Acute and subchronic toxicity assessment of the dichloromethane / methanol stem bark extract from *Erythrina senegalensis* DC (Fabaceae) in Wistar rats

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*Erythrina senegalensis* is a medicinal plant used in sub-Saharan Africa to treat pain, asthenia, inflammation, jaundice, malaria and infections. This work aimed to study the phytochemistry, the acute and subchronic toxicity of the dichloromethane / methanol stem bark extract from *E. senegalensis*. The phytochemical groups have been characterized in the extract. The content of total polyphenols and flavonoids was determined using the colorimetric methods of Folin-Ciocalteu and aluminum trichloride respectively. Acute toxicity was assessed in NMRI mice and the 28-day subchronic toxicity in Wistar rats by oral gavage. Total polyphenols and flavonoids had a high content in the extract. The DCM / MeOH extract, up to 1200mg/ Kg of body weight (bw), did not cause any mortality or modification of the behavior of the treated mice. In the subchronic toxicity study by oral administration of 60 and 120mg/ Kg of bw of extract, neither the relative weight nor the biological parameters of renal or hepatic impairment were significantly different from a group treated with the control group (p > 0.05). Anatomopathological examination revealed no signs of toxicity of the extract on the heart, lungs, spleen, liver and kidney. *E. senegalensis* is rich in polyphenols. The doses administered did not induce any significant toxicity.

**Keywords:** *E. senegalensis*; Total polyphenolic content; Acute toxicity; Subchronic toxicity

INTRODUCTION

Traditional plant-based medicine is the first resort for almost 80% of the African population (WHO, 2005) because this medicine is less expensive and still promises to cure. Unfortunately, many plants are used in traditional medicine without any knowledge of their toxicity.

*Erythrina senegalensis* DC (Fabaceae) is an ornamental and one of the oldest medicinal plants widely used by people in West Africa (Dalziel, 1948). Trunk and root bark extracts are used in the traditional treatment of pain, inflammation, rheumatism, jaundice, malaria, venereal diseases, bronchial infections and dermatoses (Nacoulma, 1996; Togola et al., 2008). The mixture of stem bark powder and leaf is taken with soup to treat threats of abortion, female infertility and fibroids (Iwu, 1993; Nacoulma, 1996). Root extracts are used in cases of asthenia. Extracts of leaves and flowers are indicated in the treatment of oral diseases (gingivitis, stomatitis), hemorrhoids and have also astringent, antiseptic and healing properties. Wood is used as a toothbrush and an aphrodisiac (Nacoulma, 1996).

Numerous studies have shown that the methanol / dichloromethane extracts from the stem bark of *E. senegalensis* are very rich in active substances for therapeutic interest.
2,3 dihydroauriculatine (Taylor et al., 1986; Linuma et al., 1994), Erythraea A (Wanjala et al., 2002) and 6,8-Diprenylgenisteine are antibacterial and antiviral (anti-HIV) isoflavonoids isolated from the plant. Sigmoidin H (Kuete et al., 2014), Alpinumisoflavone, Derrone (Matsuda et al., 2007), Erysneagalenseine (Nkengfack et al., 2001) and Erythraeana A (Rukachaisirikul et al., 2007), other flavonoids isolated from E. senegalensis have shown antiproliferative effects in various experimental cancer models.

The acute and subchronic toxicity of aqueous extracts from the stem bark of E. senegalensis has been studied. This toxicity has been considered weak by Saidu et al. (2000) and Atsamo et al. (2011). The polar extract with chloroform (CHCl₃) showed some toxicity and the lethal dose 50% (LD₅₀) intraperitoneally (ip) was estimated at 526 mg / Kg (Udem et al., 2010).

The acute and subchronic toxicity of the dichloromethane / methanol extract (1: 1) is not known to date, despite the great therapeutic potential of this extract. The objective of this work was to study the acute and subchronic toxicity by oral administration of the dichloromethane / methanol extract (1: 1) from the stem bark of E. senegalensis in order to approach the toxicity of substances biologically active isolated from the plant for therapeutic interest.

