Effect of total aqueous stem bark extract of *Spondias mombin* L. on some biochemical and anthropometric parameters in wistar albino rats

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**Abstract**

*Spondias mombin* L. (Anacardiaceae) is a medicinal plant used in the treatment of digestive disorders in Côte d’Ivoire. In order to verify its safety, the total aqueous stem bark extract is administered orally daily for 28 days, three groups of albino Wistar rats at 250, 500 and 1000 mg / kg body weight. The control group received distilled water. Measurements are made to determine the daily amount of food and the volume of water consumed, the body mass is evaluated each week. A blood sample is taken once a week to determine the effect of the extract on blood biochemical parameters. Repeated administration of total aqueous stem bark extract of *Spondias mombin* has not deeply disturbed consumption of food and water in all treated rats. Weight gain was observed in all rats throughout the study. Blood biochemical parameters such as glucose, cholesterol and Glutamate Oxaloacetate Transaminase (SGOT) were not changed in rats treated compared to the control group. In contrast, Glutamate-Pyruvate Transaminase (SGPT), creatinine and urea to a lesser degree, increased particularly in patients treated with doses of 500 and 1000 mg / kg body weight to the fourth week of treatment. These studies revealed that the total aqueous stem bark extract of *Spondias mombin* may be slightly toxic to the liver and kidney when taken in high doses prolonged way.

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Introduction

Herbal remedies, major remedies of traditional medicine, have been used for thousands of years and have made a great contribution to the maintenance of human health (Bannerman et al., 1983). This practice continues today in many parts of the world because of its biomedical benefits and cultural beliefs (WHO, 2002). However, the growing interest in herbal remedies has created the need for greater precision in their preparation and evaluation. For this purpose, WHO (2002) recommends evaluation of quality, safety and efficacy of herbal medicines. In this context, *Spondias mombin* L. (Anacardiaceae) is used for the treatment of diarrhea in Côte d'Ivoire, has been the subject of research.

*Spondias mombin* is a tree 12-25 m tall with a trunk covered with thick and rough bark (Adjanohou and Ake-Assi, 1979). The leaves alternate, odd-pinnate, pairs of opposite or alternate leaflets. The fruits are ovoid or ellipsoid drupe, yellow when ripe, sweet, more or less acidic.* Spondias mombin* is widely used in traditional medicine for many indications plant. The leaves are commonly used for the treatment of eye diseases, diarrhea, dysentery and painful colic (Kerharo and Adam, 1974). The leaves are prescribed for their diuretic, laxative proprieties and even purgative while the stem bark are used to relieve pain and prevent excessive bleeding during menstruation and also considered effective against the hemorrhoid (Adjanohoun et al., 1979). The leaves would also be effective against dental caries and abscesses (Boullard, 2001).

Many scientific studies have been made on the leaves of *Spondias mombin*. Thus, Ayoka et al. (2005) showed sedative effects, antiepileptics and antipsychotics effects of *Spondias mombin* on mice and rats. They have shown that extracts from the leaves of this plant contain GABA antagonist compounds and they do have sedative and anti-dopaminergic pronounced. Other studies have shown that the leaves and stem bark of *Spondias mombin* have antibacterial power on *Bacillus cereus*, *Streptococcus* and *Mycobacterium fortuitum pyogenes* then a sharp property with the molluscicide isolated phenolic acid ethanolic extract (Corthout et al., 1994), good activity against *Plasmodium falciparum* (Hope, 2005). Diby et al., 2012 have shown that the total aqueous stem bark extract *Spondias mombin* cause, between 397 and 794 mcg/ml, a reduction of the rhythmic contractions of the modification and a basic tone of isolated rabbit duodenum. Its 50% effective concentration (EC50) is equal to 625 mcg/ml, and this relaxant effect is concentration-dependent. Phytochemicals for their studies have highlighted the presence of large groups of chemical compounds such as tannins, saponins, flavonoids and alkaloids (Njoku and Akumefula 2007; Igwe et al, 2010).

Despite all the benefits of this plant, no study has been done to prove its safety during prolonged use. The aim of this study was to evaluate the safety of this extract on anthropometric and blood biochemical parameters in albino Wistar rats.

