

Subchronic toxicity of aqueous extract of *Cnestis ferruginea* (Connaraceae) leaves on *Wistar* rats

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Abstract

The aim of this study is to evaluate the subchronic toxicity of *Cnestis ferruginea* leaves. After preparing the aqueous extract of *Cnestis ferruginea* leaves (AECF) by maceration, 108 male and female rats divided into three groups of 24 received 1 mL of distilled water or EACF at 50 or 100 mg/kg of BW orally every day. The rats were weighed every two days and on days 15, 30 and 60 of treatment, 6 males and 6 females from each group were sacrificed. Their blood was collected in EDTA and dry tubes respectively for haematological and biochemical analyses. The results showed that the rats weight increased gradually and no significant difference was recorded between weight gains. With regard to haematological parameters, it was only after 30 days of treatment that a significant difference was observed in red blood cell and white blood cell ($p<0.05$) counts with the 50 mg/kg dose of BW. Biochemical analyses revealed a significant decrease in glucose levels of 3.83% ($p<0.05$) after 15 days with the 50 mg/kg dose of BW. In contrast, a significant increase of 7.37% ($p<0.05$) in cholesterol levels was observed after 30 days with a dose of 50 mg/kg of BW. It be noted that *Cnestis ferruginea* leaves, far from being toxic when taken orally, have pharmacological properties that make them a good candidate for the development of new drugs.

Keywords: Toxicity; *Cnestis ferruginea*; Haematological; Biochemical

1. Introduction

Traditional medicine has long been a source of acceptable and affordable healthcare. Eighty per cent of African women currently rely on traditional medicine to meet their essential healthcare needs [1]. Being inexpensive, plants represent potential natural therapeutic products [2]. However, the use of any plant extract should first be subject to toxicological analysis in order to rule out any risks associated with its use [3]. Toxicology is the branch of pharmacology that studies the adverse effects of bioactive substances on living organisms [4]. This study is of paramount importance in pharmacotherapy, as it establishes the safety and efficacy of any new drug [4]. It is in this context that this study, which aims to evaluate the subchronic toxicity of *Cnestis ferruginea* leaves *in vivo*, was conducted.

2. Material and methods

2.1. Material

2.1.1. Plant material

The plant material consists of leaves from *Cnestis ferruginea* (Connaraceae) harvested in Trawininkro, Buyo Sub-Prefecture (Ivory Coast). This part of the plant was chosen because it is traditionally used in the treatment of

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hypofertility. This plant was identified by Professor Aké Assi at the Botany and Plant Biology Laboratory of the Biosciences Training and Research Unit (TRU) at Félix Houphouët-Boigny University in Cocody-Abidjan.

2.1.2. Animal material

The rats, *Rattus norvegicus* (Muridae) *Wistar* strain (males and females), came from the *vivarium* of the HNS (Higher Normal School). These rats were used to conduct subchronic toxicity studies on the aqueous extract of *Cnestis ferruginea*. The rats were kept at a constant temperature of $24\pm2^{\circ}\text{C}$, with humidity levels of 50 to 55% and a photoperiod of 12 hours of natural light. The animals were fed Ivorian Compound Food Manufacturing Company, pellets and had free access to water.

2.2. Methods

2.2.1. Extraction method

Extraction was carried out by macerating 50 g of *Cnestis ferruginea* leaf powder in 1.5 litre of distilled water, stirring for 24 hours with a magnetic stirrer and filtering three times through cloth, cotton wool and Whatman No. 1 filter paper. The filtrate obtained is then placed in an oven (Memmert) at 50°C to produce a dry extract. The powder from *Cnestis ferruginea* leaves was obtained by drying the leaves on the bench at laboratory temperature. These leaves are then ground in a Blender (Germany) to obtain powder.

2.2.2. Subchronic toxicity

108 adult rats were divided into 3 groups of 24 animals. Each group contained an equal number of males and females. In each group, the animals were treated as follows:

- Group 1 (control); distilled water as vehicle;
- Lot 2 (treated: AECF50); aqueous extract of *Cnestis ferruginea* (50 mg/kg of body weight);
- Lot 3 (treated: AECF100); aqueous extract of *Cnestis ferruginea* (100 mg/kg of body weight).

