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Hematological, Biochemical and Histological Analysis on Wistar Rats Treated by an Herbal Medicinal Product "DAOUTRA EPIGASTRO" Marketed in Daloa City (Côte d'Ivoire)

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Abstract **Original Research Article**

Herbal products developed by traditional healers are more and more in increasing in Africa. Unfortunately, there is often no safety data on these products. That is the case of an Herbal Medicine Product (HMP) called "DAOUTRA EPIGASTRO", widely used by local population in Daloa City-Cote d'Ivoire to treat chronic gastritis without knowing its harmlessness. This study aimed to study safety of this product by evaluating its acute and sub-acute effects on Wistar rats by oral administration. Acute toxicity test was assessed with a single concentration of either 500, 1000 and 3000 mg/kg bw of the product to female rats in the goal to determine clinical signs of intoxication and LD50. However, sub-acute toxicity test was carried out by daily administration of 300, 500 and 1000 mg/kg bw for 28 days duration in order to identify eventual modifications of hematological and biochemical parameters; then to appreciate histological changes of some selected intern organs. This operation was achieved by collecting blood and isolating hearts, livers and kidneys at the end of the experiment. As results, acute study showed that from day 7 onwards, the remedy administered at 3000 mg/kg significantly increased the weight of the rats compared to the controls, no mortality, no clinical sign of intoxication and LD50 was higher than 3000 mg /kg bw. For sub-acute exposure, the animals showed a stimulation of the appetite for food after consumption of the remedy "DAOUTRA EPIGASTRO". Except the increasing of AST and ALT content at 1000 mg/kg bw, no abnormal modifications were observed for hematological and biochemical parameters, then histological analysis didn't show any alteration-of selected organs. In addition, the difference in relative weights between treated and control rats were not statistically significant for the liver and heart. However, there was a significant difference between relative weights of liver at all treated rats in comparison to control. The study showed that the remedy "DAOOUTRA EPIGASTRO" is non-toxic by oral administration at the doses under 300 mg/kg bw.

Keywords: "DAOUTRA EPIGASTRO", toxicity, LD50, acute and sub-acute.

INTRODUCTION

In recent years, there has been a renewed interest in herbal medicine. Indeed, more and more people are using traditional medicine for primary health care (WHO, 2007; Koné *et al*., 2017). This practice is a social and cultural habits for African countries where around 80% of population use medicinal plants (WHO, 2013; Lehmann H. 2013). Unfortunately, this unsupervised use could lead to dramatic situations of intoxication and death. For that reason, it is advised to ensure safety use of some herbal medicinal Products (HMP) before marketing (Atsamo *et al*., 2011; Ukwuani *et al*., 2012; Gbogbo, 2015).

Several publications talked about evaluation of toxicity of some individual plants but little literature is available on toxicity of plant mixture that is most used by traditional healers to provide treatment to patients.

For instance Otimenyin and Oguru (2006), demonstrated the acute toxicity of *Nauclea latifolia* at 852 mg/kg bw, Mindédé *et al.,* (2022) showed that in acute toxicity assay hydroethanolic extract of *Xylopia aethiopica* had DL50 greater than 5000 mg/kg bw, Obidah (2009) illustrated that *Cassia sieberiana* was toxic at 180 mg/kg bw and Benny *et al.,* (2021) published that LD50 of aqueous extract of *Zingiber officinale* was greater than 2000 mg/kg bw. All these data proved the some plants could be individually toxic and other. However, what could be the concern if these plants were mixed together to produce a single herbal medicinal product? This approach was not much developed by scientific literature.

In Côte d'Ivoire, around three to five traditional remedies based on several plants were already studied for their safety before marketing by traditional healers (Ministère de la santé et de l'hygiène publique, 2007). Amongst them, there "Nature" used in the treatment of malaria (Manda *et al*., 2017), "Noe" indicated for urinary disorders related to benign prostatic hypertrophy (Dao, 2016) and "Sanrata" traditionally used in the treatment of some pathologies (Fatto, 2016).

