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Evaluation of Hepatoprotective Activities Aqueous and Hydroethanolic Extracts from the Leaves of *Erythrococca anomala* in Rats

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Abstract: Widely used in Ivory Coast and sub-Saharan Africa in traditional medicine as anti-inflammatory, antioxidant, laxative and purgative, *Erythrococca anomala* (Euphorbiaceae), is an annual plant. In this work, the aim was to evaluate the Hepatoprotective activity of the aqueous and hydroethanolic extracts of the leaves. Carbon Tetrachloride (CCl_4) was used to induce hepatotoxicity in rats. This hepatotoxic effect causes a significant increase in the levels of liver enzymes and serum proteins. Hepatoprotective activity was assessed by assaying liver enzymes such as serum transaminases (alanine aminotransferase and aspartate aminotransferase) and proteins by spectrophotometric and electrophoretic techniques. When administered orally as a preventive and curative dose of 100 and 200 mg / kg body weight, the extracts significantly reduce significantly as silymarin, the reference hepatoprotective substance at 100 mg / kg bw, hepatotoxicity induced by Carbon tetrachloride (CCL_4) at a dose of 5 mg / kg bw after a significant reduction in liver enzyme levels, globulins and a significant increase in albumin. The possible mechanism of this hepatoprotective activity of the extracts may be due to the action of the antioxidants such as the flavonoids, the phenols present in the extracts.

Keywords: *Erythrococca anomala,* Silymarin, Carbon Tetrachloride, Hepatotoxicity, Hepatoprotective

1. Introduction

Medicinal plants are widely used in traditional medicine for the prevention and treatment of various diseases, especially in sub-Saharan Africa and in developing countries [1]. These plants currently constitute reservoirs of natural substances used in the treatment of many infections [2]. It is therefore necessary to look for highly effective, low toxic, low-cost herbal remedies [3].

Erythrococca anomala is a medicinal plant of the family Euphorbiaceae, widely used in traditional medicine in Ivory Coast and sub-Saharan Africa. Phytochemical studies have revealed the presence of phenols, flavonoids, alkaloids, sterols and saponosides in leaves [4]. These various secondary metabolites hold the attention of playing an essential role in the treatment of diseases [5]. Similarly, studies have shown that the leaves have anti-inflammatory and antioxidant activity [6, 7].

In Ivory Coast, the macerates of the leaves of this plant make it possible to fight against meningitis and malaria, while in Cameroon, decoctions and macerated leaves allow to treat dental pain; But used as laxatives and purgatives, make it possible to expel the worms; in Nigeria, bark is used against arthritis and rheumatism [8].

However, there is no information on the hepatoprotective properties of the leaves of this plant.

This study aims to evaluate the hepatoprotective activities of aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* by the determination of hepatic enzymes such as serum transaminases (alanine

aminotransferase and aspartate aminotransferase) and proteins by spectrophotometric and electrophoretic techniques.

2. Materials and Methods

2.1. Equipment

2.1.1. Plant Material

The plant material consists of leaves of *Erythrococca anomala* harvested in the Yakssé-mé region, Department of Adzopé, 15 km from Abidjan, washed and dried at room temperature, protected from the sun at the research laboratory of Inorganic and organic chemistry of the UFR of pharmaceutical and biological sciences. This plant has been identified at the National Floristic Center of Ivory Coast where a sample (OAT -ErAn) is kept.

2.1.2. Animal Equipment

Wistar albinos rats weighing 190-200 g of each sex kept for three weeks at the laboratory animal home of UFR Pharmaceutical and Biologic Sciences, University of Felix Houphouet Boigny, Ivory Coast were used. The animals were maintained under standard housing conditions: temperature (27°±1C), humidity (55-60%), light/dark cycle (12:12 h) and had free access to standard rodent pellet diet and water ad libitum.

2.2. Methods

2.2.1. Preparation of Extracts

The aqueous extract is prepared from 100 grams of *Erythrococca anomala* leaf powder in one liter of boiling distilled water for fifteen minutes. The solution thus obtained is filtered on hydrophilic cotton and then vacuumed with ordinary filter paper. The collected filtrate was placed in an oven at 40°C. A dark brown dry powder was obtained for the total crude aqueous extract of *Erythrococca anomala*.