The animals were given a daily dose of 1 mL of either distilled water or extract orally using a feeding tube (intragastric). During treatment, the rats were weighed using a Sartorius balance (BP 310P, d=0.001g, Germany) every two days and food and water intake was monitored. On days 15, 30 and 60 of treatment, 12 subjects (6 males and 6 females) are randomly selected from each batch and sacrificed by decapitation. Blood is collected in EDTA tubes for haematological parameter analysis and in dry tubes for serum collection and biochemical parameter analysis. Organs such as the liver, lungs, heart and kidneys are removed, weighed immediately using a Sartorius electronic balance (BP 310P, d = 0.001g, Germany) and some are dried in an oven at 100°C for 24 hours and then weighed again to obtain the dry weight.

2.3. Blood and serum collection technique

Blood was collected when the animals were sacrificed. Blood was collected in two types of tubes: tubes containing the anticoagulant EDTA (ethylene diamine tetraacetic acid) for complete blood count (CBC) and dry tubes. The blood in the dry tubes is centrifuged using a Vidas centrifuge (Biomerieux, France) at 3,000 revolutions per minute for 5 minutes. The serum is collected for biochemical parameter testing.

2.4. Technique for evaluating blood and biochemical parameters

2.4.1. Haematological analysis

White blood cell, red blood cell, platelet and haematocrit counts were determined immediately on blood samples collected in tubes containing EDTA anticoagulant. The analyses were performed according to the standard methods of Baker *et al.* [5] using an automatic analyser (Mindray BC-5380, China).

2.4.2. Biochemical parameter testing

All biochemical tests on serum samples were performed using an automatic analyser (Mindray BS-200E, China). The testing methods differed depending on the biochemical parameter. The protocol for each measurement was pre-established and then incorporated into the device during the measurements. Thus, blood glucose was measured using the Trinder [6] enzymatic colorimetric test, as improved by Dingeon *et al.* [7]. Triglycerides were measured using the enzymocolorimetric test [8]. Total cholesterol was determined using the enzymatic test developed by Allain *et al.* [9]. High-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol were measured by enzymatic detection. The urea test was performed using a kinetic assay [10] and the uric acid test was performed using an

enzymatic colorimetric method. The colorimetric method was used to measure creatinine. The measurements of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), they were performed using the Karmen colorimetric method and the colorimetric method recommended by the International Federation of Clinical Chemistry [11], respectively. The total protein level was determined using the Biuret method [12]. In addition, the Tietz colorimetric method was used to obtain the total and conjugated bilirubin levels.

2.5. Statistical analysis

The values were expressed as the mean followed by the standard error of the mean (M±SEM). The significance of the differences observed between the treated and control groups was assessed using analysis of variance (ANOVA 1) and Tukey-Kramer's multiple comparison test via GraphPad Prism 7 statistical software (Microsoft, California, USA). If $p<0.05$, the difference is significant, and if $p>0.05$, the difference between the values is not significant.

3. Results

3.1. Animal behaviour

During treatment, no changes in behaviour were observed in the treated rats compared to the control group.

3.2. Effect of the extract on the body weight of rats

During treatment, the weight of the rats increased gradually over time. Body weight gain in rats treated with both doses, 50 and 100 mg/kg of BW of the extract, was similar, and there was no significant difference between the treated and control groups (Figure 1).

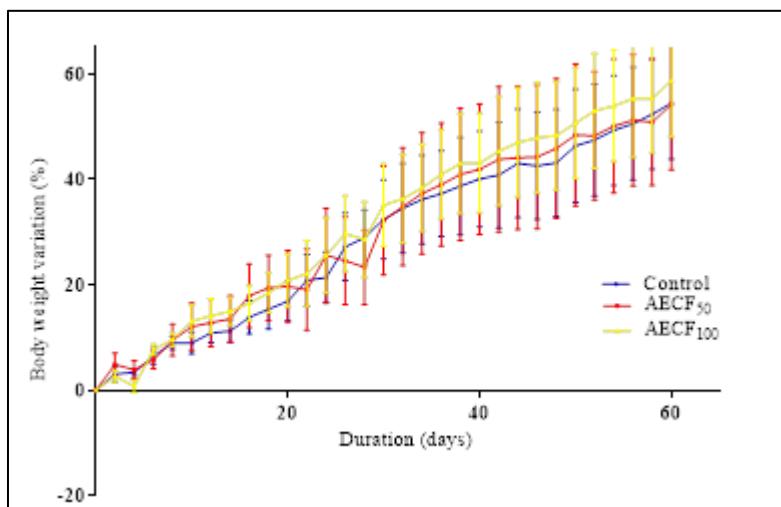


Figure 1 Change in body weight of rats during treatment with AECF

3.3. Effect of the extract on organ weight

At the end of treatment, the weight of the organs removed (kidney, liver, heart and lung) showed no significant variation compared to the control group, regardless of the dose of the extract. (Table 1).

