HEPATOPROTECTIVE ACTIVITY OF AN AQUEOUS EXTRACT OF CASHEW APPLE (*Anacardium occidentalis*) IN RATS AND MICE

ABSTRACT

Aims: Cashew apples are rich in pharmacological molecules of interest and are used against many diseases. The aim of the present work is to evaluate its hepatoprotective activity in rats and mice.
Methodology: Rats pretreated for eleven days with the aqueous extract of cashew apple or silymarin, were intoxicated with paracetamol at 2 $g.Kg^{-1}$ bw, for three days. Their blood was collected and submitted to biochemical analyses. As for the mice, they were also pre-treated with the test substance and intoxicated with paracetamol before receiving phenobarbital. Then, their sleep time was evaluated.
Results: In rats, paracetamol intoxication was materialized by the increase in serum ALT activity ranging from 109±5.19 to 571±20.28 $IU.L^{-1}$ and that of ASAT ranging from 144±5.77 to 428±14.19 $IU.L^{-1}$. Similarly, direct bilirubin level increased from 0 to 1.08± 0.58 $mg.dl^{-1}$. These increases in transaminase activity and bilirubin level were significantly decreased in rats pretreated with the aqueous extract of cashew apple at concentrations ranging from 150 to 300 $mg.Kg^{-1}$ bw or silymarin at 100 $mg.Kg^{-1}$ bw. In mice, the test with phenobarbital showed a decrease of 29.82 to 38.59% of the sleep time of mice pretreated with the aqueous extract of cashew apple for concentrations also ranging from150 to 300 $mg.Kg^{-1}$ bw or silymarin at 100 $mg.Kg^{-1}$ bw.
Conclusion: In view of these results CAJ is hepatoprotective. Therefore, this natural substance could be used in the prevention of liver diseases in traditional medicine.

Keywords: hepatoprotective- Anacrdium Occidentale L -mouse-rat

1. INTRODUCTION

The liver is an essential organ because of its multiple functions. In addition to regulating blood sugar, remodeling chylomicrons to meet the body's needs, it plays a fundamental role in the detoxification of ammonia, cholesterol and exogenous substances. During these biotransformation processes, the liver is exposed to aggressions that can lead to hepatopathies impacting its physiology and thus engaging the vital prognosis of the subject. According to studies carried out on the etiology of chronic hepatopathies diagnosed at the University Hospital of Cocody-Abidjan on 300 patients, 46% suffered from cirrhosis, 43% from chronic hepatitis and 11% from liver cancer. The first place of drug intoxications was taken by antituberculosis drugs, with 82.35%. Alcohol and viruses occupy an increasingly important place in this pathogenesis [1]. These epidemiological data revealing a diverse etiology of hepatopathies sound an alarm.

To protect this vital organ, synthetic hepatoprotective drugs are used, as well as natural substances (plants and agro-resources) with similar effects with the advantage of price and access [2, 3]. The cashew apple, containing pharmacological molecules of interest, could be an asset in the search for this protection. *Aanacardium Occidentale* is a tree of 8 to 10 meters high, belonging to the Anacardiaceae family, which also includes mango (*Mangifera indica* L.) and pistachio (*Pistacia vera* L.) trees. With a diameter of up to 12 to 15 meters, it has a dense and branched foliage. The fruit, the cashew nut, hangs from a fleshy, juicy stalk, red or yellow in color when ripe, called the cashew apple [4].
In traditional medicine, the cashew apple, is used against many ailments. The decoction of the bark of *Aanacardium Occidentale* L., is used as an antihypertensive and anti-dysenteric. The caustic oil of walnuts, treats scabs. The fruit relieves ulcers and toothache [5]. Studies carried out on the apple indicate that its aqueous extract has a self-regulating glycemic effect [6]. It is also said to be analgesic, anti-inflammatory and antipyretic [7].
To enrich these pharmacological data, this work was conducted to evaluate the hepatoprotective effects of CAJ in rats and mice intoxicated with paracetamol.

Comment [DB1]: This is not appropriate. Please delete the ''an''

Comment [DB2]: Be exact on the pretreatment substance you used. Methodology is meant to describe what you used, so state the one you used.