Unfortunately, the majority of herbal medicines sold on the market of Côte d'Ivoire have not been studied for their safety, while the populations are increasingly requesting these preparations for their health needs. That is the case of herbal medicinal product called "DAOUTRA EPIGASTRO". This traditional remedy is widely used by patients suffering of gastritis including gastro-duodenal ulcer (Choho *et al*, 2022). A preview study on this remedy highlighted its phytochemical content and its antioxidant potential. The purpose of this study aims to bring scientific data on safety use of this remedy by evaluating its acute and sub-acute toxicity in Wistar rats.

MATERIAL AND METHODS

MATERIAL

Plant Material

The plant material was an herbal medicinal product called "DAOUTRA EPIGATSRO" and marketed in Daloa city (Côte d'Ivoire) by a Non-Governmental Organization "LE DAOUTRA."

Composition of the Remedy

The Table I summarizes the different plants coming into the composition of the remedy "DAOUTRA EPIGASTRO". These plants were identified by a botanist Prof KOUASSI Kouadio Henri; Department of Plant Biology and Physiology, Faculty of Agroforestry, Jean Lorougnon Guede University.

Table I: Composition of the remedy

N°	Scientific name	Botanical family	Organs used
1	*Nauclea latifolia*	*Rubiaceae*	trunk bark
2	*Xylopia aethiopica*	*Annonaceae*	Fruits
3	*Capsicum frutescens*	*Solanaceae*	Fruits
4	*Zingiber officinale*	*Zingiberaceae*	Rhizomes
5	*Cassia sieberiana*	***Fabaceae***	trunk bark

Animal Material

Rats aged 8 to 10 weeks and with an average weight of 122 to 154.5 g were purchased from the Animal Experimental Unit (UAE) of Faculty of Pharmaceutical and Biological Sciences, Félix Houphouët-Boigny University (Côte d'Ivoire). The rats were housed in plastic cages, 6 rats per cage, and acclimatized in the local of Excellence Group of Research on Traditional Pharmacopoeia Products (GerProPhaT) of Jean Lorougnon Guédé University in Daloa (Côte d'Ivoire), for one week in standard conditions of temperature (25°C ± 2°C) and relative humidity (70% ± 5%) and a 12 hour light-dark cycle, and fed with standard food and water.

METHODS

Acute Toxicity Test

This study was designed according guidelines OECD 423 (OECD, 2001). A total of 12 nulliparous, non-pregnant female rats were randomly divided into 4 groups (n= 3rats) each group received its specific treatment of "DAOUTRA EPIGASTRO" as a single oral dose of either 500 (group A), 1000(group B), and 3000 mg/kg (bw) (group C), in proportion to 1 mL/100 g bw, respectively and the last group served as a control group (group D).

Before the experiment, the animals were weighted. The treated animals were observed at 10 min, 30 min, 60 min and 120 min then at 4 hours and 6 hours during the first day. Then, hydration and food were performed daily for 14 days. During the experiment, signs of intoxication including effects on locomotion (agitation, decreased activity and somnolence), breathing, salivation, lacrimation, cyanosis, diarrhea, refusal food, coma and death.

Sub-Acute Toxicity Test

The study was conducted according to OECD guideline 407 (OECD, 2008). A total of 24 albinos Wistar rats divided into 4 homogenous groups of 3

males and 3 females. Experimental groups 1, 2 and 3 received a single oral daily dose 300 mg/kg, 500 mg/kg and 1000 mg/kg bw respectively, then control group 4 received daily 1 mL/100 mg bw distilled water for a duration of 28 days. Throughout the experiments, the rats were fed on standard rat pellets and allowed free access to tap water ad libitum, were observed daily. The body weights of rats were recorded before the experiment and every 7 days.