MATERIALS AND METHODS

Reagents, solvents and equipment

The set of materials for this study consisted of solvents: Dichloromethane (Carlo Erba, France), Methanol (Merk, Germany); the reagents were: Folin–Ciocalteu reagent (Sigma–Aldrich, Germany), sodium carbonate (Na₂CO₃) (Carlo Erba, France), Gallic acid (Sigma - Aldrich, Germany), Aluminium trichloride (AlCl₃) (Carlo Erba, France), Quercetin (Sigma–Aldrich, Germany). The equipment and other materials that was needed in this study consisted of Rotary evaporator type ROTAVAPOR BUCHI® RE 11 coupled to a vacuum pump; Ultraviolet lamp (254 and 366nm); Analytical balance (Fischer Scientific, Analytical series, modele PAS214C, max 210 g s 0,0001 g, France); Percolator and other various glassware; Magnetic stirrer and magnetic bar; Vortex type agitator; Freeze dryer (Christ Alpha 1-2 LD plus, Germany series 19971); Ventilated oven (Memmert, W Germany series 840452); spectrophotometer (Thermo Fisher Scientific, Genesys 30 model, 9A1W198106, USA).

Laboratory animals and ethical approval for animal studies

12-week-old male (39 – 41 g) and female (37 – 39 g) NMRI mice were used for the test of acute toxicity. Subchronic toxicity was carried out on male and female 9 weeks old Wistar rats. The males weighed between 300 – 315 g and the females between 280 - 300g.

All the animals were obtained from the animal facility of the International Center for Research and Development on Livestock in Subhumid Areas (CIRDES) in Bobo - Dioulasso (Burkina Faso). The animals were placed in cages containing wood chips renewed every 2 days. They were cased according to sex in three (03) individuals per cage and maintained under standard laboratory conditions. The temperature of the experimental room was under constant ventilation and maintained at 23 ± 2 °C with an estimated relative humidity of 50%. Artificial lighting was also provided, alternating sequences of 12 hours of light (day) and 12 hours of darkness (night). All animals had free access to tap water and food. They were fed standard commercial laboratory food fortified with 29% protein (in pellet form). All the procedures used in this study were in accordance with the "principles of good laboratory practice" of the NIH (n ° 85-23) revised in 1985 and approved by the Research Institute in Health Sciences (IRSS) from Burkina Faso where this study was conducted.

Plant material

The stem bark of *Erythrina senegalensis DC* (*Fabaceae*) were collected in January 2017 in Banflawè, a village located 70 km from Bobo - Dioulasso (Burkina Faso). A specimen, after authentication by a botanical expert (Dr. GANABA Souleymane, National Center for Scientific and Technological Research) was registered under number 8709 at the National Herbarium of Burkina (HNBU). Therefore, the stem bark was dried in the open air and out of the sun for ten (10) days. They were then reduced to a coarse powder using a mortar and pestle.

Preparation of extract

A mass (150 g x 2) of the sample was placed in a glass vial with a 2,000 mL screw cap. Every time, 750 mL of extracting solvent (dichloromethane + methanol 1: 1, v / v) was added. The mixture was homogenized and left to macerate with magnetic stirring for 24 h. It was transferred to percolator, then exhausted by leaching with small amounts of the extracting solvent until to deplete the plant sample. The extract obtained was concentrated under reduced pressure on a rotary evaporator. The concentrated extract was frozen at -12 ° C, then lyophilized under high vacuum at -52 ° C for 24 h. The mass of the dry extract obtained as well as the extraction yield were determined relative to 100 g of dry vegetable drug.

Phytochemical analysis

The chemical characterization was carried out according to the method described by Ciulei (1982) and adapted by the chemistry laboratory of the Institute for Research in Health Sciences (IRSS). Thus, sterols and triterpenes were sought according to the Liebermann-Burchard (H₂SO₄ concc) reaction, alkaloids using the Dragendorff (bismuth nitrate + K iodide) and Mayer (Mercury chloride + K iodide) reagent, anthracenes according to the Bornträger (NH₄OH 25%) reaction, coumarins and derivatives according to the Feigl-Frehden-Anger reaction, flavonoids according to the Shibata reaction (Cyandin test) and anthocyanosides using sodium hydroxyde tablet (NaOH). The tannins were characterized using Stiasn reactiv (Formol 40% m/v + HCl 1N 1:1) and completed by ferric chloride (FeCl₃) at 2% in alcoholic solution. The presence of saponosides in the extracts was highlighted by the foam index which corresponds to the height of a column of foam formed by at least 1 cm and persistent for 15 minutes after vigorous stirring for 15 minutes.

