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Research Article
Curative Effect of *Terminalia superba* Engl. and Diels (Combretaceae) Trunk Bark on Paracetamol-induced Hepatotoxicity in Rats

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Abstract

Background and Objective: *Terminalia superba* is a plant used in traditional medicine to treat various ailments. This study aims at assessing the curative potential of a total aqueous extract of the trunk bark of *T. superba* (TAETs) on paracetamol-induced hepatotoxicity in rats. **Materials and Methods:** Except the control group which received distilled water, 3 groups of 6 rats were daily gavaged with 2 g kg^{-1} b.wt. of paracetamol within 3 days. Later on, distilled water, 100 mg kg^{-1} b.wt. of silymarin and 500 mg kg^{-1} b.wt. of *T. superba* extract were daily administered for10 days to the respective groups of rats by oral route. At the end of the experiment, rats were administered with phenobarbital (80 mg kg^{-1} b.wt., ip) in order to determine their sleeping time. Some blood samples were taken at the beginning of the experiment, on the 3rd day and the last day for liver biomarkers determination. The rats were sacrificed, livers were removed, macroscopically observed and weighed. **Results:** The TAETs decrease biomarkers (AST, ALT, alkaline phosphatase, total and conjugated bilirubin) and sleeping time increased by paracetamol. These results are similar to that of silymarin. **Conclusion:** The TAETs have hepatocurative effects by normalizing paracetamol-induced changes in serum liver biomarkers and reducing phenobarbital-induced sleep time in the presence of paracetamol.

Key words: *Terminalia superba*, hepatotoxicity, liver biomarkers, hepatocurative, conjugated bilirubin, TAETs

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Liver is an important organ of the digestive system. It is considered to be the "laboratory" of animal's body[1]. It fulfills many functions such as glucose metabolism, plasma protein biosynthesis and detoxification of metabolic waste products, synthesis and excretion of bile, essential for life[2]. All substances introduced into the body reach the bloodstream, pass through the liver where they undergo more or less complex transformations before being excreted. As a result, the liver is exposed to various attacks which sometimes have serious aftereffect on the whole body[3]; hence, there is a need to protect this organ.

Hepatic problems constitute a real public health problem. It's becoming more frequent because of alcoholism, self-medication, bacteria or viruses that act either directly or by the production of mycotoxins or aflatoxins[4]. These hepatopathies require long and expensive hospital stays[5].

In traditional medicine, many plants, including *Chrysanthellum indicum* and *Desmodium adscendens*, which possess anti-cholera and hepatoprotective potential[6], are used for the treatment of liver diseases. Thus, in view of the public interest in herbal treatment and the WHO recommendation[7] to make available herbal medicines of high quality efficacy to the populations, this study has been undertaken to assess the hepatoprotective effect of a total aqueous extract of *T. superba*. In fact *T. superba* is traditionally used to treat hepatitis[8]. A previous non-published work performed in our laboratory confirmed its anti- gastric ulcer effect[9]. In addition, an acute toxicity test showed that the plant is not orally toxic[10].

The present work aims at assessing the healing potential of *T. superba* on paracetamol-induced hepatotoxicity in rats *via* its effects on rats' liver biomarkers and their sleep time.

MATERIALS AND METHODS

Animal: The experiments were carried out on male and female albino rats (*Rattus norvegicus*). These rats, aged between 12 and 16 weeks and weighed between 105 and 200 g. They were fed with FACI® granules and water *ad libithum* and kept in the Laboratory of Physiology, Pharmacology and Pharmacopoeia (Nangui Abrogoua University) animal house at a temperature between 20 and 22°C with a 12 h' light/dark cycle.

Research was conducted from 10 August, 2018 to 21 February, 2019 in accordance with the internationally accepted principles for laboratory use and care as found in the European Community Guidelines[11].

Drugs: Acetaminophen (Paracetamol®, Sanofi Aventis, France), Silymarin (Legalon®, Sanofi Aventis, France), Ether (VWR International-Geldenaakfebaan464-B-3001, Leuven-Belgium) and Phenobarbital (Aventis, France) were used as drugs.