Table 1 Effects of orally administered AECF on organ weight in rats

Duration of treatments	Treatments	Weight of organs (g/100g of body weight)			
		Kidney	Liver	Heart	Lung
15 days	Control	0,270±0,005	3,730±0,220	0,3934±0,011	0,730±0,022
	AECF ₅₀	0,271±0,010	3,562±0,198	0,3938±0,019	0,827±0,094
	AECF ₁₀₀	0,269±0,013	3,646±0,231	0,3705±0,014	0,786±0,047
30 days	Control	0,276±0,098	3,192±0,103	0,3469±0,010	0,632±0,039
	AECF ₅₀	0,273±0,019	3,014±0,091	0,3470±0,013	0,700±0,057
	AECF ₁₀₀	0,261±0,014	2,939±0,078	0,3440±0,009	0,623±0,038
60 days	Control	0,273±0,010	3,831±0,169	0,3909±0,027	0,660±0,037
	AECF ₅₀	0,2743±0,010	3,878±0,124	0,3459±0,016	0,690±0,044
	AECF ₁₀₀	0,269±0,010	3,635±0,164	0,3596±0,009	0,696±0,051

Values are means ± SEM (n=12/batches). P>0.05; AECF₅₀: Aqueous extract of *Cnestis ferruginea* leaves (50 mg/kg of BW); AECF₁₀₀: Aqueous extract of *Cnestis ferruginea* leaves (100 mg/kg of BW)

3.4. Haematological parameters

At the end of treatment, haematological parameters (white blood cells, red blood cells, haematocrit, blood platelets and haemoglobin) were measured. For the 15-day treatment, the rats treated with both doses showed no significant difference compared to the control group. Conversely, the rats treated for 30 days showed a significant difference in red blood cell count (p<0.05) and white blood cell count (p<0.05) with the 50 mg/kg of BW dose. A significant difference (p<0.05) was also observed in the white blood cell count of treated rats compared to the control group after 60 days of treatment with a dose of 50 mg/kg of BW (Table 2).

Table 2 Effects of AECF on selected haematological parameters in rats.

Duration	Lots	Blood parameters				
		RBC ($\times 10^6$ μL^{-1})	WBC ($\times 10^3$ μL^{-1})	HCT (%)	PLQ ($\times 10^3$ μL^{-1})	HGB (g/dL)
15 days	Control	7,45±0,16	10,22±0,67	46,94±1,34	967,90±21,82	13,52±0,18
	AECF ₅₀	8,02±0,19	10,85±0,74	48,40±1,8	998,30±30,83	13,43±0,18
	AECF ₁₀₀	7,98±0,29	10,55±0,71	48,11±1,68	1029,00±37,25	13,72±0,19
30 days	Control	7,67±0,26	7,58±0,37	44,67±0,61	1018,00±34,64	14,00±0,06
	AECF ₅₀	6,47±0,27*	8,95±0,09*	38,87±0,87	1079,00±15,59	13,77±0,09
	AECF ₁₀₀	7,46±0,35	8,10±0,12	41,77±1,81	934,30±24,54	13,62±0,37
60 days	Control	7,74±0,59	8,09±0,35	44,33±0,68	987,70±17,32	13,87±0,15
	AECF ₅₀	7,33±0,61	10,58±0,57*	43,87±1,94	989,30±48,55	13,47±0,41
	AECF ₁₀₀	7,37±0,19	8,32±0,69	43,90±2,29	941,30±41,57	13,53±0,35

Values are means ± SEM (n=12/batch). *p<0.05. For values without (*), p>0.05; AECF₅₀: Aqueous extract of *Cnestis ferruginea* leaves (50 mg/kg of BW); AECF₁₀₀: Aqueous extract of *Cnestis ferruginea* leaves (100 mg/kg of BW); RBC: Red blood cell, WBC: White blood cell, HCT: Haematocrit, PLQ: Blood platelet, HGB: Haemoglobin

3.5. Biochemical parameters

3.5.1. Glucose and lipid levels

Table 3 summarises the glucose and lipid parameter values measured in the animals' serum after 15, 30 and 60 days of treatment with AECF. This extract induces a significant decrease in serum glucose concentration of 3.83% (p<0.05) after 15 days of treatment with a dose of 50 mg/kg of BW. This decrease was also observed in serum triglyceride levels, which fell by 7.64% (p<0.01) after 30 days and 11.69% (p<0.05) after 60 days of treatment with doses of 50 and 100 mg/kg of BW, respectively. In contrast, a significant increase of 7.37% (p<0.05) in cholesterol concentration was observed after 30 days of treatment with a dose of 50 mg/kg of BW. HDL and LDL cholesterol did not show any significant variation during this study.