Estimation of total phenolic

The Folin–Ciocalteau method was used to determine total phenolic content as described by Meda et al (2005) with slight modifications. The extract was first solubilized in methanol (1.0 mg / mL). The working solution (0.1mg/mL) was obtained by taking 0.5 ml of this solution to 5.0 ml by adding distilled water. This working solution (0.125 ml) was then mixed with 0.625 ml of 0.2 N Folin–Ciocalteau reagent for 5 min and 0.5 ml of 75 g/L sodium carbonate (Na₂CO₃) was then added. After incubation at room temperature for 2 h, the absorbance of the reaction mixture was measured at 760 nm against a blank (distilled water at 1%
methanol).

The gallic acid (0 to 200mg/l) standard calibration curve was used to graphically determine the concentration of total phenolic in the extract \(y=4.668.10^{-2}x-0.034\), \(r^2=0.9991\). The result is expressed in mg of Gallic Acid Equivalent per 100 milligrams of the extract (mg EAG / 100 mg).

**Estimation of total flavonoid contents**

The total flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand et al. (1994). Briefly, 0.625 ml of 2% aluminium trichloride (AlCl\(_3\)) in methanol was mixed with 0.625 ml of extract (0.01mg/ml) in methanol. Absorbance reading at 415 nm was taken after 10 min against a blank sample consisting of 1.25 ml of methanol without AlCl\(_3\).

The total flavonoid content was determined using a standard curve of quercetin \(y=1.259.10^{-2}x\), \(r^2=0.9990\) from a range of concentrations from 0 to 50 mg / L. The results expressed in mg of Quercetin Equivalent per 100 mg of the extract (mg EQ / 100 mg).

**Assessment of the acute toxicity of the extracts**

The acute toxicity of the extracts from *E. senegalensis* was evaluated by oral gavage of the NMRI mice as described by Atsamo et al. (2011). Six groups were made up of equal numbers of mice, five (05) males and five (05) females. The animals were acclimated for one week before the start of the tests. Fourteen (14) hours before treatment, they were fasted.

Each of the six (06) groups received respectively a dose equal to 0, 150, 300, 600, 900 and 1200 mg / Kg of body weight in the form of an oral suspension prepared in 400 μl of isotonic glucose solution at 2% of Tween 80. This volume was administered twice 20 μl at 30 minute intervals via oral gavage, using an appropriate curved, ball-tipped stainless-steel feeding needle connected to a syringe at the appropriate concentration. The control group (group 1) received only the solvent used to prepare the extract suspensions (400 μl IGS at 2% Tween 80). After treatment, the animals were placed under observation for a period of 14 days. Behavioral changes (aggression, drowsiness, refusal to eat) and mortality were noted.

**Assessment of the subchronic toxicity of extracts of *E. senegalensis***

Three groups were made up of equal numbers of rats, five (05) males and five (05) females. The doses were prepared by suspending the appropriate masses (60 or 120 mg/Kg of bw) in 500 μl (10 mL/Kg of bw) of isotonic saline at 2% Tween 80 (IS at 2% Tween 80). The control group (group 1) received only the solvent used to prepare the extract suspensions (500 μl IGS at 2% Tween 80). The other 2 groups received the DCM / MeOH extract suspension of *E. senegalensis* at doses of 60 and 120 mg / Kg of body weight. All treatments were administered orally only once a day for 28 days. The animals were observed over the entire period and cases of abnormal behavior were noted.

At the end of the treatment, the animals were put to 12 hours of fasting except free access to water. The blood was taken and the animals sacrificed after petroleum ether anesthetia.

**Determination of biochemical parameters of toxicity**

Whole blood (2.0 - 2.5 mL) was collected by intracardiac puncture using a sterile 5ml syringe with a 24G needle on top of rats etheral with petroleum ether. One (01) milliliter was transferred to heparinized tubes (lithium heparinate gel). The samples were kept at rest for 2 hours at room temperature and then centrifuged at 4000 rpm for 5 minutes. The collected plasma was aliquoted for biochemical analyzes using the Architect-type automatic analyzer (c4100, Abbott diagnostics, France). The parameters measured were: alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (PAL), total cholesterol, triglycerides, total protein, glucose, creatinine, urea, total bilirubin, conjugated bilirubin and blood ions (sodium, potassium, calcium and chloride).

