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Evaluation of the acute toxicity and hemostatic properties of the aqueous extract of *Cola cordifolia* seeds.

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ABSTRACT

Cola cordifolia is a plant whose seeds are rich in nutrients and bioactive compounds. This plant is used in traditional medicine to treat certain ailments and prevent certain metabolic diseases in order to enhance its value. Objective of this study was to evaluate the toxicity and hemostatic effect of the aqueous extract of *Cola cordifolia* seeds with a view to its valorization in the dietary and medicinal fields. Seeds were fermented for 6 days then dried for 15 days in the sun. Flour obtained was used to obtain the aqueous extract. Acute toxicity of the extract was evaluated at doses 300 mg/Kg bw and 2000 mg/Kg bw on rats. Subsequently, the hemostatic properties were evaluated. Acute toxicity results showed that *Cola cordifolia* seeds are not toxic at a dose of 2000 mg/Kg bw. Extract caused an increase in the weight of rats by 10.34 g and 9.34 g respectively at doses of 300 mg/Kg and 2000 mg/Kg bw. Aqueous extract reduced activated partial thromboplastin times from 44.4 to 18.6 seconds and prothrombin times from 6.5 to 4.5 seconds. Aqueous extract of *Cola cordifolia* promotes blood coagulation by the intrinsic and extrinsic pathways.

Keywords: Acute toxicity, hemostatic, aqueous extract, *Cola cordifolia*

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INTRODUCTION

Hemostasis is a protective mechanism that depends on an important balance between procoagulant and anticoagulant processes [1]. When a blood vessel is injured, platelets and fibrin are involved in blood clot formation and stopping blood loss from the damaged vessel [2]. Hemorrhage is the leading cause of death among women in most developing countries. Over the past 15 years, pharmaceutical industries have developed new hemostatic agents [3]. Several studies have shown that many medicinal plants have hemostatic activity, which could guide us towards new, more effective substances [4]. Given the high cost of modern medicines, the populations of these countries have a growing interest in herbal medicine [5]. Among these plants we have *Cola cordifolia* which belongs to the *Malvaceae* family. Leaves are used in the treatment of fever, diarrhea, pain, leprosy and eye problems[6,7]. Fruit seed contains significant quantities of minerals, polysaccharides, phenolic compounds, tannins, flavonoids and has good anti-radical activity [8]. Despite their richness in bioactive compounds, *Cola cordifolia* seeds, there is no scientific data on the effect of these seeds on blood coagulation. Hence the objective of this study to evaluate the toxicity and hemostatic effect of the aqueous extract of *Cola cordifolia* seeds with a view to its valorization in the dietary and medicinal fields.

MATERIALS AND METHOD

Vegetables material

Vegetables material used in this study consists of *Cola cordifolia* fruits collected from the botanical garden of the Université Peleforo Gon Coulibaly in Korhogo.

Photography 1: *Cola cordifolia* fruit and seeds

Animal material

Animal material consisted of female Winstar strain rats (*Rattus norvegicus*) with an average mass of 115.33 ± 1.57 g and rabbits (*Oryctologus cuniculus*) with an average mass of 2 ± 0.4 kg. These animals were obtained from the Animal House of the Laboratory of Biology and Health of UFR Biosciences at Cocody University in Abidjan (Côte d'Ivoire).

Seeds treatment

Seeds treatment was performed according to the method described [9]. *Cola cordifolia* fruits were manually opened, and the seeds were separated from the shells. Seeds were fermented for 6 days on banana leaves, with mixing occurring every 2 days. After fermentation, the seeds were sun-dried for 8 days, then hulled and ground using an electric grinder (Stainless Steel). Ground material was sieved through a 10 µm mesh, and the flour was stored in a plastic container at 4°C.

Extraction aqueous

Aqueous extraction was carried out according to the method [10]. 200 g of *Cola cordifolia* flour were stirred in 1.5 L of distilled water for 12 hours. Mixture obtained was filtered on Whatmann No. 4 filter paper. Filtrate obtained was filtered through hydrophilic cotton then dried in an oven at 50°C for 72 hours. Dry residue obtained was packaged in a plastic box and stored at 4°C.