Methods

Preparation of the aqueous extract of *Terminalia superba*: The bark of the trunk of *T. superba* were harvested and washed with distilled water. It was cut into small pieces and dried in an oven at 45°C. It was pulverized using an electric grinder of the RETSH brand, type SM 100 (Haan, Germany). One hundred grams of this powder were infused for 15 min in 1 L of boiled distilled water. The aqueous solution obtained was filtered on hydrophilic cotton and on filter paper Whatman. The filtrate was dried in an oven (Friucell, Germany) at 45°C and weight 11.56 g of brown powder was obtained. This powder was the total aqueous extract of trunk bark of *T. superba* (TAETs).

Preparation of the doses of *Terminalia superba* extract and the drugs: A dose of 500 mg kg^{-1} b.wt. of the total aqueous extract of the trunk bark of *T. superba* was prepared. This dose was chosen in accordance with the results found by Goze *et al.*[9]. In fact, according to these researchers, 500 mg kg^{-1} b.wt. of *T. superba* exhibited the highest pharmacological effect on gastric mucosa.

The 100 mg kg^{-1} b.wt. of silymarin (5 mg mL^{-1}), 2 g kg^{-1} b.wt. of paracetamol and 80 mg kg^{-1} b.wt. of phenobarbital (20 mg mL^{-1}) were prepared.

Experimental design: The evaluation of hepatocurative effect and sleeping time of *T. superba* were carried out respectively according to the methods of Walker *et al.*[12] and Girish *et al.*[13] with a slight modification. Thus, 30 rats (15 males and 15 females) were fasted for 18 h. They were divided into 5 groups of 6 rats with 3 males and 3 females each.

Group 1 : **Negative control:** Rats were orally administered with distilled water (10 mL kg^{-1} b.wt.) for 13 days

Group 2 : The 2 g kg^{-1} b.wt. of paracetamol was orally administered to rats for the first 3 days followed by distilled water (10 mL kg^{-1} b.wt.) for 10 days

Group 3 : Rats were gavaged with paracetamol (2 g kg^{-1} b.wt.) for the first 3 days and thereafter the total aqueous extract of *T. superba* (500 mg kg^{-1} b.wt.) for 10 days

Group 4 : **Positive control:** The rats were gavaged with paracetamol (2 g kg^{-1} b.wt.) for the first 3 days followed by silymarin (100 mg kg^{-1} b.wt.) for 10 days

Group 5 : Rats were gavaged with distilled water for 3 days. The animals were thereafter orally administered with the aqueous extract of *T. superba* (500 mg kg^{-1} b.wt.) for 10 days

The 24 h later, 80 mg kg^{-1} b.wt. of phenobarbital was administered by intraperitoneal route to the rats in order to assess their sleeping time. The sleeping time corresponds to the time rats start sleeping till the time they get up.

Blood sampling and biochemical parameters determination: Blood samples of the animals were taken at different period of the experiment: At the beginning, after three days paracetamol administration and at the end of the treatments. Before blood sample collection, the animals were weighed and anesthetized with ether. Blood was kept into tubes from the retro orbital sine of the animals.

Tubes were centrifuged at 3000 rpm for 5 min. The serum obtained was stored at -20°C until the time of the biochemical analyzes which are made with the aid of a Coulter AC.T diff 2 type automatons. Serum samples were used for the assessment of the liver biomarkers such as total bilirubin (TB), conjugated bilirubin (CB), alanine amino transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total proteins (TP).

Liver sampling: Livers of the rats were removed, cleaned with normal saline solution (0.9%), weighed and photographed at the end of the experiment. Livers were fixed in 10% formalin. The relative liver-body weight (RLBW) was calculated in relation to the body weight according to the formula given by Islam *et al.*[14]:

$$\text{RLBW (\%)} = \frac{\text{Liver weight}}{\text{Body weight}} \times 100$$

Macroscopic examination: Gross observations were done on the external structure of the whole liver. Color, consistency and texture were considered. Livers of the rats were photographed using a Kodak camera.