The Guédé-Guina method [9] was used to obtain the 70% hydroethanolic extract of *Erythrococca anomala*. A 70% hydroethanolic solution (Et OH / H_2O, 70: 30) was used for the preparation of Hydroethanolic extract of *Erythrococca anomala* in a vial. One liter of the hydroethanolic solution and 100 g of *Erythrococca anomala* powder were used for this purpose. The mixture obtained was homogenized with a magnetic stirrer for 24 hours. The homogenate is filtered on hydrophilic cotton and then under vacuum. The collected filtrate was concentrated in a rotary evaporator and then placed in an oven at 40°C. for complete drying. The 70% hydroethanolic extract of *Erythrococca anomala* appears in the form of a dark green paste after drying.

2.2.2. Preventive Treatment

The determination of the Hepatoprotective properties of the extracts was carried out according to the method described by [10] with some modifications. It is a method that demonstrates the preventive properties of aqueous and hydroethanolic extracts of leaves of *Erythrococca anomala* against carbon tetrachloride (CCL_4) poisoning in comparison with silymarin (Sil).

The animals received the following treatments:

- Lot 1 received distilled water for 7 days and then 5 ml / kg bw of CCl_4 poisoning solution intraperitoneally on the seventh day of treatment.
- Lot 2 received Olive Oil for 7 days by intraperitoneal on the seventh day.
- Lot 3 received silymarin (Sil) at 100 mg / kg bw for 7 days then 5 ml / kg bw of the CCl_4 intoxication solution by intraperitoneal route on the seventh day of treatment.

The rats of batches 4 and 5 respectively received 100 and 200 mg / kg bw of aqueous extract for 7 days then 5 ml / kg bw of the CCl_4 intoxication solution by intraperitoneal route on the seventh day of treatment.

The rats of batches 6 and 7 received respectively 100 and 200 mg / kg of hydroethanolic extract for 7 days then 5 ml / kg bw of the CCl_4 intoxication solution by intraperitoneal route on the seventh day of treatment.

2.2.3. Curative Treatment

The curative treatment demonstrates the curative properties of aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* in comparison with that of Sil.

- Lot 9 received 5 mL / kg bw of CCl_4 intraperitoneally for 7 days.
- Lot 10 received Olive Oil per os for 7 days.
- Lot 11 received intramuscularly 5 mL / kg bw of CCl_4 per day for 3 days followed by silymarin (Syl) at 100 mg / kg bw per os for 4 days.

The rats of lots 12 and 13 received intramuscularly 5 ml / kg bw of CCl_4 per day for 3 days and then 100 mg / kg and 200 mg / kg bw of the aqueous extract of the leaves of *Erythrococca anomala* per os, during 4 days.

The rats of batches 14 and 15 received intramuscularly 5 mL / kg bw of CCl_4 per day for 3 days and 100 mg / kg and 200 mg / kg bw of the hydroethanolic extract of the leaves of *Erythrococca anomala* per os, during 4 days.

Blood was collected by caudal puncture on day 8 to estimate serum levels of transaminases (Aspartate Aminotransferase (ASAT), Alanine Aminotransferase (ALAT)), proteins and globins by electrophoresis.

2.2.4. Statistical Analysis

The values expressed as mean ± SD from 6 animals. The results were subjected to statistical analysis by using one way ANOVA followed by Dunnett's test to significant, p values less than 0.05 were considered significant.

3. Results

3.1. Preventive Effect of Different Extracts on the Concentrations of Transaminases and Total Proteins During Hepatotoxicity

The results of the assay show that before the experiment (D0) there was no significant difference in the concentration of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and total proteins (PRO) between the different Lots. After 7 days of experiments, the assay

showed that CCl_4 increased transaminase concentrations and decreased the total proteins in the untreated intoxication batch (distilled water + CCl_4) significantly *(p <0.001*) compared to that of the non-intoxicated batch Treated Olive Oil, intoxicated by Silymarin (Sil) and aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* (Table 1).

In general, the preventive effect of aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* over concentrations of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and total proteins (PRO) is similar to that of Sil (Table 1).

Table 1. *Preventive effect of extracts on the concentrations of transaminases and total proteins during hepatotoxicity.*

TREATMENT	DO			D7		
	ASAT (UI/L)	ALAT (UI/L)	TOTAL PROT (g/L)	ASAT (UI/L)	ALAT (UI/L)	TOTAL PROT (g/L)
Olive Oil	259.7±19,63	229.3± 19	82± 35.54	259.7±4.0***	228.5±35,54**	83.8±37.17***
Water+ CCl_4	222.3±27.43	206.3±5.68	88.67 5.5	372± 22.5	389.54± 5.85	39.7± 5.03
Sil+CCl_4	252± 38.69	224.67±9.07	83.33±16.86	258± 9.6***	226.67±19.2***	80.67± 33***
A. E 100 mg/kg +CCl_4	238± 86.03	229.3± 1.52	82.14± 2,03	271.3±8.61**	227.49±3.2***	78.47±3.7***
A. E 200 mg/kg +CCl_4	227± 20.3	223± 49.96	82.4±2.22	253.3±10.2***	225.33± 5.8***	79.86±3.8***
H. E 100 mg/kg +CCl_4	246± 24.56	226± 31.61	83.08±3.94	287.3±9.25**	230.93±10.4***	77.81±1.9***
H. E 200 mg/kg +CCl_4	254.7±17.67	227± 41.55	81.47±3.83	275.7±5.50**	228.93±17.8***	78.12±0.78***