Acute toxicity of the aqueous extract

Study of the acute toxicity of the aqueous extract of *Cola cordifolia* was carried out according [11]. Wistar strain rats were previously fasted for 12 hours before administration of the aqueous extract. This extract was tested in a sequential process in which three female rats were used at each stage. Test design used in the study was that using an initial dose of 300 mg/kg bw. Absence of mortality or the absence of manifestation of mortality linked to the aqueous extract at the dose of 300 mg/kg bw after 14 days made it possible to determine the next step which is the administration of the immediately higher dose (2000 mg/kg bw) to three additional female rats followed by 14 days of observation to note clinical signs.

Preparation of platelet-poor plasma

Obtaining platelet-poor plasma was carried out according to the method described by the professional order of medical technicians of Quebec [12]. Blood from rabbits was collected in a centrifuge tube containing citrate. Mixture was centrifuged at 2500 rpm for 15 min. The supernatant obtained was removed without disturbing the pellet and a second centrifugation was carried out at 2500 rpm for 10 min. Resulting plasma was collected and stored at -20°C.

Intrinsic Pathway Exploration Test (TCA)

Exploration of the intrinsic coagulation pathway was carried out according to the method [13]. 43 µL of plasma were introduced into coagulation tubes then heated for 2 to 3 min at 37°C. Then, 7 µL of the aqueous extracts (10^{-4} - 1 mg/mL) were added. 50 µL of cephalin-koaline reagent and 50 µL calcium chloride (2.5 10-3 M) previously heated at 37 °C for 2 to 3 min were added successively to the mixture. Clotting time was recorded with a coagulometer (CyanCoag, Belgium). Distilled water was used as a control.

Extrinsic Pathway Exploration Test (PT)

Method [13] was used to explore the extrinsic coagulation pathway. 43 µL of plasma were introduced into coagulation tubes then heated for 2 to 3 min at 37°C. Then, 7 µL of the aqueous extracts (10^{-4} - 1 mg/mL) were added. 100 µL of prothrombin reagent previously heated at 37°C for 2 to 3 min were added to the mixture. Clotting time was recorded with a coagulometer (CyanCoag, Belgium). Distilled water was used as a control.

Statistical analysis

Statistical processing of the data was carried out with Microsoft Excel Office version 2016 and GraphpadPrism version 7 software. The values were expressed as mean plus standard error on the mean.

RESULTS AND DISCUSSION

Results of the study of the acute oral toxicity of the aqueous extract of *Cola cordifolia* in *Rattus norvegicus* are presented in Figure 1 and 2.

On day 0, the female rats which were to receive the dose of 300 mg/kg bw (figure 1) had an average weight of 108.33 ± 1.53 g. After administration of the aqueous extract to female rats at a dose of 300 mg/kg bw, their average weights increased to 113.53 ± 1.57 and 118.67 ± 1.53 g respectively on the 7th and 14th days. . A weight gain of 5.2 g was recorded on the 7th day. From the 7th to the 14th day, 5.14 g of weight gain was recorded. A total weight gain of 10.34 g was recorded over 14 days.

As for the female rats which were to receive the dose of 2000 mg/kg bw (figure 2), they had an average weight of 121.33 ± 3.51 g on day 0. After administration of the aqueous extract to female rats at a dose of 2000 mg/kg bw, their average weights increased to 125.00 ± 3.61 and 130.67 ± 3.05 g respectively on the 7th and 14th days. . A weight gain of 3.67 g was recorded on the 7th day. From the 7th to the 14th day, 5.67 g of weight gain was recorded. A total weight gain of 9.34 g was recorded over 14 days.

