Antihepatotoxic Potential of Three Bamun Folk Medicinal Plants

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ABSTRACT

A survey for medicinal plants with antihepatoxic activity from the Bamun popular and traditional medicine was done and 56 plants species were recorded. They were then tested for their antihepatoxic potential activity; their ability to inhibit lipid and microsomal protein oxidation. Ten of them were selected on those basis. We present herein an evaluation of the in vitro antihepatoxic activity for three of the plants. Entada africana, Erythrina senegalensis and Khaya grandifoliola were shown to possess antihepatoxic potential properties, as indicated by the ability of their respective stem bark extracts to inhibit microsomal lipid peroxidation initiated with ascorbate (respective IC<sub>50</sub> values: 50.67 ± 0.46; 33.11 ± 3.78 and 81.70 ± 3.30) and enzymatically with NADPH (respective IC<sub>50</sub> values: 18.33 ± 0.76; 31.75 ± 3.65 and 102.04 ± 2.52). These extracts also inhibited protein oxidation (respective IC<sub>50</sub> values: 9.85 ± 0.66; 8.57 ± 0.23 and 42.04 ± 0.16) and exhibited antiradical activity when tested in the presence of 2,4-dinitrophenyl-1-picrylhydrazyl (2,4-DNPH) (respective EC<sub>50</sub> values: 17.08 ± 0.24; 123.53 ± 9.57 and 12.14 ± 0.04). Using rat liver slices system, extracts were effective in decreasing LDH leakage at the dose of 100μg/ml in the 4 in vitro hepatitis models with the following respective protective percentage (carbene etrachloride: 63.33, 65.26 and 65.26 %; D-galactosamine: 59.29, 64.17 and 63.33 %; paracetamol: 96.67, 93.89 and 94 %; tert-butylhydroperoxide: 80, 72.73 and 68.75 %). Phytochemical studies of these extracts revealed the presence of flavonoids and polyphenols. These classes of compounds are known to be antioxidant and therefore support the observed properties.

Key words: medicinal plants; lipid peroxidation; protein oxidation; antiradical; hepatitis in vitro.

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INTRODUCTION

Entada africana, Erythrina senegalensis and Khaya grandifolia are three plant species used in the Bamun region for the treatment of hepatitis and other liver related hazards (Moundipa et al., 2002).

Since hepatitis and others degenerative diseases are often associated with the oxidative destruction of lipids and proteins (Dean et al., 1997; Anuoma, 1998; Kehrer, 2000), the plants used by the Bamun in order to cure this disease may contain antioxidant compounds which have been suggested as prophylactic agents (Anuoma, 1997, 2002). Thus, these plants may possess antihepatotoxic properties. However, no systematic pharmacological screening of most of these plants has been reported so far.

This work aimed to evaluate the in vitro antihepatotoxic activity of the three above mentioned plant species by testing their efficiency as antioxidant and hepatoprotective.

MATERIALS AND METHODS

Chemicals: All reagents used in this study were purchased from Sigma Chemicals Company (St. Louis, MO, USA) and Prolabo (Paris, France).

Plant extracts: Stem bark powders methanol-methylene chloride (1:1 v/v) were used and tested at the doses 10, 100 and 200μg/ml.

Antioxidant activities screening

Lipid peroxidation assay: Liquid peroxidation assays procedure as described by Garle and Fry (1989). Experiments were carried out following the method described by Ulf et al., (1989). Lipid peroxidation was non-enzymatically initiated using ascorbate or enzymatically by NADPH. The samples were assayed for thiobarbituric acid-reactive substances (TBA-RS) as described by Wills (1987).

BSA oxidation assay: BSA was oxidised by a Fenton-type reaction and its carbonyl content determined as 2,4-dinitrophenylydrazine (DNPH) derivatives by the method described by Martinez et al., (2001).

Antiradical effect assay: The 2,4-DPPH in methanol was used and decolorated according to the method described by Brand-Williams et al., (1995).

Hepatoprotective properties study

The hepatoprotective action of the extracts was studied by assessing the LDH leakage from rat liver slices according to the method described by Benford and Hubbard (1987). Liver slices were intoxicated either with carbon tetrachloride (CCI4 40mM), D-galactosamine (D-galN 100mM), paracetamol (APAP 30mM) or tert-butyl hydroperoxide (tBH 15mM) as indicated by Wormser and Zakine (1990).

Phytochemical study

Groups of phytochemical compounds (flavonoids, polyphenols, leucoanthocyanins, alkaloids, tannins, triterpenes and steroids, anthranoids) were tested.

Calculations and statistical analysis

Different IC50 values were estimated using the EPA probit analyses. LDH leakage percentages were analysed by ANOVA test and the probabilities P<0.05, P<0.001 considered as significant.

RESULTS

Antioxidant activities screening

For lipid peroxidation and protein oxidation, all the extracts strongly inhibited these biochemical phenomena as indicated by their low IC50 values in Figures 1 and 2. Values were less than 200μg/ml irrespective of the mode of lipid peroxidation initiation. The same observation was done with regard to their antiradical activities (Figure 3).