E. A= Aqueous Extract; E. H= Hydroethanolic Extract. Each value was the average ± SD, N = 6 rats, the data were analyzed by One Way ANOVA followed by the Dunnett test *** *p <0.001,* ** *p <0.01* where the extracts, olive Oil and silymarin (Sil) were compared with water + CCl_4.

3.2. Curative Effect of Extracts on the Concentrations of Transaminases and Total Proteins During Hepatotoxicity

The results of the assay show that before the experiment (D0) there was no significant difference in the concentrations of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and total proteins (PRO) between different lots.

After 7 days of experiments, the assay shows that CCl_4 increases transaminase concentrations and decreases the total protein in the untreated intoxication lot (distilled water + CCl_4) significantly (*p <0.001*) compared to that of the non-intoxicated lot, Untreated with olive oil, intoxicated with Silymarin (Sil) and aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* (Table 2). In general, the curative effect of aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* on the serum concentration of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and total proteins (PRO) is similar to that of Sil (Table 2).

Table 2. *Curative effect of extracts on the concentrations of transaminases and total proteins during hepatotoxicity.*

TREATMENT	DO			D7		
	ASAT (UI/L)	ALAT (UI/L)	TOTAL PROT (UI/L)	ASAT (UI/L)	ALAT (UI/L)	TOTAL PROT (UI/L)
Olive Oil	259.7±19.63	241± 37.32	82± 35.54	240.7±7.5***	242 ±35.54***	84.8±37.17***
Water+ CCl_4	252.3±27.43	242.3±5.68	82.67 5.5	324.8± 22.5	389.54± 5.85	38.7± 5.03
Sil+CCl_4	253± 38.69	244.67±9.07	83.33±16.86	247.5±9.6***	248.67±19.2***	82.67± 33***
A. E 100 mg/kg +CCl_4	258± 86.03	241.3± 1.52	81.14± 2.03	248.3±7.5***	247.49±3.2***	79.47±3.7***
A. E 200 mg/kg +CCl_4	257± 20.3	240.7±49.96	84.4±2.22	245.3±15.8***	246.33± 5.8***	81.86±3.8***
H. E 100 mg/kg +CCl_4	256± 24.56	241.8±31.61	83.08±3.94	249.3±7.3***	249.93±10.4***	78.81±1.9***
H. E 200 mg/kg +CCl_4	255.7±17.67	247.9±41.55	85.47±3.83	247.3±5.80***	248.93±17.8***	82.12±0.78***

A. E= Aqueous Extract; H. E= Hydroethanolic Extract. Each value was the average ± SD, N = 6 rats, data were analyzed by One Way ANOVA followed by the Dunnett test *** *p < 0.001;* Where the extracts, olive Oil and silymarin (Sil) were compared with water + CCl_4.

3.3. Preventive Effect of Different Treatments on Protein Electrophoresis

The results of protein electrophoresis show that before the experiment (D0) there was no significant difference in the concentration of the globulins and the albumin between the different batches.

After 7 days of experience, protein electrophoresis showed that CCl_4 increased globulin concentrations and decreased albumin in the untreated intoxicated lots (distilled water + CCl_4) significantly *(p <0.001)* compared to that of the non-intoxicated lot untreated Olive Oil, intoxicated by Silymarin (Sil) and the aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala.*

This increase was corroborated by the albumin / Globulin (Alb / Glb) ratio, which significantly decreases *(p <0.001)* in the untreated intoxication batch (Distilled water + CCl_4) compared to the other batches (Table 3).

In general, the preventive effect of aqueous and hydroethanolic extracts of leaves of *Erythrococca anomala* on protein electrophoresis is similar to that of Sil.