**Measurement of relative body and organ weights**

Wistar rats' body weights were recorded on day 0 (P0), day 7 (P7), day 14 (P14), day 21 (P21) and day 28 (P28) after treatment.

The average relative body weight was calculated using the body weight P0 of each group as the denominator according to the following formula according to Atsamo et al. (2011) [17]:

\[\text{Relative body weight (rBW)(%)} = \left(\frac{P_i}{P_0}\right) \times 100\]

Where P0 is the weight of the rat before the start of treatment \(i = \text{ordinal number of the weighing days (0, 1, 7, 14, 21, 28)}\) and Pi the weight of the treated rat measured on day 1, 7, 14, 21 and day 28.

By appropriate dissection, the heart, liver, spleen, lungs and kidneys of each animal were removed and weighed (Sartorius type balance, Germany, sensitivity 0.001g). The relative weight of each organ (rOW) was determined according to the following formula:

\[\text{rOW(in%) = \left(\frac{\text{organ weight}}{\text{animal body weight}}\right) \times 100}\]

**Histopathological analysis**

Using petroleum ether, the animals were anesthetized and then sacrificed. The carefully removed and weighed organs (heart, liver, lungs, spleen and kidneys) were examined for a morphological anomaly and then fixed for one (1) week in NaCl buffer at 10% formaldehyde and immediately transported to the laboratory of pathological cytology and anatomy at the Souró SANOU University Hospital Center for anatomopathological analyzes.

Slices of section made perpendicular to the axis of each organ, allowed to obtain three (03) samples in healthy and lesiona areas. These samples were subjected to impregnation using the VIP6 automated device, then coated with paraffin. Thin sections 4 μm thick with a microtome were spread on microscope slides and then stained with hematein - eosin - safron (HES). The observation was made under an optical microscope (Olympus brand at 400 x magnification).

**Statistical analysis**

Data were presented as mean ± standard deviation from the mean (m ± SEM).

The values of the treated groups were compared with those of the control group analyzing the variances (ANOVA) at the significance level of 0.05 using Microsoft Excel 2016 for Mac. In the event of a significant difference, the precision on the different groups was provided by the Bonferroni test.

**RESULTS**

**Phytochemistry**

The dichloromethane / methanol (1: 1) extraction of 300 g of *E. senegalensis* stem bark made it possible to obtain a total mass of
Table 1. Phytochemical groups characterized in the dichloromethane / methanol extract (1: 1) from the stem bark of E. senegalensis.

<table>
<thead>
<tr>
<th>Chemical groups sought</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids and triterpenes</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids (aglycones and glycosides)</td>
<td>++</td>
</tr>
<tr>
<td>Saponosides</td>
<td>++</td>
</tr>
<tr>
<td>Emodols</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins et derivatives</td>
<td>+</td>
</tr>
<tr>
<td>Anthracenosides</td>
<td>+</td>
</tr>
<tr>
<td>Anthocyanosides</td>
<td>nd</td>
</tr>
<tr>
<td>Alkaloids (base and salt)</td>
<td>nd</td>
</tr>
<tr>
<td>Cardiotonic heterosides</td>
<td>nd</td>
</tr>
</tbody>
</table>

++ = abundant; + = scarce; nd = not detected

干 extract equal to 57.70 g (extraction yield of 19.23%). In the extract, the color reactions allowed to characterize the presence of flavonoids, tannins, saponosides, coumarins, anthracenosides, emodols, triterpenes and sterols (Table 1). Total phenolics were estimated at 82.0 ± 1.0 mg gallic acid equivalent per 100 mg of extract and total flavonoids at 47.1 ± 0.5 mg quercetin equivalent per 100 mg of extract. Alkaloids (base or salt), anthocyanosides and cardiotonic heterosides were not detected in the extract of stem bark of E. senegalensis with DCM / MeOH (1: 1).