Comment [DB3]: How many rats, how many mice ? How was the grouping done ? Give a very brief description of your experimental design, so that the role of silymarine will be understood.

Example :
Twenty female rats of 50kg body weight were pretreated with 200mg/kg bw of cashew apple aqueous extract for eleven days, Silymarin served as the standard……
…..

Comment [DB4]: Is this AST or ASAT ?

Comment [DB5]: The description of the exact substance you use dis a bit confusing.

Silymarin is a standard drug used as part of your control, so that should not be a reference statement in describing the effect. Silymarine should be used in comparison of the casew apple extract.

Comment [DB6]: Reflect as explained in comments above.

Comment [DB7]: This is not a standard abbreviation, please write in full

Comment [DB8]: The biomarkers tested are not sufficient to have such conclusion on its hepatoprotective potency.

It should rather be suggested that Cashew apple may posses some hepatoprotective properties.

Comment [DB9]: Journal stipulâtes 4-8 keywords. Please adhere accordingly.

Comment [DB10]: Not a standard abbreviation, please write in full.

2. MATERIALS AND METHODS

2.1 Material

2.1.1 Plant material

The cashew apples harvested from the trees or picked up after they fell in December 2021 come from Korhogo, a town located in the far north of the Ivory Coast. Apples of red or yellow color without any injury were selected. They are then carefully separated from the nuts. Then, they were transported to the laboratory.

Comment [DB11]: Please rephrase your sentence structure with addition of punctuation where necessary for better understanding.

Example :

Cashew apples of red or yellow color, without dents were selected for this study, from Korhogo community in Far North of the Ivory Coast. They were harvested from the trees while some were picked after they fell in December, 2021.

2.1.2 Animal material

Wistar albino rats were used for hepatoprotective testing. They weigh between 200 and 300 grams. They come from the Pasteur Institute of Adiopodoumé, and were raised at the Felix Houphouët Boigny University animal house.
Male and female mice of the species *Mus musculus* weighing between 20 and 30 g were used in the sleep tests using phenobarbital. They also came from the animal house of the Pasteur Institute of Adiopodoumé located on the road to Dabou (Ivory Coast). Transported to the animal house of the University Peleforo GON COULIBALY, they were raised at an average temperature of 28°C with a relative humidity of 70% and fed with pellets from IVOGRAIN.

2.1.3 Chemicals and physiological fluid

- Glibenclamide arrow: (SANOFI-AVENTS (France).
- D (+) Glucose anhydrous: E. MERCK, Darmstadt (Germany).
- Nacl 0,9%:

2.2 Methods

2.2.1 Extraction

Comment [DB12]: Extraction of what ?

Comment [DB13]: were

Comment [DB14]: Since you are describing what you did, it should be in past tense, so replace ‘’are’’ with ‘’were’’.

Comment [DB15]: were

The apples collected are cleaned, washed and then disinfected for 30 min with 100 ppm of active chlorine in tanks. They are then rinsed with water before being squeezed. Pressing and spinning are done manually. The cakes, separated from the juice, were dried in the shade between 25 and 28°C. Dried, these cakes are crushed and reduced to powder. This powder was used in the preparation of the aqueous extract of cashew apple. Fifty grams (50) of crushed cashew apple are mixed on a magnetic stirrer of the AGIMATIC-N type for 24 hours in a liter of distilled water. The resulting solution is filtered through cotton wool and Wattman paper. The same operation is repeated. Distilled water is added to the pellet, then mixed for 2 hours and also filtered. The filtrates are collected in a flask and dried in the study at 60° C. The powder obtained, perfectly soluble in water, is used as the aqueous extract of cashew apple.

Comment [DB16]: The process used here to prepare the aqeous extract is not scientifically justifiable. This is because, some of the bioactive compounds would have been contained in the juice that was discarded, so the cake contains just the fiber aspect which may not contain sufficient bioactive component to justify your claims.

However, you need to cite a reference to justify the methodology used in getting your aqeous extract.

