248-269.

Garle MJ, Fry JR. 1989. Detection of reactive metabolites *in vitro*. *Toxicol.*, **54**: 101-110.

Gilani AH, Janbaz KH. 1995. Preventive and curative effects of *Berberis arista* fruit extract on paracetamol-and CCl_4-induced hepatotoxicity. *Phytother. Res.*, **9**: 489-494.

Gornall AA, Bardwill GS, David MM. 1949. Determination of serum proteins by means of the Biuret reaction. *J. Biol. Chem.*, **177**: 751-766.

Gupta VK, Sharma SK. 2010. *In vitro* antioxidant activities of aqueous extract of *Ficus bengalensis* Linn. root. *Int. J. Biol. Chem.*, **4**(3): 134-140.

Harbourne JB. 1984. *Phytochemical Methods. A Guide to Modern Techniques of Plant*

Analysis (2nd Edn). Chapmann and Hall: London.

Husain SR, Cillard J, Cillard P. 1987. Hydroxy radical scavenging activity of flavonoids. *Phytochem.,* **26**: 2489-2491.

Iniaghe OM, Malomo SO, Adebayo JO. 2008. Hepatoprotective effect of the aqueous extract of leaves of *Acalypha racemosa* in Carbon tetrachloride-treated rats. *J. Med. Plants Res.* **2**(10): 301-305.

Kang EH, Kown TY, Oh GT, Park WF, Park SI, Park SK, Lee YL. 2006. The flavonoids ellagic acid from a medicinal herb inhibits host immune tolerance induced by hepatitis B virus antigen. *Antiviral Res.,* **72**(2): 100-106.

Kessler M, Ubeand G, Jung L. 2003. Anti and pro-oxidant activity of rutin and quercetin derivatives. *J. Pharm. Pharmacol.,* **55**: 131-142.

Khalid HJ, Sheik AS, Anwar HG. 2002. Protective effect of rutin on paracetamol and CCl_4-induced hepatotoxicity in rodents. *Fitoter.*, **73**(7): 557-563.

Magassoub FB, Diallo A, Kouyate M, Mara O, Bangoura O, Camara A, Traore S, Diallo AK, Zaoro M, Lamah K, Diallo S, Camara G, Traore S, Keita A, Camara MK, Barry R, Keita S, Oulare K, Barry MS, Donzo M, Camara K, Tote K, Vanden BD, Totte J, Pieters L, Vleitinck AJ, Balde AM. 2007. Ethnobotanical survey and antibacterial activity of some plants used in Guinean traditional medicine. *J. Ethnopharmacol.,* **114**: 44-53.

Malgras D. 1992. *Arbres et Arbustes Guérisseurs des Savanes Maliennes.* Ed. Karthala-ACCT: Paris.

Martinez GM, Giuliani A, Leon-Fernandez OS, Davison GP, Nunez-Selles AJ. 2001. Effect of *Mangifera indica* L. extract (QF808) on protein and hepatic microsome peroxidation. *Phytother. Res.,* **15**: 581-585.

Middleton MJR, Kandaswami CK, Theoharides CT. 2000. The effects of plant flavonoids on mammalian cells: Implications on inflammation, heart disease and cancer. *Pharmacol. Rev.,* **52**: 673-751.

Mortier F. 1990. Préparation des extraits destinés à l'évaluation pharmacologique. In *Ethnopharmacologie: Sources, Méthodes, Objectifs*, Fleurentin J, Cabaillon P, Mazarj G, Santos JD, Younos C (eds). Actes du 1er colloque Européen d'Ethnopharmacologie, Metz (France) – ORSTOM; 199-209.

Moundipa FP, Njayou FN, Yanditoum S, Sonke B, Mbiapo T. 2002. Medicinal plants used in the Bamoun region of the Western province of Cameroon against jaundice and other liver disorders. *Cam. J. Biochem. Sc.,* **12**: 39-46.

Njayou FN, Moundipa FP, Tchana AN, Tchouanguep MF. 2004. Antihepatotoxic potential of three Bamun folk medicinal plants. *J. Cam. Ac. Sc.,* **4**: 325-330.

Olusin SO. 2002. Obesity is an essential independent risk factor for plasma lipid peroxidation and detection of erythrocyte cytoprotenic enzyme in human. *Int. J. Obes.,* **26**: 1159-1164.

Priyashree S, Pattanayak SP. 2008. Hepatoprotective activity of the leave extracts from *Dendrophthoe falcate* (L. F) Ettingsh against Carbon tetrachloride-induced toxicity in Wistar albino rats. *Phcog Mag.,* **4**(15): 218-222.

Recknagel RO, Glende EAJ, Dolack JA, Walke RL. 1989. Mechanisms of carbon tetrachloride. *Pharmacol. Ther.,* **43**: 139-154.

Rodier J, Mallein R. 1983. Enzymologie. Transaminases sériques (S.G.O.T et S.G.P.T). In: *Manuel de Biochimie Pratique*, Maloine SA (ed). Paris ; 396-400.

Saidu K, Onah J, Orisapide A, Olusola A, Wambebe C, Gamaniel K. 2000. Antiplasmodial, analgesic and anti-inflammatory activities of the aqueous extract of the stem bark of *Erythrina senegalensis. J. Ethnopharmacol.,* **71**: 275-280.

Togola A, Diallo D, Dembele S, Barsett H, Paulsen BS. 2005. Ethnopharmacological

survey of different uses of seven medicinal plants from Mali (West Africa) in the regions of Dioila, Kolokani and Siby. *J. Ethnobiol. Ethnomed.*, **1**: 7-15.

Togola A. 2008. Ethnopharmacology, Phytochemistry and Biological Activities of Malian Medicinal Plants. PhD thesis, Oslo University, Norway, p.36-47.

Ulf AN, Lars-I O, Gunnar C, Fallenius ACB. 1989. Inhibition of lipid peroxidation by spin labels. *The J. Biol. Chem.*, **264**: 11131-11135.

WHO. 1993. *Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines*. Regional Office for Western Pacific: Manila. ISBN 9290611103; 1-2.

Wills EO. 1987. Evaluation of lipid peroxidation and biological membranes. In *Biochemical Toxicology, a Practical Approach Series*, Snell K, Mullock B (eds). IRL Press: Oxford, Washington; 127-151.

Wolf PL. 1999. Biochemical diagnosis of liver disease. *Indian J. Clin. Biochem.*, **14**: 59-65.

Wormser U, Zakinen BS. 1990. The slice liver system: An *in vitro* acute toxicity test for assessment of hepatotoxins and their antidotes. *Toxicol. In Vitro*, **4**: 449- 451.

Zheng LP, Gao LW, Zhou JQ, Sima YH, Wang JW. 2008. Antioxidant activity of aqueous extract of a *Tolypocladium* sp. Fungus isolated from wild *Cordiceps sinensis*. *Afr. J. Biotech.*, **7**(17): 3004-3010.

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