Acute toxicity

After 2 hours of treatment, the higher doses of the extract (900 and 1200 mg / Kg of bw) reduced the mobility and the aggressiveness of the mice which appeared calmer compared to the control group. The administration of increasing doses (from 150 to 1200 mg / Kg) of the DCM / MeOH (1: 1) stem bark extract of E. senegalensis did not cause any mortality in the six (06) groups of mice. The median lethal dose (LD₅₀) of E. senegalensis was then greater than 1200 mg / Kg of bw.

Sub-chronic toxicity

Relative body and organs weights during the treatment

Weight gain was gradual in each group during the four (04) weeks of treatment (Figure 1). The mean relative weight in the treated groups was not significantly different from that of the control group (p> 0.05; one-way ANOVA) regardless of the sex of the rats. In order to detect a potential influence of sex on the response (variation in weight) of the animals to the extract of E. senegalensis, males and females were monitored separately. Body weight gain was significantly greater in female rats than male rats regardless of the week considered or the dose received (p <0.012; 2-way ANOVA for sex and doses comparison followed by Bonferroni correction).

In order to explore a possible morphological alteration of the heart, liver, kidneys, lungs and spleen their relative weights were determined. In the group treated with DCM / MeOH extract (1: 1) at a dose of 120 mg / Kg of body weight, a slight non-statistically significant increase in the mass of the kidneys (Table 2) was noted compared to the control group (p = 0.058).
Table 2. Effect of doses of E. senegalensis DCM / MeOH (1: 1) extract on relative weights of organs in rats treated for 28 consecutive days

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control</th>
<th>60 mg/kg/day</th>
<th>120 mg/kg/day</th>
<th>ANOVA test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.32 ± 0.02</td>
<td>0.31 ± 0.08</td>
<td>0.36 ± 0.02</td>
<td>0.37</td>
</tr>
<tr>
<td>Liver</td>
<td>3.54 ± 0.25</td>
<td>3.56 ± 0.22</td>
<td>3.88 ± 0.26</td>
<td>0.44</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.70 ± 0.04</td>
<td>0.71 ± 0.02</td>
<td>0.82 ± 0.05</td>
<td>0.058</td>
</tr>
<tr>
<td>Lung</td>
<td>0.63 ± 0.07</td>
<td>0.64 ± 0.06</td>
<td>0.66 ± 0.04</td>
<td>0.92</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.23 ± 0.02</td>
<td>0.24 ± 0.05</td>
<td>0.27 ± 0.01</td>
<td>0.29</td>
</tr>
</tbody>
</table>

The results are presented in the form m ± SEM. The comparison between the control group and the treated groups was carried out using One way ANOVA. There is no statistically significant difference (p > 0.05) between the values of the relative organ weight in treated groups and those in control group.

Table 3. Biochemical parameters in rats treated with E. senegalensis DCM / MeOH (1: 1) extract for 28 consecutive days.

<table>
<thead>
<tr>
<th>Blood parameters</th>
<th>Values of parameters according to doses</th>
<th>ANOVA test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>60 mg/kg/day</td>
</tr>
<tr>
<td>Renal function parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>56.08 ± 2.98</td>
<td>55.50 ± 2.28</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>5.35 ± 0.16</td>
<td>4.93 ± 0.60</td>
</tr>
<tr>
<td>Hepatic function parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase (UI/L)</td>
<td>171.00 ± 14.39</td>
<td>181.00 ± 9.37</td>
</tr>
<tr>
<td>Alanine aminotransferase (UI/L)</td>
<td>41.17 ± 2.80</td>
<td>39.17 ± 3.33</td>
</tr>
<tr>
<td>Alkaline phosphatase (UI/L)</td>
<td>98.50 ± 9.25</td>
<td>93.50 ± 4.22</td>
</tr>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>2.27 ± 0.12</td>
<td>2.32 ± 0.05</td>
</tr>
<tr>
<td>Conjugated bilirubin (µmol/L)</td>
<td>3.00 ± 0.79</td>
<td>2.90 ± 0.55</td>
</tr>
<tr>
<td>Metabolic function parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>7.27 ± 0.31</td>
<td>7.33 ± 0.95</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>55.67 ± 4.12</td>
<td>56.00 ± 3.42</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>1.73 ± 0.09</td>
<td>1.70 ± 0.06</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.88 ± 0.15</td>
<td>0.94 ± 0.13</td>
</tr>
<tr>
<td>Ionogramm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (Na+) (mmol/L)</td>
<td>145.67 ± 0.92</td>
<td>145.00 ± 1.46</td>
</tr>
<tr>
<td>Potassium (K+) (mmol/L)</td>
<td>6.48 ± 0.98</td>
<td>4.92 ± 0.47</td>
</tr>
<tr>
<td>Calcium (Ca2+) (mmol/L)</td>
<td>2.72 ± 0.11</td>
<td>2.75 ± 0.07</td>
</tr>
<tr>
<td>Chlorure (Cl-) (mmol/L)</td>
<td>103.67 ± 0.76</td>
<td>103.50 ± 1.25</td>
</tr>
</tbody>
</table>