Table 3 Effects of AECF on serum glucose and lipid concentration

Duration	Lots	Glucose	Triglycerides	Total cholesterol	HDL	LDL
15 days	Control	0,983±0,032	1,360±0,022	0,920±0,025	0,405±0,009	0,258±0,035
	AECF ₅₀	0,915±0,022	1,332±0,027	0,927±0,018	0,399±0,014	0,258±0,021
	AECF ₁₀₀	0,880±0,007*	1,366±0,0196	0,923±0,014	0,409±0,010	0,225±0,006
30 days	Control	1,027±0,021	1,361±0,026	0,908±0,019	0,468±0,009	0,290±0,007
	AECF ₅₀	1,039±0,024	1,257±0,021**	0,975±0,017*	0,445±0,010	0,287±0,006
	AECF ₁₀₀	0,995±0,026	1,283±0,020	0,938±0,018	0,450±0,009	0,291±0,008
60 days	Control	1,004±0,043	1,300±0,021	0,957±0,022	0,410±0,018	0,372±0,014
	AECF ₅₀	1,038±0,024	1,220±0,023	0,852±0,041	0,393±0,008	0,380±0,009
	AECF ₁₀₀	1,068±0,034	1,148±0,049*	0,933±0,041	0,388±0,011	0,377±0,009

Values are means ± SEM (n=12/batch). *p<0.05. For values without (*), p>0.05; AECF₅₀: Aqueous extract of *Cnestis ferruginea* leaves (50 mg/kg of BW); AECF₁₀₀: Aqueous extract of *Cnestis ferruginea* leaves (100 mg/kg of BW)

3.6. Renal parameters

The results of renal parameter analyses (urea, uric acid and creatinine) after 15, 30 and 60 days of treatment with the extract are shown in Table 4. These results show a significant decrease of 23.31% (p<0.05) and 37.5% (p<0.05) in serum urea concentration for the respective durations of 15 and 60 days with the 100 mg/kg dose of BW. There was also a significant decrease of 37.5% (p<0.05) in serum creatinine concentration after 15 days of treatment with a dose of 100 mg/kg of BW.

Table 4 Effects of AECF on serum concentration of renal parameters

Duration	Lots	Urea (g/L)	Uric acid (mg/L)	Creatinine (mg/L)
15 days	Control	0,163±0,013	23,830±1,721	6,000±0,408
	AECF ₅₀	0,140±0,007	22,830±1,014	5,000±0,408
	AECF ₁₀₀	0,125±0,006*	24,170±1,701	3,750±0,479*
30 days	Control	0,193±0,009	23,920±1,228	5,833±0,207
	AECF ₅₀	0,200±0,006	22,830±0,716	5,917±0,229
	AECF ₁₀₀	0,203±0,004	25,080±0,596	5,750±0,250
60 days	Control	0,180±0,017	22,000±1,414	6,000±0,577
	AECF ₅₀	0,167±0,009	26,000±2,191	5,333±0,667
	AECF ₁₀₀	0,123±0,009*	22,800±1,158	5,000±0,577

Values are means ± SEM (n=12/batch). *p<0.05. For values without (*), p>0.05; AECF₅₀: Aqueous extract of *Cnestis ferruginea* leaves (50 mg/kg of BW); AECF₁₀₀: Aqueous extract of *Cnestis ferruginea* leaves (100 mg/kg of BW)

3.7. Liver parameters

The mean values of liver parameters measured in the blood of animals at the end of the various treatments are presented in Table 5. The aqueous extract of *Cnestis ferruginea* leaves induced a significant reduction of 21.35% (p<0.05) in AST concentration at a dose of 100 mg/kg of BW after 15 days of treatment. In animals treated with a dose of 50 mg/kg PC, a significant decrease of 20.38% (p<0.05) and 11.07% (p<0.05) in serum ALT concentration was observed for 15 and 30 days, respectively. In animals treated with a dose of 100 mg/kg of BW for 15 days, a significant increase of 19.90% in serum concentration was observed. ALT was observed. However, total protein levels increased significantly by 20.06% (P<0.05) after 60 days of treatment at a dose of 100 mg/kg, and conjugated bilirubin levels increased by 36.60% (p<0.05) after 15 days of treatment at a dose of 50 mg/kg of BW. There was no significant change in the concentration of ALP and total bilirubin.