- **Blood and Organ Sampling**

At the end of the treatment period, the rats were anesthetized with Cooper ether by inhalation. Then, blood samples were collected by sacrificing the rats.

Blood was collected in two tubes with or without pro-coagulant (facilitating serum formation) for hematological and biochemical analysis. The tubes containing the pro-coagulant were centrifuged at 4000 rpm for 5 min and the serum obtained was stored at -20°C for biochemical analysis (Manda *et al*., 2017). The parameters were measured using automated biochemical and hematological analysis systems.

In addition, an autopsy was carried out in all rats, and hearts, livers and kidneys were isolated, weighted and preserved in 10% formalin for further histological analysis. The relative weight of each organ was calculated using the formula (Etame *et al*., 2017):

$$\mathbf{W_r = \frac{W_0}{W_b} \times 100}$$

Wr: relative weight of the organ (g/100 g)
Wo: organ weight (g)
Wb: rat body weight (g).

- **Biochemical and Hematological Analysis**

Concentrations of Creatinine, Urea, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) were determined by using auto-analyser (Cabas® U411, suisse). Hematological parameters such as red blood cells (RBC), white blood cells (WBC), hematocrit (HCT), hemoglobin (HGB), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined by an hematological auto-analyser (CYANHemato -Socimed, France).

- **Histological Analysis**

After dehydrating selected organs, they were embedded in paraffin wax to be sectioned 5 µm thick, and stained for the histological evaluation using Eosin and hematoxylin stains. The histological evaluation in this study was performed using a light microscope with camera and tablet (MSC-B102T, Siedentopf) (Ouahchia *et al*., 2017) and pictures were taken. An experienced pathologist who was unaware of the experiment groups to which section belonged conducted the analysis.

Ethical Approval

The experimental procedures and protocols from the beginning to the end of this study, were conducted according to the agreed guidelines of European Council Legislation 87/60/EEC for the protection of Laboratory animals. The maintenance and care of the animals were followed every day.

Statistical Analysis

The results were expressed as means plus or minus standard deviation at risk $\alpha = 0.05$. The statistical analysis of the results was carried out using the analysis of variances (ANOVA ONE WAY). Differences between means were determined using Dunnett's multiple comparison test. *** $p < 0.001$: very highly significant difference; ** $p < 0.01$: very significant difference; * $p < 0.05$: significant difference and $p > 0.05$: non-significant difference.

RESULTS

Acute Toxicity Test

Acute oral toxicity study showed no mortality and no clinical signs of intoxication after administration of the various doses of 500, 1000 and 3000 mg/kg bw of the remedy. At the end of the experiment, there was no change in the amount of water consumed.

However, food consumption of the test animals was increased compared to the controls. Weight of group C (3000 mg/kg bw) test group increased significantly compared to the controls between the 7th and 14th day (Figure 1).

The variations in body weight (bw) of the four groups of rats are reported in Figure 1.

Sub-Acute Toxicity Test

This study was carried out by evaluating body weights, relative organ weights, biochemical and hematological parameters.

• Evolution of Weight Gain

Weight of treated rats was significantly increased overall compared to controls from day 7th at 500 and 1000mg/kg and at all doses from day 21th to 28th of treatment.

The body weight variation curves of the four groups of rats are shown in Figure 2.