The results are presented in the form m ± SEM. The comparison between the control group and the treated groups was carried out using One way ANOVA. There is no statistically significant difference (p > 0.05) between the values of the biological parameters of the treated groups and those of the control group.

Biochemical effects of repeated doses of E. senegalensis extracts

The purpose of the evaluation of the blood concentration of biochemical parameters (Table 3) was to identify a possible subchronic toxicity of the DCM / MeOH extract (1: 1) like the hepatic cytolysis (ASAT and ALAT), the cholestasis syndrome (PAL, bilirubins), the kidney damage (creatinine, urea), the ionic disorders (ionogram) and the metabolic disorder (total proteins, glucose, cholesterol).

There was no statistically significant difference (p > 0.05) between the biochemical parameters of the groups treated with DCM / MeOH extract (1: 1) at doses of 60 mg / Kg and 120 mg / Kg of body weight compared to the control group (Table 3).

The DCM / MeOH extract appeared to induce no liver cytolysis, no cholestasis syndrome, no kidney damage, no ion disorder or no metabolic disorder in rats at doses of 60 mg / Kg and 120 mg / Kg of body weight.

Histopathological analysis of organs

A histological analysis of major organs was carried out in order to explore possible organ toxicity. On macroscopic examination, the major axis of all the organs was measured. These were hearts measuring between 1.8 - 2 cm, liver 4 cm, lungs measuring 3.5 cm, kidneys measuring 2 cm and spleen 3.5 cm long axis. Microscopic examination of the histological sections and cross-sections showed an absence of histological alteration (Figure 2). The histological structure of the heart, liver (without Councilman’s body, portal inflammation or fibrosis), kidneys, lungs and spleen was normal.

DISCUSSION

Erythrina senegalensis is a medicinal plant whose extracts are widely and long used in the traditional treatment of many
Flavonoids, tannins, saponosides, sterols and triterpenes were characterized in this extract as in previous work by Taylor et al. 1986 and Wandji et al. 1994 and 1995. Total phenolics were estimated at 82 ± 1 mg GAE / 100 mg of extract. The flavonoid content was estimated at 47 ± 0.5 mg QE /100 mg of extract. The flavonoids isolated from trunk extracts of *E. senegalensis* are by far the best studied (Taylor et al., 1986; Linuma et al., 1994; Matsuda et al. 2007; Kuete et al., 2014). Alkaloids have not been characterized in the stem bark of *E. senegalensis* by Dragendorff and Mayer reagent. These results once again corroborate a previous work that did not highlight alkaloids (Fofana et al., 2016).

The extraction yield (19.23%) with the dichloromethane / methanol mixture (1: 1) of the stem bark of *E. senegalensis* was better than those found with dichloromethane alone or methanol alone using the soxhlet (3.9% and 1.2%) (Togola et al. 2009) or percolation (4.24% and 2.2%) respectively. The dichloromethane / methanol mixture (1: 1) offered an intermediate polarity between the non-polar and polar solvents which could extract a large number of chemical principles.