Statistics analysis: All assays were repeated 3 times. Comparisons between groups versus controls were made using one way ANOVA[15] test and values of $p<0.05$ were considered statistically significant using GraphPad Prism 5.01 (San Diego, California, USA) software. The analysis was completed by multiple comparisons of the average values of the different parameters using the Tukey Kramer[16] *post hoc* test, if significant differences were revealed between the tested averages.

RESULTS

Effects of (TAETs) on paracetamol-induced liver toxicity in rats

Variation of rats' body weight, relative liver-body weight and gross observations of livers: The body weight of the control group rats (treated with distilled water) were increased from 118.2±3.3 to 155.3±2.2 g, i.e., a gain of 37.1 g while the relative liver-body weight was 2.78±0.07%. As for the body weight of the group of animals administered with paracetamol (2 g kg^{-1} b.wt.), it decreased significantly ($p<0.05$) from 177.3±2.2 to 157.7±7.35 g, i.e., a loss of 19.6 g ($p<0.05$). The relative liver-body weight also decreased and got to 1.89±0.01. Both TAETs and silymarin promoted significant weight gain of rats pretreated with paracetamol. In fact, TAETs and silymarin induced significant ($p<0.05$) rats' weights gain of 23.63 and 33.1 g, respectively. The relative livers-body weight of these groups of rats were also increased significantly ($p<0.05$) to 3.55±0.3% for TAETs and 3.4±0.04% for silymarin against 2.78±0.07% for distilled water and 1.89±0.01% for paracetamol+distilled water (Table 1).

Figure 1 shows the photographs of the livers of the rats. The aspect of the liver of animals treated only with distilled water is normal. In this group, Livers were light brown, soft and firm when it's touched (Fig. 1a). On the other hand, the liver of rats gavaged with paracetamol has a nutmeg aspect, namely, granite, variegated with reddish marks (Fig. 1b). Paracetamol-treated rats administered with either silymarin (100 mg kg^{-1} b.wt.) or TAETs (500 mg kg^{-1} b.wt.) have a light brown liver with normal consistency and firmness when touched (Fig. 1c, d).

Effect of $TAET_S$ on the sleeping time of paracetamol-induced hepatotoxicity rats: The results of the effect of TAETs after 80 mg kg^{-1} b.wt. of phenobarbital administration on rats' sleeping time were shown in Fig. 2. It indicates that the

Table 1: Variation of the body weights and relative liver-body weights of the different groups of rats

Groups	Initial weight (g)	Final weight (g)	Liver weight (g)	Relative weight (%)
Control (distilled water)	118.20±3.3	155.3±2.2	4.34±0.24	2.78±0.07
2 (Paracetamol+distilled water)	177.30±2.2^{r}	157.7±7.35^{z}	2.99±0.31^{h}	1.89±0.01^{d}
3 (Paracetamol+silymarin)	110.70±7.3^{m}	143.8±6.4^{q}	4.89±0.44^{j}	3.40±0.04^{e}
4 (Paracetamol+TAETS)	105.67±1.8^{n}	129.3±5.3^{w}	4.59±0.66^{b}	3.55±0.03^{a}

Values in the same column with different letters are statistically different from the control paracetamol group (Group II) at $p<0.05$, n = 6 rats