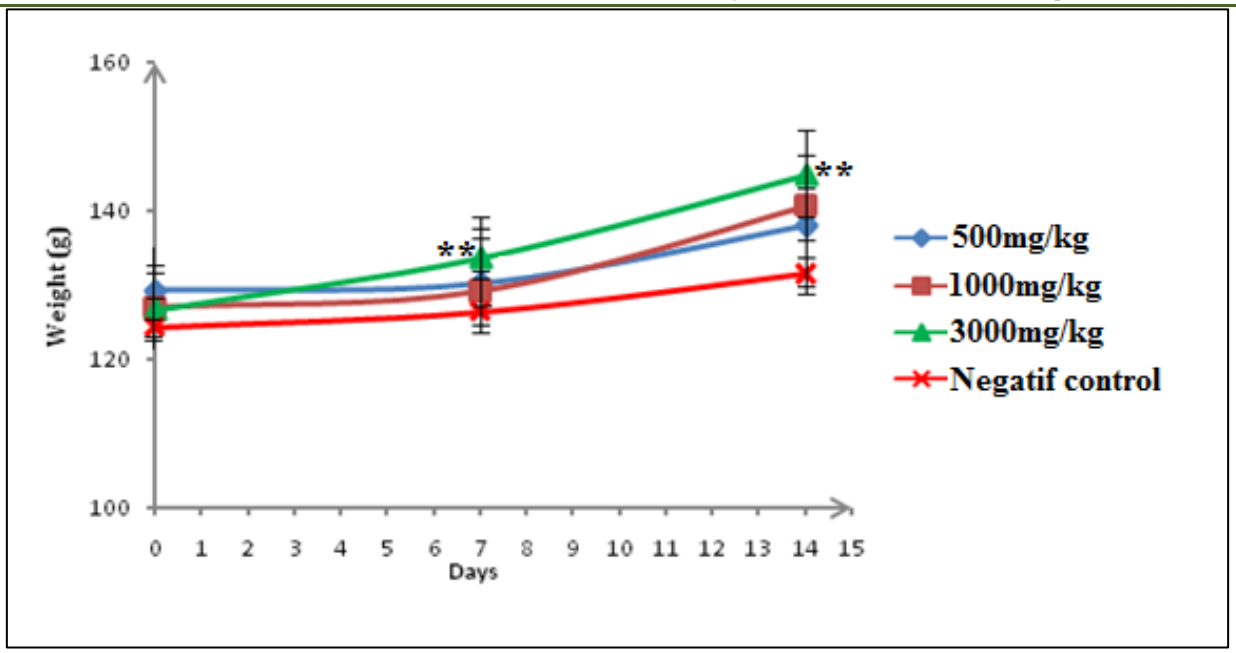

Figure 1: Evolution of the body weight of rats during the acute toxicity test

Data are expressed as mean ± S.E.M at risk α = 5%. N=6 per dose. Data are analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's test. ** p < 0.01: very significant difference; * p < 0.05: significant difference.