The study of acute toxicity by single oral administration of increasing doses (150 to 1200 mg / Kg of bw) of DCM /MeOH extract of *E. senegalensis* did not cause any mortality after 24 hours or after observation for 14 days. The toxicity profile of the DCM / MeOH extract (1: 1) was similar to that of the aqueous extracts reported in the works of Saidu et al. and Atsamo et al. (2011). The 50% lethal oral dose of DCM / MeOH extract (1: 1) is likely to be in excess of 1200mg / kg of body weight. The DCM / MeOH extract (1: 1) administered orally seemed less toxic than the a polar extract with chloroform reported by Udem et al. (2010). The chloroform extract at a dose of 1200 mg / kg i.p. had induced the death of all the animals in the group (Udem et al. 2010). In the present study, the signs of toxicity were fairly simple but marked by a reduction in the mobility and aggressiveness of mice as noted in the work of Atsamo et al. (2011). This behavior of mice was considered to be a depressive effect by this author. However, Matsuda et al. (2007) isolated from the stem bark extract of *E. senegalensis*, Alpinumisoflavone, an isoflavonoid endowed with inhibitory properties of mouse brain monoamine oxidase (Han et al. 2005). This effect would rather be considered as antidepressant. It was simply fear and reaction of animals to the administration by a feeding tube and not a sign of toxicity.

For subchronic toxicity, doses of 60 and 120 mg / kg were administered orally against a control group who received solvent (IS at 2% tween 80). No mortality and no behavioral abnormalities were observed during the study. These results are in agreement with those of Saidu et al. (2000) and Atsamo et al. (2011) who have worked on aqueous extracts. A longer observation (84 days) after oral administration of chloroform extract did not show unusual behavior in animals (Udem et al., 2010).

A gradual increase in body weight that was not statistically significant (p> 0.05) compared to the control group was noted in animals during the experiment. These signs did not appear to be related to the toxicity of the DCM / MeOH extract but to young rats that had not yet completed their growth. Apart from the slight non-statistically significant increase in renal mass in the group treated with DCM / MeOH extract at doses of 120mg/kg/day, there were no morphological particularities of the organs analyzed. There is no renal function impairment assessed through the appearance of the histological structure of the renal parenchyma and the values of the blood concentration of

![Figure 2: Histological sections of rat organs in untreated and treated groups with DCM / MeOH extract (1: 1) for 28 days. Hematein - Eosin - Saffron (HES) staining; Magnification 400. There is no lesion in treated group compared to control group.](image-url)
compared to the control group, the toxicity was observed with the doses administered after 28 days of treatment and monitoring.

The DCM / MeOH extract (1: 1) did not cause hepatic cytolysis or cholestasis syndrome in rats treated for 28 days. The activity of transaminases (ASAT and ALAT) is increased in hepatic cytolysis (Bénichou et al., 1996). The activity of alkaline phosphatase and the blood concentration of bilirubin (total and conjugate) are increased in cholestasis syndromes (Bénichou et al., 1996). In the present study, in addition to the normal histological structure of the liver, these hepatic markers in the treated groups were not significantly different from those in the control group (p > 0.05). There was no hydro electrolytic disorder or metabolic syndrome. In fact, the blood concentrations of glucose, total cholesterol, triglycerides, total proteins and ions were not significantly different (p > 0.05) in the groups treated with the extract of E. senegalensis for 28 days. Compared to the control group, the extract studied did not exhibit metabolic toxicity.

The pathology study did not show any morphological or microscopic abnormalities of the sections of the lungs, spleen and heart. The kidney, lungs and liver are major organs in the metabolism of drugs and xenobiotic administered by general route. That is why, an impairment of these functions would considerably modify the pharmacology or toxicology of these substances (Ramaiah et al., 2011).

The low toxicity of the extract seemed to indicate a certain harmlessness of the main phytochemical groups which are characterized therein. Indeed, some of these phytochemical groups are known to be non-toxic (Gao et al., 2017; Valiki et al., 2017; Qin et al., 2009; Cerdaă et al., 2003; Zhang et al., 2012) and others are even considered to be hepatoprotective (Rodriguez et al., 2001; Ning et al., 2018) or nephroprotective (Wang et al., 2018).

**CONCLUSION**

This study showed that the dichloromethane / methanol extract (1: 1) of *Erythrina senegalensis* contain various phytochemicals such as sterols / triterpenes, flavonoids, tannins and saponosides.

Acute and subchronic toxicity studies revealed that the plant extract was not very toxic. Oral administration of a single dose of the extract did not cause any mortality in the treated animals. No significant variation in biological and pathological signs of organ toxicity was observed with the doses administered after 28 days of treatment and monitoring.

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**Conflicts of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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