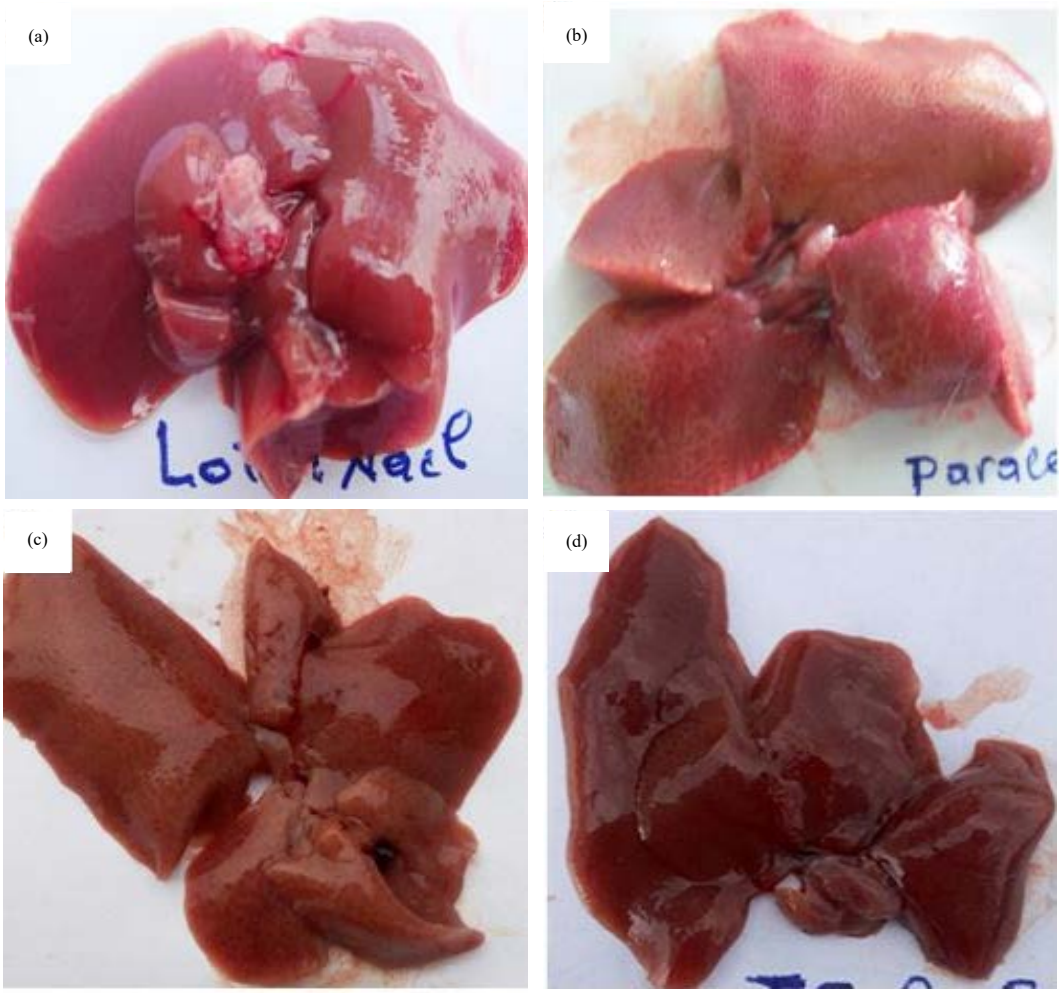

Fig. 1(a-d): Photographs of the livers of the rats at the end of the experiments, (a) Aspect of livers of rats treated with distilled water, (b) Paracetamol+distilled water, (c) Paracetamol+silymarin and (d) Paracetamol+TAETs
Source: Original photographs (Goze, 2019)

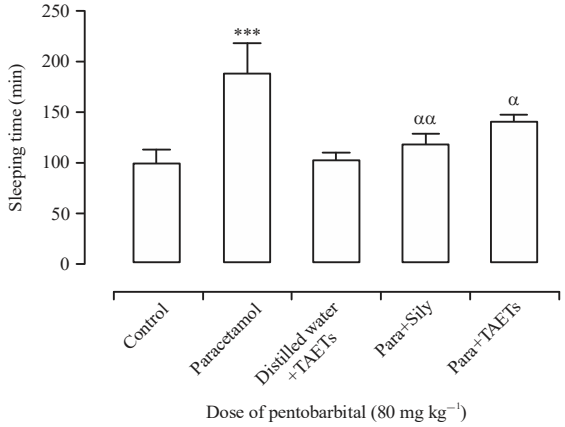

Fig. 2: Effect of silymarin and $TAET_S$ on the sleeping time of paracetamol-induced hepatotoxicity rats
***p<0.001, n = 6, Significant difference when compared to the control group, $^{\alpha}p<0.05$, $^{\alpha\alpha}p<0.01$, n = 6, significant difference when compared to the paracetamol group, Sily: Silymarin (100 mg kg^{-1} b.wt.), $TAET_S$: Total aqueous extract of the trunk bark of *T. superba* (500 mg kg^{-1} b.wt.), Para: Paracetamol (2 g kg^{-1} b.wt.)