2.2.2 Effect of aqueous extract of cashew apple (CAJ) on biochemical parameters of rats intoxicated with paracetamol.

For the determination of the hepatoprotective properties of the aqueous extract of cashew apple, was recommended among other methodological approaches that of Rao KS and Mishra SH [8] slightly modified. For this purpose, fifteen (15) rats were used for the manipulations. These rats were weighed and deprived of food for 18 hours, with free access to water only one hour before the manipulations. Then, they were divided into 5 batches of three rats, as follows:
Batch 1 (negative control): received only distilled water (10 ml.Kg^{-1} bw) orally for 14 days
Batch 2: Distilled water (10 ml.Kg^{-1} bw) administered orally for 11 days, followed by the oral administration of paracetamol at 2 g.Kg^{-1} bw for 3 days

Comment [DB17]: Please adhere to the journal format for reference citation.

Comment [DB18]: This should be study, not manipulation

Comment [DB19]: Administration should be used rather than manipulation

Batch 3: Silymarin 100 mg.Kg^{-1} bw administered orally for 11 days, followed by oral administration of paracetamol 2 g.Kg^{-1} bw for 3 days
Batch 4: CAJ 150 mg.Kg^{-1} bw administered orally for 11 days, followed by oral administration of paracetamol 2 g.Kg^{-1} bw for 3 days.
Batch 5: CAJ 300 mg.Kg^{-1} bw administered orally for 11 days, followed by oral administration of paracetamol 2 g.Kg^{-1} bw for 3 days.

2.2.3. Blood sampling technique for biochemical parameters analysis

24 hours after the end of the treatments, the animals were weighed and anesthetized with ether. Then, their blood collected from the retro orbital sinus of the animals' eyes was transported in dry tubes to the laboratory for biological analysis. The blood from the dry tubes was centrifuged at 3000 rpm and the supernatant was used for biochemical analysis.

Comment [DB20]: Numericals are not appropriate to start a sentence.

2.2.4. Transaminase assay

The determination of aspartate aminotransferase (ASAT/GOT) and alanine aminotransferase (ALAT/GPT) was performed in the biochemistry laboratory of the Faculty of Medicine of Abidjan-Cocody. It was performed with commercial Cobas kits bearing technical information ALTL: ACN 685; ALTPL: ACN 684 for ALAT/GPT and ASTL: ACN 687; ASTPL: ACN 686 for ASAT/GOT. The Cobas C111 analyzer was used for the different determinations, according to the manufacturer's recommendations. The concentration of these enzymes is expressed in international units per liter of substrate (IU/L).

2.2.5 Conjugated bilirubin assay

The analyses were carried out according to the Malloy-Evelyn principle [9], with the commercial cobas kit bearing the technical name BILD2: ACN 734. Cobas C111 was the analyzer used for the assays.

2.2.6 Liver sampling

At the end of all treatments, the liver was harvested and weighed. The relative weight (RW) of the liver was calculated in relation to the body weight according to the formula given by Islam et al [10]:

$$RW = \frac{\text{Liver weight (LW)}}{\text{Body weight (BW)}} \times 100$$

2.2.7 Study of the effect of CAJ on the sleep time of mice intoxicated with paracetamol and given phenobarbital

This study was performed according to the method of Girish et al [11]. After 12 hours of fasting, 30 mice were divided into five batches of six. They were then treated as follows:
Batch 1 (negative control): received only distilled water (0.1 ml.Kg^{-1} bw)
Batch 2: distilled water (0.1 ml.Kg^{-1} bw) administered orally for 11 days, followed by intraperitoneal (IP) administration of paracetamol at 200 mg.Kg-1 PC for 3 days
Batch 3: CAJ 100 mg.Kg^{-1} bw administered orally for 11 days, followed by IP administration of paracetamol 200 mg.Kg^{-1} bw for 3 days.
Batch 4: CAJ 200 mg.Kg^{-1} administered orally for 11 days, followed by IP administration of paracetamol 200 mg.Kg^{-1} bw for 3 days.
Batch 5: Silymarin 100 mg.Kg^{-1} bw administered orally for 11 days, followed by IP administration of paracetamol 200 mg.Kg^{-1} PC for 3 days
24 hours after these different treatments, 80 mg.Kg^{-1} bw of phenobarbital was administered orally to the mice in order to evaluate the effect of CAJ on the sleep time of the animal.
The biological tests were carried out in accordance with the internationally accepted principles of the European Community Directive (Council Directive 86/609/EEC of 24 November 1986).