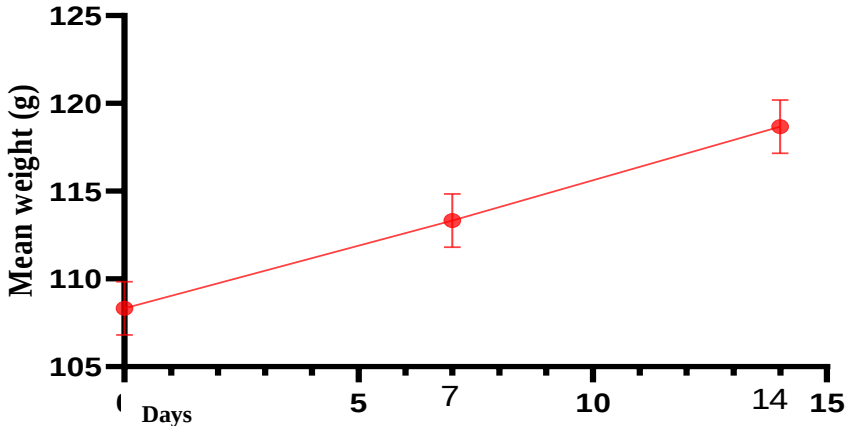

Figure 1: Evolution of the mean weight of rats treated at the dose of 300 mg/kg bw by the aqueous extract of Cola Cordifolia

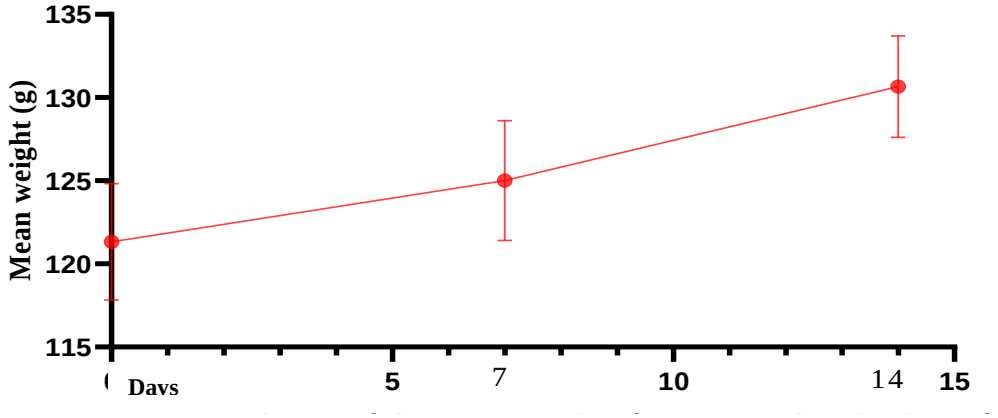

Figure 2: Evolution of the mean weight of rats treated at the dose of 2000mg/kg bw by the aqueous extract of Cola Cordifolia

Figure 3 presents the effect of Cola cordifolia aqueous extract on the coagulation time of the intrinsic and extrinsic pathway in vitro. Concerning the intrinsic pathway, the aqueous extract led to the reduction of activated partial thromboplastin time (ACT) at different concentrations compared to the control. This reduction is more pronounced at the concentration of 1 mg/mL. This reduction in coagulation time was also observed by Kpahe et (2022) with the aqueous extract of Garcinia kola seeds. Regarding the extrinsic route, the aqueous extract led to a reduction in coagulation time. This drop is significantly significant from 0.1 mg/mL.