Materials and methods

Plant material

The stem bark of *Spondias mombin* were collected in December 2011 in Kokumbo in the department of Toumodi (Ivory Coast) located about 200 km from Abidjan. The identification of this plant was made by us and confirmed at Floristic National Center of University of Cocody (Abidjan), where a sample of herbarium is registered under number 15778.

Animal material

Rats of the species *Ratus norvegicus*, Wistar, aged six weeks and weighing between 100 and 104 g were used for testing. All animals were subjected to a temperature of 25 ± 2 °C and alternating 12 hours of light and 12 hours dark. The diet consists of pellets IVOGRAIN® and were available to the tap water in bottles without interruption.

Preparation of total aqueous stem bark extract of *Spondias mombin*

The stem bark of *Spondias mombin* are dried in the laboratory at a temperature of 25 ± 2 °C for two weeks and then ground into a fine powder using a
grinder brand RETSH, SM100 kind. Fifty grams (50g) of the resulting particles are macerated in 1L of distilled water with magnetic stirring for 24 hours at a temperature of 25 ± 2 °C (Guede-Guina et al., 1993) water. The solution obtained is filtered through cotton wool and Watman N°1 paper. The filtrate is then concentrated under reduced pressure at 60 °C, using a rotary evaporator brand Type R110 MKE i Buch 6540/2. After drying in an oven at 45 °C for 48 hours, the resulting powder was stored at -5 °C for use in experiments.

Subacute toxicity studies
The study of subacute toxicity is determined from the Guideline 407 line (OECD, 1995) which comprises administering the extract orally daily, increasing to four groups of animals doses due to one dose per lot for 28 days.

Forty (40) rats were randomly divided into four groups of 10 animals including three (3) attempts groups and a control batch. Each batch consisted of five (5) female rats and five (5) male rats. Three (3) doses are prepared in accordance with the doses used in the work of Diby et al., 2012. Doses of 250, 500 and 1000 mg / kg of body weight are administered respectively to groups B, C and D. Group A received distilled water. Prior to administration of the extracts, the animals of each lot individually marked and weighed. They received orally, a solution volume of 2 ml/100 g body weight. The amount of food consumed and the volume of water are recorded daily while the weighing is done every week.

Blood biochemical examination
At the end of each week, animals anesthetized with ether, blood was collected early in the morning thanks to the technique of amputation of the tail tip (5 mm from the end ) previously disinfected with the alcohol 96 ° ( Kraus , 1980) . The blood drawn in dry tubes was centrifuged at 3000 revs / min for 5 min and the obtained serum was used for the determination of certain blood biochemical parameters. Transaminases Glutamate - Pyruvate (SGPT ) and Oxaloacetate (SGOT ) were determined by the kinetic method (Gella et al., 1985) , urea and total cholesterol by the enzymatic method (Allain et al., 1974 ; Gutmann and Bergmeyer , 1974; Talke and Schubert, 1965) , creatinine by the colorimetric method (Fabiny and Ertingshausen , 1971). The aforementioned parameters are determined using the Fully® automate. Glucose is measured directly from whole blood using a glucometer Accu-Chek® (Roche Diagnostics) according to the method of glucose oxidase (Tietz , 1987).

Statistical analysis method
Statistical analysis is performed using the XLSTAT-PRO 7.1 computer software from the test repeated measure ANOVA with the mixed model followed by Dunnett’s test for comparison of groups with the control group. Values are given as mean standard error followed on average. Statistical significance is expressed at p < 0.05.

Results
Effect of total aqueous stem bark extract of Spondias mombin on anthropometric parameters in rats
The evaluation of anthropometric parameters generally indicated an increase in food and water consumption in all animals during the four weeks. However, the amount of food consumed has remained uneven across groups (Figure 1). Indeed, the consumption of food in the D group remained lower than in group A with a highly significant difference (p < 0.001) throughout the study. All animals normally consumed tap water with a net increase in the weeks (Figure 2). However, the rats in the control group had the volume of water ingested higher from the second week of study with a highly significant difference (p < 0.001), especially in the fourth week. Just like the food and water consumption, there has been an increase in body weight in all subjects of the study (Figure 3). However, the weight gain did was not uniform across all groups. Body weight of the rats of Group B significantly increased the second week compared to the group A, prior to being near the last two weeks (Figure 3) . Reverse against group D and particularly the group C showed low weight gains compared to the
control group from the second week of treatment until the end of the study.