Table 3. *Preventive effect of extracts on serum protein electrophoresis during hepatotoxicity.*

Treatments	D0					
	Alb	α_1Glb	α_2Glb	βGlb	γGlb	Alb/Glb
Olive Oil	23.70±3.5	8.5±2.3	9.3±5.16	9.33±3.8	15.5±7.6	0.55
Water+CCl4	24.58±2.9	7.53±6.2	8.93±0.1	8.73±1.3	14.97±5.1	0.61
Sil+CCl4	23.57±0.4	8.33±3	7.6±3.5	9.96±1.0	15.1±3.13	0.57
A. E 100 mg/kg+CCl4	24.17±1.2	7.1±3.3	8.15±1.3	10.23±4	13.30±5	0.62
A. E 200 mg/kg+CCl4	25.63±1.6	9.11±2.7	7.7±37	9.7±3.6	14.27±13	0.62
H. E 100 mg/kg+CCl4	24.98±1.6	8.13±4.1	8.6±2.5	8.00 ±1.8	13.37±2.6	0.65
H. E 200 mg/kg+CCl4	27.90±5.1	7.1±3.7	9.6±2.1	10.5±0.7	12.8±3.6	0.70

Table 3. *Continue.*

Treatments	D7					
	Alb	α_1Glb	α_2Glb	βGlb	γGlb	Alb/Glb
Olive Oil	23.47±1***	8.0±1***	9.73±3**	9.73±1***	15.6±5**	0.55***
Water+CCl4	11.45±3.04	18.4±1,5	16.47±2.1	19.13±2	27.33±5	0.14
Sil+CCl4	22.13±3***	10.5±5***	9.43±2***	10.9±4***	17.36±4***	0.45***
A. E 100 mg/kg+CCl4	21.13±8***	9.0±4***	10.5±2***	12±0.95**	16.6±5***	0.43***
A. E 200 mg/kg+CCl4	23.4±0.7***	11.1±5***	9.3±3***	10.7±2***	15.5±14***	0.50***
H. E 100 mg/kg+CCl4	21.17±1***	9.9±0.5***	10.6±2***	11.9±1***	15.92±5***	0.43***
H. E 200 mg/kg+CCl4	22.0±1.4***	8.5±0.3***	9.8±1***	10.7±4***	14.17±1***	0.50***

A. E= Aqueous Extract; H. E= Hydroethanolic Extract. Each values was average ± SD; N= 6 rats; data were analyzed by one way Anova following the Dunnett test ***$p< 0.01$. Where the extracts olive oil and control the distilled water was compared with silymarin (Sil). α_1Glb = α_1Globulin; α_2Glb = α_2Globulin; βGlb =βGlobulin; γGlb = γGlobulin; Alb = Albumin; Alb/ Glb = Ratio Albumin/ Globulin.

3.4. Curative Effect of Different Treatments Protein Electrophoresis

The results of protein electrophoresis show that before the experiment (D0) there was no significant difference in the concentration of the globulins and the albumin between the different lots. After 7 days of experience, protein electrophoresis showed that CCl_4 increased globulin concentrations and decreased albumin in the untreated intoxication lots (Distilled water + CCl_4) significantly ($p <0.01$) compared with To that of the non-intoxicated untreated lot Olive Oil, intoxicated treated with Silymarin (Sil) and the aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala.*

This increase was corroborated by the albumin / Globulin (Alb / Glb) ratio, which decreased significantly ($p <0.01$) in the untreated intoxicated batch (Distilled Water + CCl_4) compared to the other batches (Table 4).

In general, the curative effect of aqueous and hydroethanolic extracts of *Erythrococca anomala* leaves on protein electrophoresis is similar to that of Sil.

Table 4. *Curative effect of extract on protein electrophoresis during hepatotoxicity.*

Traitements	D0					
	Alb	α_1Glb	α_2Glb	βGlb	γGlb	Alb/Glb
Olive Oil	23.70±3.5	8.5±2.3	9.3±5.16	9.33±3.8	15.5±7.6	0.55
Water+CCl4	24.58±2.9	7.53±6.2	8.93±0.1	8.73±1.3	14.97±5.1	0.61
Sil+CCl4	23.57±0.4	8.33±3	7.6±3.5	9.96±1.0	15.1±3.13	0.57
A. E100 mg/kg+CCl4	24.17±1.2	7.1±3.3	8.15±1.3	10.23±4	13.30±5	0.62
A. E 200 mg/kg+CCl4	25.63±1.6	9.11±2.7	7.7±3.7	9.7±3.6	14.27±13	0.62
H. E 100 mg/kg+CCl4	24.98±1.6	8.13±4.1	8.6±2.5	8.00 ±1.8	13.37±2.6	0.65
H. E 200 mg/kg+CCl4	27.90±5.1	7.1±3.7	9.6±2.1	10.5±0.7	12.8±3.6	0.70