sleeping time of the animals that received distilled water (Control group) was 101.3±4.88 min with a value of 105±5.38 min, the sleeping time of those gavaged with ($p<0.001$) $TAET_S$ only was statistically the same like that of the control group. Results also showed that paracetamol increased significantly ($p<0.001$) the sleeping time of rats to 190±2.22 min. Meanwhile, silymarin and TAETs reduced significantly ($p<0.001$) the sleeping time of paracetamol-induced hepatotoxicity rats when compared to those which received paracetamol only. Values were 120±3.96 min for silymarin and 143±4.73 min for TAETs.

Effect of $TAET_S$ on biochemical parameters

Effect of $TAET_S$ on transaminases activity: Figure 3 shows the effect of different treatments to groups of rats on their serum transaminases (AST and ALT) levels. The ASAT and ALT levels were 233±13.6 and 152.4±7.28 U L^{-1}, respectively for rats gavaged with distilled water for 3 days (control group). These values which do not vary significantly after 10 days were 235±12.18 (ASAT) and 157±16.1 (ALT). However, after 3 days' paracetamol administration, AST and ALT levels rose significantly to approximately 460 U L^{-1} for AST and 220 U L^{-1} for ALT. Daily administration of silymarin and $TAET_S$ for 10 days to paracetamol-induced hepatoxicity rats reduced significantly ($p<0.001$) the levels of transaminases. In fact, Silymarin dropped the AST levels from 462±21 to 242±11 U L^{-1} and the ALT from 223±16 to 159.80±14 U L^{-1}. As for the $TAET_S$, results indicated a reduction of AST from 453±23 to 335±7 U L^{-1} and from 221.4±19 to 184±13 U L^{-1} the ALT. Thus, silymarin and TAETs induced normalization in serum transaminase levels of paracetamol induced hepatotoxicity rats.

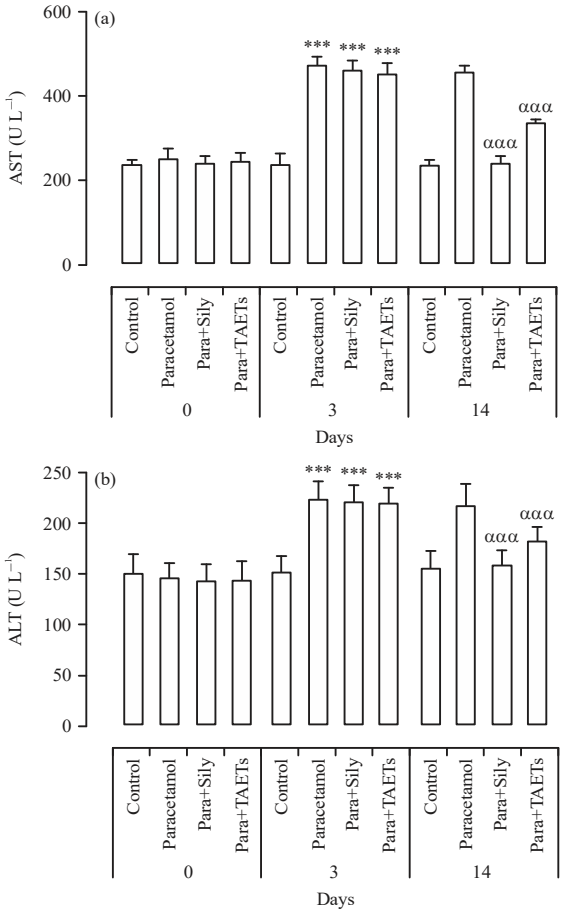

Fig. 3(a-b): Effect of TAETs on serum transaminases activities (a) AST and (b) ALT