Hepatoprotective activity study

Generally, the inhibition of the enzyme leakage was dose dependent and all the three plant extracts significantly increased (P<0.05) the LDH leakage in

<p>| Table 1: Phytochemical composition of plant extracts |
|---------------------------------|---------------|-------------|-----------|----------|-------------|---------|-----------|----------|</p>
<table>
<thead>
<tr>
<th>Classes of compounds</th>
<th>Species</th>
<th>Flavonoids</th>
<th>Triterpenes</th>
<th>Sterols</th>
<th>Alkaloids</th>
<th>Polyphenols</th>
<th>Tannins</th>
<th>Anthranoids</th>
<th>Leucoanthocyanins</th>
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<tbody>
<tr>
<td>Erythrina senegalensis</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Khaya grandifolia</td>
<td>+</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>+</td>
</tr>
<tr>
<td>Entada africana</td>
<td>+</td>
<td>-</td>
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<td>+</td>
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</tbody>
</table>
Figure 1: Half inhibiting concentration of plant extracts depending on the mode of lipid peroxidation initiation.

Figure 2: Half inhibiting concentration of plant extracts in protein oxidation.

Figure 3: Half effective concentration of plant extracts in DPPH discoloration.
Figure 4: LDH leakage from rat liver slices incubated in the presence of CCl4 and different doses of plant extracts. Values are means ± SD of two experiments in duplicate and significantly different from intoxicated control to *p<0.05; **p<0.001

Figure 5: LDH leakage from rat liver slices incubated in the presence of D-galN and different doses of plant extracts. Values are means ± SD of two experiments in duplicate and significantly different from intoxicated control to *p<0.05

Figure 6: LDH leakage from rat liver slices incubated in the presence of tBH and different doses of plant extracts. Values are means ± SD of two experiments in duplicate and significantly different from intoxicated control to *p<0.05; **p<0.001

Figure 4: LDH leakage from rat liver slices incubated in the presence of CCl4 and different doses of plant extracts. Values are means ± SD of two experiments in duplicate and significantly different from intoxicated control to *p<0.05; **p<0.001
all the four models of hepatitis. Leakage percentages values were not significantly different from that of silymarine. Data are presented in figures 4 to 7.

**Phytochemical study**

Phytochemical studies of active plant extracts revealed the presence of flavonoids polyphenols among others classes of compounds as shown in table I.

**DISCUSSION**

The low values of different IC₅₀ allow us to suggest that these extracts were all active against lipid peroxidation process (non-enzymatically or enzymatically induced) and BSA oxidation. The protection of hydroxyl-mediated oxidation of BSA takes place essentially by reducing the H₂O₂ concentration, a fundamental component in Fenton-type reaction, by chelating iron or by scavenging the hydroxyl formed on the immediate side during oxidation on the target protein (Kingu and Wei, 1997). Thus, it may suggest that plant extracts tested in this study were able to scavenge hydroxyl or chelate iron.

Plant extracts were otherwise tested for their antiradical activity in order to appreciate their efficiency to scavenge free radicals. According to the EC₅₀ values obtained, *Entada africana* and *Khaya grandifoliol* extracts may be classified as extracts that quickly discolores the DPPH solution and that of *Erythrina senegalensis* as the one which slowly discolorates it. Since the DPPH discoloration takes place by H⁺ atoms transfer to the DPPH° radical, structural differences of chemical compounds bearing the transferable hydrogen atom are present in each extract may reasonably explain the varied behaviour of the extracts as suggested by Brand-Williams et al., (1995) when analysing the antiradical efficacy of 20 phenolic compounds.

The liver slice system in four different models was used to assess the hepatoprotective effect of the extracts. Considering the results model by model, all the 3 plant extracts, at the dose of 100μg/ml, exhibited significant(p< 0.05) protective effect, compared to intoxicated control, against injuries induced in rat liver slices by any of the toxins used in this study. Since there are differences between the mechanisms of CCl₄, D-galN, APAP and tBH toxicity (Recknagel and Glende, 1973; Mandl et al., 1989; Masaky et al., 1989; Fleurentin and Joyceux, 1990), these extract may contain a mixture of hepatoprotective compounds. Some of these compounds may protect against certain toxins and others against others toxins as it was shown for other hepatoprotective plants (*Hikino* and kiso, 1988) and *Melothria naderaspanata* (Thabrew et al., 1995).

Flavonoids and polyphenols were identified in these 3 plant extracts. In another studies (Wendji et al., 1995), flavonoids had been detected in *Erythrina senegalensis* extract. Thus, the observed inhibitory effect against lipid peroxidation, free radical-mediated degradation of BSA, LDH leakage from intoxicated rat liver slices by *Entada africana*, *Erythrina senegalensis* and *Khaya grandifoliola* extracts and their antiradical effect may be attributed to the presence of flavonoids and polyphenols as many of these phytoconstituents are known to be antioxidants and antiradical (Fauré et al., 1990, Markus et al., 1996).

**CONCLUSION**

*Entada africana*, *Erythrina senegalensis* and *Khaya grandifoliola* extracts exhibited strong antioxidant and hepatoprotective effects in four different in vitro hepatitis models. These extracts also contain flavonoids and polyphenols. The potential antipherotoxic of these species is therefore likely. The confirmation of this antipherotoxic potential activity by in vivo studies is indicated.

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**REFERENCES**