Comment [DB21]: Your batches here do not agree with the batch arrangement and tag in your result tables. Please correlate the both.

2.3 Statistical analysis

Statistical data expressed as means ± standard error were obtained from the (n=5) separate experiments. The averages calculated were compared from Student's test (t). When $p \leq 0.05$, the difference is said to be significant. The curves and statistical analysis were performed using Graph Pad Prism 5.1 San Diego, CA, USA.

3. RESULTS

3.1 Effect of aqueous extract of cashew apple (CAJ) on biochemical parameters of rats intoxicated with paracetamol

Table 1, presents the results of the effect of aqueous extract of cashew apple (CAJ) on biochemical parameters of rats intoxicated with paracetamol.

In the control rat given only distilled water as treatment, ALAT and ASAT activities were 109 and 144 $IU.L^{-1}$, respectively. The bilirubin level is zero (0) $mg.dL^{-1}$. ASAT activity is 428 $IU.L^{-1}$ ($P < 0.0001$) and ALAT activity is 571 $IU.L^{-1}$ ($P < 0.0001$) in the control rat intoxicated with paracetamol. In the latter, the bilirubin level was 1, 08 ± 0.5 $mg.dL^{-1}$ ($P < 0.0001$). Paracetamol at 2 $g.Kg^{-1}$ bw, therefore, caused a very significant increase in serum transaminase activity, and in direct bilirubin level. In animals pretreated with silymarin followed by intoxication with paracetamol, serum transaminase activities decreased significantly, they are now 173 ± 11.55 $IU.L^{-1}$ for ALAT and 198 ± 4.56 $IU.L^{-1}$ for ASAT ($P < 0.01$). Those pretreated with CAJ followed by paracetamol intoxication, show serum transaminase activities reminiscent of the normal control. The bilirubin level is at its initial value of 0 $mg.dL^{-1}$ in all rats treated with silymarin or CAJ.

Table 1 Effect of aqueous extract of cashew apple (CAJ) on biochemical parameters of rats intoxicated with paracetamol

Treatment		ASAT (IU/L)	ALAT (IU/L)	DBil (mg/dL)
Batch 1: normal (distilled water)		144 ± 5,77	109± 5,19	0
Batch 2: PCM intoxicated (2 g/Kg bw)		428 ±14,19****	571 ±20,28****	1,08 ± 0,58****
Batch 3: pre-treated with silymarin and intoxicated with PCM (2 g/Kg bw)		198 ± 4,56**	173± 11,55**	0
Batch 4 and 5: pre-treated with CAJ and intoxicated with PCM (2 g/Kg bw)	Batch 4: CAJ (150 $mg.Kg^{-1}$ bw)	132 ± 17,32	94± 5,25	0
	Batch 5: CAJ (300 $mg.Kg^{-1}$ bw)	157 ± 11,55	99± 8,33	0

The ASAT/ALAT ratio (428/571) in rats intoxicate with paracetamol is 0.74. Lower than 1, this ratio indicates a hepatic cytolytis. Values are given as means ± standard deviation (N=3). Student's t test: **P < 0.01; ***P <0.0001 compared to normal control values. PCM: paracetamol. CAJ: cashew apple aqueous extract

3.2. Effect of cashew apple aqueous extract (CAJ) on body weight, liver weight and relative weight of paracetamol intoxicated rats.

Table 2 shows the results of the effect of CAJ on body weight, liver weight and relative liver weight of rats.

During treatment, all animals, including the normal control (treated only with distilled water), experienced live weight losses. The control intoxicated with paracetamol and untreated had the greatest weight loss of 30±6.33 grams ($P < 0.05$). Animals pretreated for 11 days with silymarin followed by intoxication with paracetamol have a less significant weight loss of seven (07) grams. In animals pretreated with CAJ and then intoxicated with paracetamol, this weight loss varied between eleven (11) and thirteen (13) grams.