***$p<0.001$, n = 6, significant difference when compared to the control group, $^{\alpha\alpha\alpha}p<0.001$, n = 6, significant difference when compared to the paracetamol group, Sily: Silymarin (100 mg kg^{-1} b.wt.), $TAET_S$: Total aqueous extract of the trunk bark of *T. superba* (500 mg kg^{-1} b.wt.), Para: Paracetamol (2 g kg^{-1} b.wt.)

Effect of $TAET_S$ on some other biochemical parameters: Graphs plotted in Fig. 4 show the serum total protein, alkaline phosphatase and bilirubins levels in paracetamol induced hepatotoxicity rats treated with the total aqueous extract of *Terminalia superba* (TAETs) or Silymarin (Syl).

As for serum total protein, results show that the levels were about 6 g dL^{-1}, at the beginning of the experiment (Day 0) in the different groups. The level of this parameter in the group of rats treated with distilled didn't significantly change throughout the study. But those gavaged with paracetamol for 3 days showed a significant ($p<0.05$) decrease of their total protein to about 4.3 g dL^{-1}. After 13 days of TAETs or silymarin administration to paracetamol induced toxicity rats, though the levels of total protein rose, they were not statistically different from those treated with paracetamol only. In fact, on the day 14, the levels were 5.72±0.62 (Para+Syl) and 5.31±0.95 g dL^{-1} (Para+TAETs) (Fig. 4a).

The gavage of paracetamol for 3 days to rats increased significantly ($p<0.001$) the levels of alkaline phosphatase, total and conjugated bilirubin from about 365-500 U L^{-1}, 1.1-4 and 0.75-1.15 mg dL^{-1} in rats' serum. The levels of these biochemical parameters of rats gavaged with distilled water (Control group), on the 14th day, didn't significantly change throughout the study compared to the values after 3 days' paracetamol administration. Silymarin and TAETs reduced significantly ($p<0.001$) the levels of alkaline phosphatase and bilirubins (Fig. 4b-d).