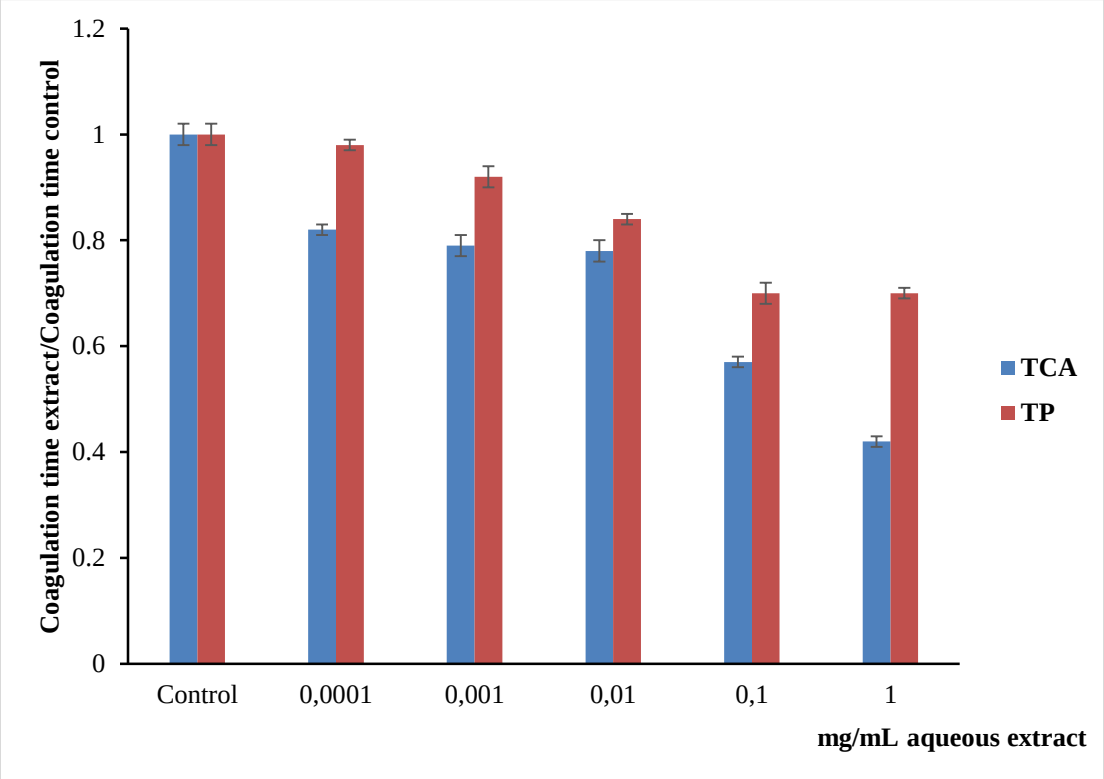

Figure 3: Effect of the aqueous extract on the coagulation time of the intrinsic (TCA) and extrinsic (TP) pathways

DISCUSSION

Weight gain after administration of the aqueous extract was also observed respectively[14] and[15] during the administration of the aqueous extract of the leaves of *Combretum micranthum* and *Ruta montana* to the dose of 2000 mg/Kg in *Rattus norvegicus*. Weight gain could be due to the stimulation of appetite by taking the aqueous extract in rats [16]. Study of the acute oral toxicity of the aqueous extract of *Cola cordifolia* in *Rattus norvegicus* showed that the extract is not toxic up to a dose of 2000 mg/kg of body weight (bw). Its toxicity would be beyond 2000 mg/kg of body weight.

Concerning the hemostatic properties, this reduction in coagulation time was also observed [17] with the aqueous extract of *Garcinia kola* seeds. Reduction in activated partial thromboplastin time could be due to the activation of plastic proteins and prekallikrein by the aqueous extract of *Cola cordifolia* [18]. Aqueous extract could act on blood coagulation through the intrinsic pathway. Regarding the extrinsic pathway, our results are different from those [10] whose aqueous extract of *Garcinia kola* did not affect prothrombin time. Reduction in prothrombin time by the aqueous extract of *Cola cordifolia* could be explained by the increase in the number of platelets by the extract.

CONCLUSION

Study showed that the aqueous extract of *Cola cordifolia* is not toxic at a dose of 2000 mg/Kg bw. Extract caused a reduction in activated partial thromboplastin and prothrombin times. Extract promotes blood clotting through the intrinsic and extrinsic coagulation pathways. Seeds of *Cola cordifolia* have hemostatic properties.

ACKNOWLEDGMENT

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ETHICS APPROVAL

Experimental procedures and protocols used in this study were approved by ethical committee of Health Sciences, University Felix Houphouet-Boigny of Cocody-Abidjan. , Health Sciences Committee, Félix Houphouët-Boigny University. These guidelines were in accordance with those of the European Council Legislation 87/607/EEC for the protection of experimental animals. Every effort has been made to minimize animal suffering and reduce the number of animals used.

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