The relative liver weight of the control animal treated with distilled water was 4.19 ± 0.35 grams. In the control rat intoxicated with paracetamol and not treated, the relative liver weight of 3.09 ± 1.25 grams, shows a decrease. In rats treated with silymarin or CAJ and then intoxicated with paracetamol, the relative liver weight increased and was close to that of the normal control.

Comment [DB22]: on

Comment [DB23]: Use standard abbreviations. Standard abbreviations for these transaminases are ALT and AST.

All ALAT should be changed to ALT,
All ASAT should be changed to AST.

Comment [DB24]: 1.The format of presentation of your treatment appears so ambigious and confusing, please use a simplified format.
Example : Batch 3 : Silymarin + PCM (2g/kg BW)

2. The Standard deviation values should have a decimal point, not commas. Please change all commas to decimal points.

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Table 2. Effect of cashew apple aqueous extract (CAJ) on body weight, liver weight and relative weight of rats intoxicated with paracetamol

groups	Weight loss (g)	Weight of liver (g)	Relative liver weight (%)
batch 1: Normal (distilled water)	05±3,15	8,189±2,35	4,19±0,35
Batch 2: PCM Intoxicated	30±6,33*	7,046±2,89	3,06±1,25
Batch 3: Silymarin + PCM	07±5,01	8,086±1,68	4,07±0,89
Batch 4: CAJ (150 mg/Kg bw) + PCM	13±4,15	7,655±2,67	3,73±1,23
Batch 5: CAJ (300 mg/Kg bw) + PCM	11±4,15	7,700±3,89	3,88±0,95

Values are given as means ± standard deviation (N=3). Student's t test: *P <0.05; compared with normal control values. PCM: paracetamol (2 g/Kg bw). CAJ: cashew apple aqueous extract.

Comment [DB27]:
The Standard deviation values should have a decimal point, not commas. Please change all commas to decimal points.

3.3. Sleep test in mice intoxicated with paracetamol and given phenobarbital

Table 3 shows the results of sleep time of mice intoxicated with paracetamol and given phenobarbital.

The non-intoxicated mouse (normal control), having received only water and phenobarbital at 80 $mg.kg^{-1}$ bw was put into a sleep that lasted 160 minutes. The mouse intoxicated (intoxicated control) with paracetamol a 200 $mg.kg^{-1}$ bw and not treated, was plunged into a sleep of longer duration, 250 minutes ($P < 0.01$). The paracetamol-intoxicated mouse treated with silymarin had a sleep duration of 165 minutes, approximately equal to that of the normal control. Indeed, this sleep time was shortened by 85 minutes compared to that of the intoxicated control. The mouse intoxicated with paracetamol and treated with CAJ at 300 $mg.Kg^{-1}$ bw followed by intraperitoneal administration of phenobarbital, was plunged into a sleep whose duration was 175 minutes, a value close to those of the normal mouse and the mouse intoxicated and treated with silymarin. The reduction in sleep time under these conditions was 38.59%. The mouse of lot 4, treated with CAJ at 150 $mg.Kg^{-1}$ bw had a relatively long sleep time of 200 minutes, which corresponds to a reduction of 29.82% compared to the sleep time of the mouse of lot 2 (intoxicated and untreated control).

Table 3. Effect of cashew apple aqueous extract (CAJ) on sleep time of mice intoxicated with paracetamol and given phenobarbital

Comment [DB28]:
The Standard deviation values should have a decimal point, not commas. Please change all commas to decimal points.

Treatment ($mg.Kg^{-1}$ bw)	Batch 1 Water + PHE	Batch 2 Water + PCM + PHE	Batch 3 Silymarin 100 mg/Kg PC + PCM + PHE	Batch 4 CAJ 150 mg/Kg PC+ PCM + PHE	Batch 5 CAJ 300 mg/Kg PC+ PCM + PHE
Sleep time (minutes)	160±33,09	285± 23,09**	165± 18,78	200± 20,56	175± 19,84
Percentage reduction in sleep time (%)	-	-	42,10	29,82	38,59

Values are given as means ± standard deviation (N=3). Student's t test: **P < 0.01 compared with normal control values. PCM: paracetamol **(200 mg/Kg bw)**; PHE: phenobarbital **(80 mg/Kg bw)**; CAJ: cashew apple water extract.