**Effect of total aqueous stem bark extract of Spondias mombin on the biochemical parameters in rats**

Concerning the effect of the administration of the total aqueous extract of *Spondias mombin* on some biochemical parameters of rat, urea rate remained lower in animals in group B than in controls throughout the study period (Table 1). The animals in group C had a first week low of urea, before knowing a greater increase significantly (p < 0.05) than controls at week four. In group D, their urea was high the first week and less than that in group A the past three weeks. Apart from the fourth week, creatinine levels of rats treated remained in all lower than those of the control rats. No disturbance of total cholesterol were observed in treated rats compared with controls (Table 1). The rats in groups A and B were serum transaminase SGPT close throughout the study (Table 2). Those of groups C and D had an increase in the experience with a significant difference from the second week compared to controls. In contrast, the total aqueous extract had no effect on glucose and transaminases SGOT (Table 2) in all subjects.

**Table 1. Effect of total aqueous stem bark extract of Spondias mombin on the biochemical parameters in rats.**

<table>
<thead>
<tr>
<th>Doses (mg/kg de pc)</th>
<th>Urea (g/l)</th>
<th>Creatinine (mg/dl)</th>
<th>Total Cholesterol (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
<td>Week 3</td>
</tr>
<tr>
<td>0</td>
<td>2.09 ± 0.93</td>
<td>2.66 ± 0.41</td>
<td>2.16 ± 0.13</td>
</tr>
<tr>
<td>250</td>
<td>1.40 ± 0.27</td>
<td>1.98 ± 0.13</td>
<td>1.09 ± 0.58</td>
</tr>
<tr>
<td>500</td>
<td>1.92 ± 0.02</td>
<td>2.63 ± 1.26</td>
<td>2.65 ± 0.80</td>
</tr>
<tr>
<td>1000</td>
<td>2.49 ± 0.28</td>
<td>2.23 ± 0.13</td>
<td>1.34 ± 0.01</td>
</tr>
</tbody>
</table>

Values are given as mean standard error followed on average. n = 10 animals in each group for the same period and for a given parameter: * = statistically higher rate (p < 0.05) than the control.

**Table 2. Effect of total aqueous stem bark extract of Spondias mombin on the biochemical parameters in rats.**

<table>
<thead>
<tr>
<th>Doses (mg/kg de pc)</th>
<th>SGPT (UI/L)</th>
<th>SGOT (UI/L)</th>
<th>Glycaemia (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
<td>Week 3</td>
</tr>
<tr>
<td>0</td>
<td>13.02 ± 0.06</td>
<td>12.83 ± 0.28</td>
<td>12.60 ± 0.28</td>
</tr>
<tr>
<td>250</td>
<td>13.10 ± 0.11</td>
<td>12.73 ± 0.75</td>
<td>12.73 ± 0.14</td>
</tr>
<tr>
<td>500</td>
<td>12.71 ± 0.16</td>
<td>12.98 ± 0.17</td>
<td>13.40 ± 0.23*</td>
</tr>
<tr>
<td>1000</td>
<td>12.81 ± 0.32</td>
<td>13.56 ± 0.06*</td>
<td>13.19 ± 0.44*</td>
</tr>
</tbody>
</table>

Values are given as mean standard error followed on average. n = 10 animals in each group for the same period and for a given parameter: * = statistically superior (p < 0.05) than the control rate.

SGPT: Glutamate-Pyruvate Transaminase, SGOT: Glutamate oxaloacetate transaminase.
Discussion

Effect of total aqueous stem bark extract of Spondias mombin on anthropometric parameters in rats

Weight changes are an indication of the overall health of the animal. The observation of relatively low weight especially in rats treated with a dose of 500 and 1000 mg/kg of body weight gain suggests that the total aqueous extract of the stem bark of *S. mombin* slows weight gain in treated animals. Furthermore, the quantities of food and water consumed in the groups treated rats may be correlated with low weight gains. These results are similar to those obtained by Salawu *et al.*, (2009) which showed a difference in food and water consumption in rats treated with the ethanol extract of *Crossopteryx febrifuga* bark at doses of 500 and 1000 mg/kg body weight.