Table 5 Effects of AECF on serum concentrations of liver parameters

Duration	Lots	AST (UI/L)	ALT (UI/L)	Total protein (g/L)	Total bilirubin (mg/L)	Conjugated bilirubin (mg/L)
15 days	Control	57,00±3,04	51,50±2,33	59,50±2,04	4,80±0,36	1,94±0,31
	AECF ₅₀	44,83±1,35*	41,00±2,86*	56,80±56,80	5,93±0,19	3,06±0,18*
	AECF ₁₀₀	53,50±1,84	41,25±0,48*	54,40±1,84	5,18±0,54	2,12±0,29
30 days	Control	86,08±2,20	42,08±1,05	56,08±1,59	3,767±0,09	2,07±0,15
	AECF ₅₀	74,75±3,35	37,42±1,05*	57,83±1,16	3,80±0,59	2,20±0,06
	AECF ₁₀₀	86,42±2,52	44,50±1,20	58,33±1,18	4,43±0,59	1,70±0,06
60 days	Control	80,17±4,04	40,67±2,03	43,83±2,21	4,27±0,29	2,00±0,15
	AECF ₅₀	84,83±3,59	40,33±0,88	45,33±2,33	4,20±0,64	1,77±0,24
	AECF ₁₀₀	91,17±1,87	42,00±2,65	54,83±2,32*	4,20±0,35	2,10±0,20

Values are means ± SEM (n=12/batch). *p<0.05. For values without (*), p>0.05; AECF₅₀: Aqueous extract of *Cnestis ferruginea* leaves (50 mg/kg of BW); AECF₁₀₀: Aqueous extract of *Cnestis ferruginea* leaves (100 mg/kg of BW)

4. Discussion

The subacute toxicity study showed that treatment of rats with AECF had no significant effect on the body weight of treated rats. Similarly, the organs (kidney, liver, lung, heart) removed at the end of the various treatments showed no significant variation in weight. This absence of change in body weight and organ weight can be explained by the harmlessness and non-toxicity of *Cnestis ferruginea* leaves.

As for the haematological analysis, it revealed no significant changes in the parameters measured in rats treated with doses of 50 and 100 mg/kg of BW after 15 days of treatment. However, after 30 days, the results showed a significant decrease in red blood cell count compared to the controls with the 50 mg/kg of BW dose. This decrease in red blood cell count could be explained by the presence of saponosides in AECF. Indeed, haemolysin, a saponoside substance isolated from *Passiflora quadrangularis* (Passifloraceae), has haemolytic effects [13]. AECF may contain this substance or similar substances that cause red blood cell haemolysis. These results are similar to those obtained by Bleu *et al.* [14]. After administering an aqueous extract of *Passiflora foetida* (Passifloraceae), this author recorded a decrease in red blood cell count.

Regarding white blood cell count, after 30 and 60 days of treatment, the 50 mg/kg of BW dose caused a significant increase in white blood cell count. This could be the result to stimulation of the immune system by the plant extract. Indeed, the tannins found in this extract have immunostimulatory activities [15]. Other authors have observed similar results. This is the case of Gupta *et al.* [16] with the aqueous extract of *Clerodendrum phlomidis* (Verbenaceae) leaves.

During this study, biochemical analyses focused on glucose and lipids, renal parameters and hepatic parameters. Glucose testing revealed a significant decrease in its concentration after 15 days of treatment at a dose of 50 mg/kg of BW. This result could be linked to the alkaloids present in the plant extract. Alkaloids have hypoglycaemic properties. This hypoglycaemic activity is consistent with the results obtained by Adisa *et al.* [17]. These authors worked on the

methanolic and acetate extract of the leaves of this plant. Similar results were obtained by Gupta *et al.* [16] with the aqueous extract of *Clerodendrum phlomidis* (Verbenaceae) leaves.