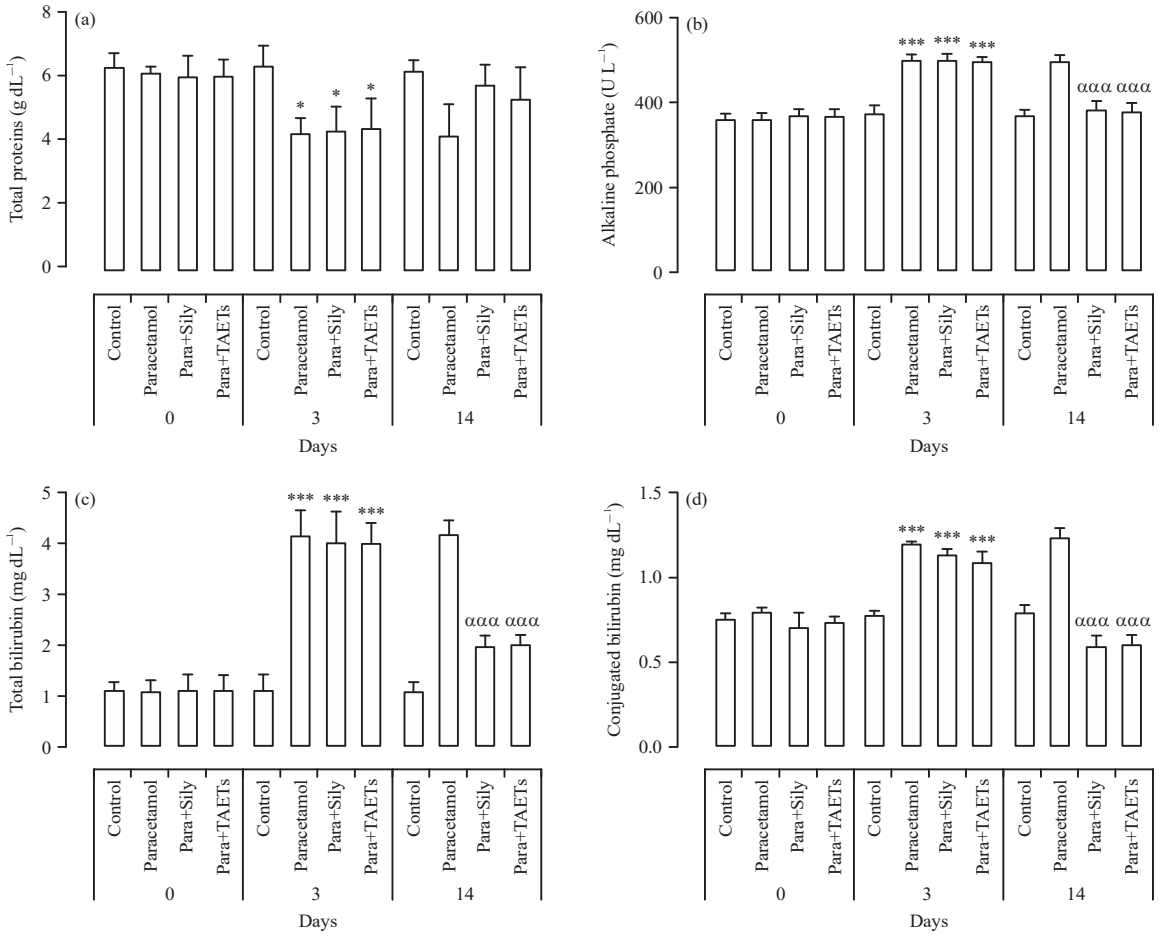

Fig. 4(a-b): Effect of TAETs on (a) Total protein, (b) Alkaline phosphatase, (c) Total bilirubin and (d) Conjugated bilirubin

***p<0.001, n = 6, significant difference when compared to the control group, $^{\alpha\alpha\alpha}$p<0.001, n = 6, significant difference when compared to the paracetamol group, Sily: Silymarin (100 mg kg^{-1} b.wt.), $ETAT_5$: Total aqueous extract of the trunk bark of *T. superba* (500 mg kg^{-1} b.wt.), Para: Paracetamol (2 g kg^{-1} b.wt.)

DISCUSSION

The results of the administration of the total aqueous extract of the stem bark of *Terminalia superba* (TAETs) over 10 days' paracetamol-induced hepatotoxicity in rats revealed that the extract possesses a curative effect because it normalizes the level of serum alkaline phosphatase (ALP), total bilirubin (TB) and conjugated bilirubin (CB), transaminases (AST and ALT) and total protein (TP) disturbed by paracetamol.

The TAETs induced an increase in the weight of the animals except those which were paracetamol-poisoned and which were not treated. This observed weight gain could be due to the good functioning of the liver repaired following the repeated administration of TAETs. As regards the percentage of relative weights, the results obtained compared to the control batches do not allow to make an assessment in relation to the effects of the tested extract. It is known that paracetamol, used at the therapeutic dose as antipyretic and analgesic is safe for the organism[17]. However, a high dose of paracetamol can cause severe hepatotoxicity[18]. This hepatotoxicity is observed by the presence of an increase in the serum level of some hepatic biomarkers (AST, ALT and ALP) due to a cellular lesion; particularly the cells of the liver[19]. These liver biomarkers are enzymes that have significant metabolic activity inside the cells. They are synthesized in cells' cytoplasm and discharged into the circulation in case of cells damage[20]. They are considered to be good indicators of hepatic cytolysis.

The significant decrease in liver enzymes observed after silymarin and TAETs administration to animals could mean a protection of the liver by these substances against paracetamol damage.

The ability of hepatoprotective substances to reduce the harmful effects or preserve the mechanisms of liver functioning against hepatotoxin disturbances is an indication of its protective effect[21]. In addition, repeated administration of the TAETs at a dose of 500 mg kg b.wt. for 10 days protects against hepatotoxicity caused by paracetamol with an efficacy closer to that of silymarin.