Table 4. *Continue.*

Traitements	D7					
	Alb	α_1Glb	α_2Glb	βGlb	γGlb	Alb/Glb
Olive Oil	25,47±1,8**	9,60±5**	10,4±35**	9,53±3**	11,8±5**	0,60**
Water+CCl4	19,32±2,4	18,4±1,5	17,53±2,1	18,15±2,8	19,37±5**	0,26**
Sil+CCl4	29,83±3,2**	10,56±1**	12,4±2**	11,1±4**	12,27±3**	0,64**
A. E100 mg/kg+CCl4	29,15±0,1**	10,16±4**	12,5±2**	12,5±3**	13,9±7**	0,59**
A. E 200 mg/kg+CCl4	28,4±0,8**	12,1±0,5**	11,2±3**	10,3±2**	12,5±15**	0,61**
H. E 100 mg/kg+CCl4	26,27±2,4**	10±0,2**	13,63±2**	13,96±1**	13,2±5**	0,51**
H. E 200 mg/kg+CCl4	28,50±5,7**	10±8,2**	12,8±2**	11,4±1**	12, 8±9**	0,60**

A. E= Aqueous Extract; H. E= Hydroethanolic Extract. Each values was average ± SD; N= 6 rats; data were analyzed by one way Anova following the Dunnett test ***$p< 0.01$. Where the extracts olive oil and control the distilled water was compared with silymarin (Sil). α_1Glb = α_1Globulin; α_2Glb = α_2Globulin; βGlb =βGlobulin; γGlb = γGlobulin; Alb = Albumin; Alb/ Glb = Ratio Albumin/ Globulin.

4. Discussion

The results of the preventive and curative tests are similar in general and show that the treatment with aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* protect the liver and repair the lesions caused by CCl_4. The protective effects of the various extracts were due to an increase in the activity of the antioxidant enzymes. Indeed, CCl_4 is a dose-dependent hepatotoxic and its toxicity is mainly due to the appearance of free radicals or toxic forms of oxygen which induce lipid peroxidation leading to the destruction of cell membranes. The increase in serum levels of transaminases, total proteins, globulins and decreased serum albumin after CCl_4 injection is evidence of significant hepatic involvement. CCl_4 induced hepatic injury is commonly used as a model for liver drug screening and the extent of damage is assessed by the level of cytoplasmic transaminases (ALAT and ASAT), total proteins, globulins, and albumin [11]. The reduction in the level of liver enzymes, globulins and albumin, total protein, albumin / globulin ratio by extracts is an indicator of the regeneration of the repair process of the lesions of the liver. [12]. These results corroborate those of the studies, which reported that transaminases and serum proteins are restored with hepatocyte regeneration and hepatic parenchyma restructuring [13].

It has been shown that the ability of hepatoprotective substances to reduce the harmful effects or preserve the mechanisms of liver functioning against hepatotoxin disturbances is an indication of their protective effect. [13]. It can therefore assert that repeated administration of the aqueous and hydroethanolic extracts of *Erythrococca anomala* leaves protect against the hepatotoxicity caused by CCl_4 with an efficiency close to that of Sil. Since, as a result of CCl_4 induced lesions, it is seeing a substantial increase in the values of ASAT, ALAT, and globulins. A decrease in albumin and total protein levels, which is a clear sign of cell lysis and loss of functional integrity of the hepatocyte membrane, the decrease in CCl_4-induced morphological lesions may be a sign of repair of the hepatocytes, a reinforcement of the parenchyma, following the treatment with the extracts. The decrease in ASAT serum levels, of ALAT, globulins, and increased albumin and total protein levels is therefore indicative of an improvement in liver function. This work has shown that the aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala* act as preventive as well as curative. Flavonoids and phenols are recognized for their hepatoprotective activities [14]. The antioxidant and hepatoprotective activities of these extracts can therefore be due to the presence of flavonoids and phenols.

5. Conclusion

The work performed in this study confirmed the hepatotoxic effect of CCl_4. Its effects result in cell lysis and loss of functional integrity of the hepatocyte membrane following the intensive production of free radicals causing an increase in enzymes, globulins and a decrease in hepatic proteins and albumin. Aqueous, hydroethanolic extracts and silymarin (a reference hepatoprotectant) play a protective role in the oxidative stress produced in the liver by CCl_4 by decreased enzymes, globulins and increased protein and albumin. The results show that the protective effect of the aqueous and hydroethanolic extracts of the leaves *of Erythrococca anomala* is due to the antioxidant phenolic compounds free radical scavengers that protect the hepatocytes against the oxidizing effect of CCl_4.

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