![Fig. 1. Effect of total aqueous stem bark extract of *Spondias mombin* consumption food rats over time](image)

*Statistically significant difference (p < 0.05) **: highly significant statistical difference (p < 0.01) ***: highly significant statistical difference (p < 0.001).

Effect of total aqueous stem bark extract of *Spondias mombin* on the biochemical parameters in rats

The study of blood biochemical parameters used to assess the effects of repeated administration of aqueous extract of *S. mombin* orally on markers of vital organs such as the liver, kidney and heart. Creatinine and urea are markers of renal function and reflects an increase of the renal dysfunction. In general, the levels of creatinine and urea of treated rats remained lower compared to controls during the first three weeks of study. The fourth week is marked by a significant difference with the increase of creatinine levels in rats treated with a dose of 500 and 1000 mg/kg body weight compared to the rate observed in the controls. It appears that signs of nephrotoxicity occur from the fourth week of experience. These results are similar to those reported by Adebayo *et al.* (2010) who showed in their study a slight increase in serum creatinine and urea in the rats treated with the ethanol extract of the leaves of *Ageratum conyzoides*. This assessment of the glomerular filtration function must be supported by renal histopathology to confirm or contradict a nephrotoxic effect of the total aqueous extract of stem bark of *S. Mombin*.

![Fig. 2. Effect of total aqueous stem bark extract of *Spondias mombin* consumption water rats over time](image)

*Statistically significant difference (p < 0.05) **: highly significant statistical difference (p < 0.01) ***: highly significant statistical difference (p < 0.001).

Serum transaminase assay is the evaluation of the most widespread (Assah - Hondius, 2004) liver function mode. Transaminases are enzymes that are important in metabolic activity of cells. Their serum increase reflects cellular injury, particularly in the liver (Kew, 2000). Our study showed a gradual rise in the value of transaminases SGPT patients treated at 500 and 1000 mg/kg body weight. Indeed, this rise starts from the third week in rats treated with the dose 500 mg/kg body weight as she begins the second week in rats treated with 1000 mg/kg body weight. The increase in SGPT is dose-dependent and may suggest hepatotoxic nature of the total aqueous extract of stem bark of *S. mombin*. Moreover, Ali and Bashir (1998) showed in their work that high levels of SGPT indicate an early achievement of liver function with or without necrosis. Our results are similar to those reported by Gatsing *et al.*, (2005) who observed elevated transaminases in both sexes after subacute administration of a dose of 4800 mg/kg body weight of the aqueous extract *Allium sativum* rats. The transaminases SGOT are less specific than the SGPT as an indicator of liver function. However, they provide valuable information on the state of skeletal muscle and myocardium (Rosalki *et al.*, 2004).
Overall, the SGOT did not show high values in the test groups compared with values observed in the control group during the study. These results show that the total aqueous stem bark extract of S. mombin did not cause disturbances in myocardial and skeletal muscle cells of rats. Glycemic and lipid determinations have revealed that treatment of rats with aqueous total extract of S. mombin does not affect blood sugar and cholesterol compared to the control group over time. These observations imply that the carbohydrate and lipid metabolic functions are not impaired or under the alterations are not extended as to alter certain biological functions. These results are in agreement with those of KONE et al., (2009) which showed that daily administration of aqueous extract of Sacoglottis gabonensis (Baille) Urban (Humiriaceae) for 28 days in rats causes no modification of carbohydrate and lipid metabolism.

**Fig. 3.** Effect of total aqueous stem bark extract of *Spondias mombin* body mass in rats over time *": Statistically significant difference (p <0.05) ** Difference statistically highly significant (p <0.01).

**Conclusion**

This study has highlighted the potential toxicity of the total aqueous stem bark extract of *Spondias mombin* when administered orally daily for 28 days at doses of 500 and 1000 mg/kg body weight in rats. Should therefore observe extreme caution when using this phytomedicinal by the population because it could probably be toxic to humans if administered repeatedly at high doses over a long period. Histopathological investigations are needed to confirm or refute the hepatotoxicity and nephrotoxicity of the probable total aqueous extract.

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