Triglycerides are among the biochemical parameters whose increased serum levels are associated with cardiovascular disease [18]. In this study, the AECF₅₀ and AECF₁₀₀ doses induced a significant decrease in the level of this parameter after 30 days and 60 days of treatment, respectively. This could be explained by the richness of this plant extract in chemical compounds such as alkaloids. Indeed, these compounds have the ability to positively influence the cardiovascular system by reducing fat mass, for example [19]. These results differ from those obtained by Bleu *et al.* [14] and Pillai *et al.* [20], who observed no significant variation in serum triglyceride concentration after administering extracts of *P. foetida* (Passifloraceae) and *Plectranthus amboinicus* (Lamiaceae) respectively. Conversely, Gupta *et al.* [16] observed an increase in this parameter with the aqueous extract of *C. phlomidis* (Verbenaceae) leaves. The measurement of lipid parameters such as HDL and LDL showed no significant variation. However, AECF significantly increased blood cholesterol levels at a dose of 50 mg/kg of BW after 30 days of treatment. This increase in cholesterol concentration is linked to an increase in the cardiovascular disease index [20]. However, this increase could be beneficial for steroidogenesis, in which cholesterol is the precursor [21] for the synthesis of steroid hormones. This result contradicts that obtained by Bleu *et al.* [14]. These authors administered a dose of 800 mg/kg of BW of aqueous extract of *Passiflora foetida* (Passifloraceae) to rats and observed a decrease in blood cholesterol levels.

The concentration of urea, uric acid and creatinine is an important marker for the diagnosis of renal function [12; 23]. Analysis of these different renal parameters revealed a significant decrease in serum urea concentration after 15 days and 60 days of treatment with a dose of 100 mg/kg of BW and creatinine on the 15th day of treatment with a dose of 100 mg/kg of BW. The decrease in creatinine on day 60 with a dose of 100 mg/kg of BW is not significant. Creatinine and urea are mainly eliminated from the blood by glomerular filtration, which itself depends on blood pressure in the glomerular capillaries. In these capillaries, normal arterial pressure is equal to 30 mmHg [24]. AECF acts on capillary arterial pressure by facilitating glomerular filtration of creatinine and urea, thereby reducing their blood levels. These results confirm the work of Adisa *et al.* [17]. These authors demonstrated that methanolic extract and acetate of *Cnestis ferruginea* reduce creatinine and urea levels in streptozotocin-induced diabetic rats. No significant variation in uric acid was observed.

In this study, transaminase (AST and ALT) concentrations decreased significantly in animals treated for 15 days with AECF₅₀ (AST and ALT) and AECF₁₀₀ (ALT). After 30 days of treatment, EAFCF50 also induced a significant decrease in ALT concentration. Significant reductions in AST and ALT levels have already been achieved in rats by Bleu *et al.* [14] with the aqueous extract of *Passiflora foetida* (Passifloraceae) at a dose of 800 mg/kg of BW.

Transaminases are liver markers, and their activity increases in cases of liver toxicity [25]. Their concentrations in serum provide information about hepatocyte damage [19]. ALT is a liver-specific enzyme in dogs, rats, rabbits, cats and primates. It can provide a quantitative assessment of the degree of damage to the liver [26]. As for AST, in addition to the liver, it is also present in the heart and skeletal muscles [27]. It can therefore be said that AECF has a beneficial effect on the liver and, by extrapolation, on the heart and skeletal muscles.

If it has been demonstrated that a substance's ability to reduce harmful effects or preserve the mechanisms of liver function against disruption is an indication of its protective effect [28], then the administration of AECF would therefore protect the liver. This hepatoprotective property of AECF could be linked to the presence in this extract of flavonoids such as apigenin and naringenin [29], which are hepatoprotective molecules [30]. This hypothesis is confirmed by the work of Akharaiyi *et al.* [31]. Indeed, these authors demonstrated that the ethanolic extract of *Cnestis ferruginea* leaves has a hepatoprotective effect in cases of paracetamol-induced hepatotoxicity in mice.

Abbreviations

- BW: Body Weight
- CBC: Complete Blood Count
- EDTA : Ethylene Diamine Tetra-Acetic Acid
- HDL: High-density lipoprotein
- HNS : Higher Normal School
- kg: kilogram
- LDL: low-density lipoprotein
- mg: milligram
- ml: millilitre
- TRU: Teaching and Research Unit

5. Conclusion

With regard to toxicological studies conducted on the aqueous extract of this plant, it should be noted that oral administration over several days revealed a decrease in red blood cell count. This study also revealed that this plant may have hypoglycaemic, immunostimulant, cardiovascular protective, hepatoprotective and renal protective effects. These pharmacological effects, which promote improved health, would justify the numerous therapeutic uses of this plant in traditional medicine by populations living in areas where it is found.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest and they are alone responsible for the accuracy and integrity of the paper content.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

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