Indeed, the macroscopic observation of the liver of the animals treated with the TAETs and the silymarin shows the same types of lesions. The decrease in morphological lesions could be a sign of hepatocyte repair, a strengthening of the parenchyma after treatment with the extract or silymarin. It can therefore be assumed that the TAETs and silymarin possess hepatotoxic protective activity of the antitoxic type directed against paracetamol-induced hepatotoxicity. The decrease in the serum level of ASAT, ALAT and PAL is therefore a sign of an improvement in liver function. These results are similar to those obtained by and on the effects of hepatoprotective plant extracts[22,23].

Indeed, the extracts of *Rosemarinus officinalis* and the silymarin of *Silybum marianum* act better in repeated administration as well as the TAETs[22]. The ethanolic extract of the stem bark of *Mammea africana* significantly reduces the serum level of liver biomarkers (AST, ALT and ALP), total proteins and bilirubins

The mechanism by which this extract act is not clearly understood yet, according to these authors[23].

The significant decrease in total protein in animals administered with paracetamol without any treatment compared to healthy animals and treated animals means that the liver of these animals is affected, resulting in malnutrition and mal-absorption of the ingested food. The administration of the TAETs above all repeatedly would have made it possible to correct this dysfunction whence the increase in the serum level of the total proteins. These results are similar to those who showed that administration of the ethanol extract of *Moringa oleifera* leaves to carbon tetrachloride poisoned rats would increase serum total protein levels[24].

Although ALAT is the best indicator of liver malfunction[3,25], total bilirubin and conjugated bilirubin provide information on the proper functioning of the liver[26]. The TAETS reduced the levels of total bilirubin and conjugated bilirubin, that confirms its protective effects and also its effectiveness on the functioning of liver cells.

In order to investigate the role of hepatic microsomal enzymes as an indicator of hepatic function in the early elucidation of a likely mechanism of hepatoprotective effect, phenobarbital-induced sleep time was determined in rats. The results of this study showed that the animals treated with TAETs and Silymarin after paracetamol-induced-hepatotoxicity reduced the sleep time thus confirming the protective effect of these substances against the damage caused by paracetamol to the hepatocytes. These results are similar to those obtained with the methanolic extract of *Solanum nigrum* in rabbits[27].

Phenobarbital is a barbiturate intensively metabolized in the liver and its degradation is delayed in case of liver damage[27,28]. However, hepatic microsomal enzyme inhibitors also delay the release of barbiturates and increase phenobarbital-induced sleep time[13-27]. The fact that the TAETs did not prolong phenobarbital-induced sleep time suggests that it has no inhibitory effect on hepatic microsomal enzymes. We wish to point out that due to lack of adequate material we do not have to realize the histological sections of the liver to better appreciate the repairing effect of our extract. We note, however, that the results of analyzes of biochemical parameters of blood show a more or less perfect agreement with regard to the hepatocurative activity of the extract, which is expressed by an elevation or diminution of these parameters in the blood.

CONCLUSION

The study of the effect of the total aqueous extract of the trunk bark of *Terminalia superba* on the hepatic toxicity induced with paracetamol for 3 days revealed that this extract has a hepatocurative potential. This extract normalizes paracetamol-induced changes in serum liver biomarkers. This extract reduces phenobarbital-induced sleep time in the presence of paracetamol, suggesting a resumption of hepatic function. This hepatocurative activity of the extract is probably due to its phytochemical composition.

SIGNIFICANCE STATEMENTS

This study will allow new treatments development based on plant extracts against hepatotoxicities induced by paracetamol. This study will help the researcher to uncover the critical areas of the treatment of hepatotoxicities induced by paracetamol by using the efficacy dose of plant extract that many researchers were not able to explore. Thus a new theory on plant extracts and their possibility to treat liver injuries may be arrived at new natural anti-hepatotoxicity drugs for the population.

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