4. DISCUSSION

The objective of this work was to evaluate the hepatoprotective activity of an aqueous extract of cashew apple in rats and mice intoxicated with paracetamol.
The liver is an organ with several functions. One of them, the protein metabolic function, is partly characterized by transaminations reactions, producing precursors of gluconeogenesis from amino acids. These reactions are mainly catalyzed by ALAT and ASAT, which are found in the liver, but also in the muscle. Both present in the cytosol and in the mitochondria for ASAT, the increase in serum activity of these enzymes in rats intoxicated with paracetamol at 2 $g.Kg^{-1}$ bw, as indicated by the results, suggests that cytolysis is taking place. The ASAT/ALAT ratio being lower than one, shows that this cytolysis concerns the liver and not the muscle, given the specificity of ALAT for the latter [12]. The digestive function of the liver is partly reflected in the detoxification of bilirubin from the degradation of hemoglobin in red blood cells, in the form of conjugated bilirubin, which is mainly eliminated through the bile. The serum level of unconjugated (direct) bilirubin increased from 0 to 1.08 mg.dL-1, after ingestion of paracetamol, showing that this hepatic function is affected [13]. Also, although less formal according to statistical analysis, the loss of live weight and the decrease in the relative liver ratio after administration of paracetamol, are also indicators of intoxication of the animal. These results are reminiscent of those of Sangaré et al, who used carbon tetrachloride as a hepatotoxic agent in rats [14].

In the presence of silymarin or CAJ at concentrations ranging from 150 to 300 $mg.Kg^{-1}$ bw, serum ALAT and ASAT activities were significantly decreased, direct bilirubin level returned to its initial value of zero. In addition, the weight of intoxicated and treated rats showed relative improvement. These data suggest that CAJ, like Silymarin, has a hepatoprotective effect. Thus, CAJ and silymarin may act through similar mechanisms by inhibiting hepatic cytochrome P450 oxidase [15]. Also, it could be that CAJ, given its molecular content indicating the presence of vitamin C and flavonoids [4], scavenges and deactivates free radicals including NAPQI, a toxic product derived from the biotransformation of paracetamol. *Rhamnus alaternus* L., and *Ananas comosus*, hepatotropic plant substances, are thought to act in part by these same mechanisms [16, 17].
Phenobarbital is an antiepileptic drug that increases the sleep time of mice intoxicated with paracetamol, as shown by the results. This increase, could be attributed to a dysfunction of the liver and the life span of this antiepileptic molecule. In the presence of CAJ for concentrations ranging from 150 to 300 $mg.Kg^{-1}$ bw, this sleep time decreases from 28.82 to 38.59%. This decrease is synonymous with an improvement of the liver physiology and thus a confirmation of the hepatoprotective effect of this natural substance. Similar results were obtained with *Terminalia superba* in rats [18]. However, the rat would be more resistant to the toxic effect of paracetamol than the mouse in view of the sleep times recorded and the toxic doses of paracetamol used.

5. CONCLUSION

The liver is a vital organ with multiple functions. Although resistant, it must be protected from the constantly recurring aggressions of the biotransformation processes of xenobiotics (administered drugs, pollutants, alcohol) and of endogenous substances harmful by their nature (ammonia), because of their concentration (cholesterol) and their activity (steroid hormone). CAJ with its ability to reduce serum transaminase activities and biturbin levels, as well as the duration of sleep in mice intoxicated with paracetamol and subjected to the phenobarbital test, could therefore play a hepatoprotective role. The hepatoprotective effect of this natural substance would be partly related to its phytochemical and nutritional content, revealing the presence of vitamin C and polyphenols. In sum, the use of CAJ in traditional medicine to prevent liver diseases can be considered.

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Comment [DB29]: Use the standard abbreviation as commented above for all transaminases

Comment [DB30]: These preliminary sentences should not be in the conclusion. Conclusion should contain just the summary of your findings.

Comment [DB31]: Check all reference and correct according to Journals stipulated format

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