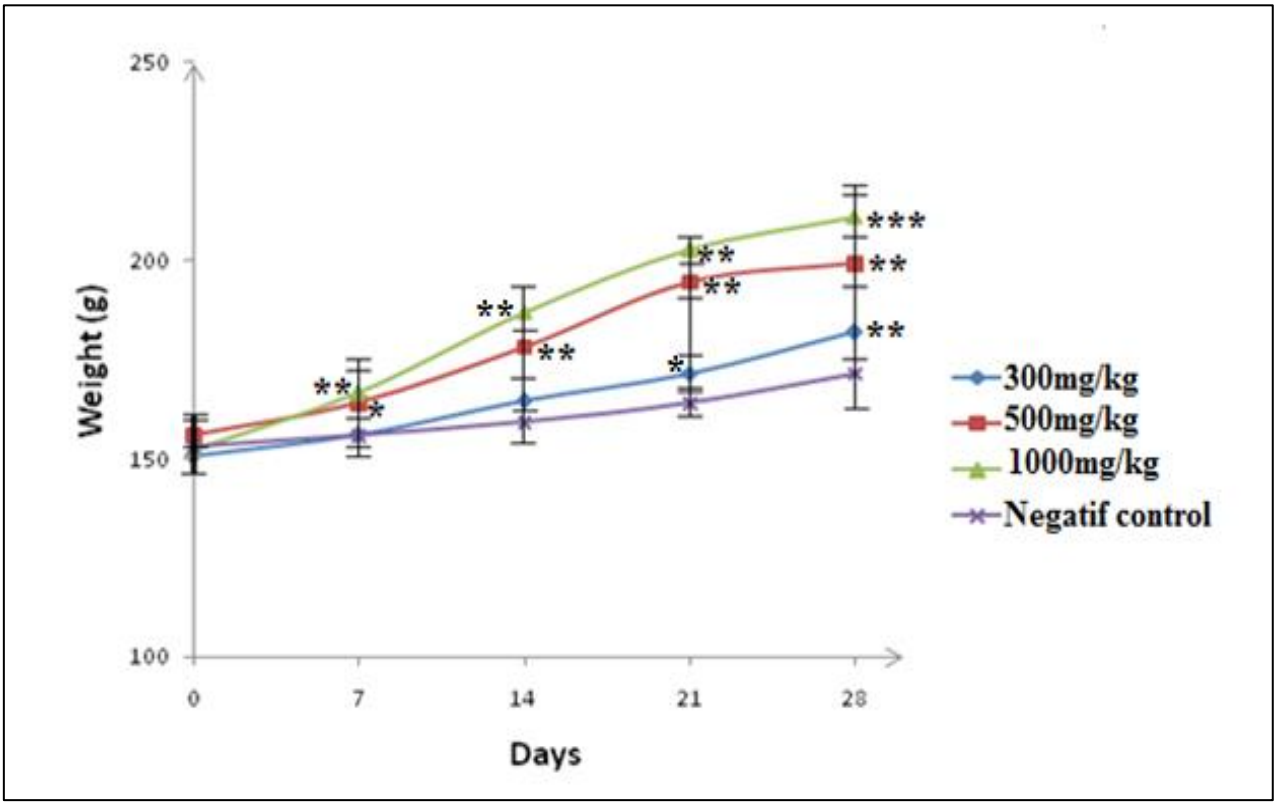

Figure 3: Evolution of the body weight of rats during the sub-acute toxicity test

*Data are expressed as mean ± S.E.M at risk α = 5%. N=6 per dose. Data are analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's test. *** p < 0.001: very highly significant difference; ** p < 0.01: very significant difference; * p < 0.05: significant difference.*

• Effects of the Remedy on Relative Organ Weights in Rats

The relative weights of the organs were reported in Table II.

Table II: Effects of the administration of the remedy on the weights of the target organs

		Relative weights of selected organs Mean ± standard Déviation		
Groups	**Body Weights**	**RLW**	**RKW**	**RHW**
Group 4 control	182.66 ± 18.44	4.40± 0.41	0.72± 0.15	0.37± 0.026
Group 1 300 mg/kg	202.00 ± 20.66	3.63± 0.20 *	0.70± 0.055	0.34± 0.00
Group 2 500 mg/kg	203.33± 20.23	3.56± 0.25 *	0.67± 0.035	0.35± 0.00
Group 3 1000mg/kg	221.00 ± 17.43	3.52± 0.16 *	0.76± 0.035	0.33± 0.028

RLW= relative liver weight; RKW = relative kidney weight; RHW= relative heart weight.
*Data are expressed as mean ± S.E.M at risk α = 5%. N=6 per dose. Data are analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's test. * p < 0.05: significant difference compared to the control group.*

- **Biochemical and Hematological Evaluations**

Figure 3 showed level of four biochemical markers (Urea, Creatinine, AST and ALT) of 300, 500 and 1000 mg/kg remedy treated rats as compared with those of rats in the normal control group. About urea and creatinine, there was no significant difference between treated rats and control for the concentrations. However, at 1000 mg/kg bw, a significant difference was observed for AST and ALT between treated rats and control group.

As for the hematological assessment (Red blood cells (RBC), white blood cells (WBC), hematocrit (HCT), hemoglobin (HGB), platelets (PLT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration (MCHC).The remedy did not induce any significant change in the groups of rats treated at the different doses administered compared to the control (Table III).

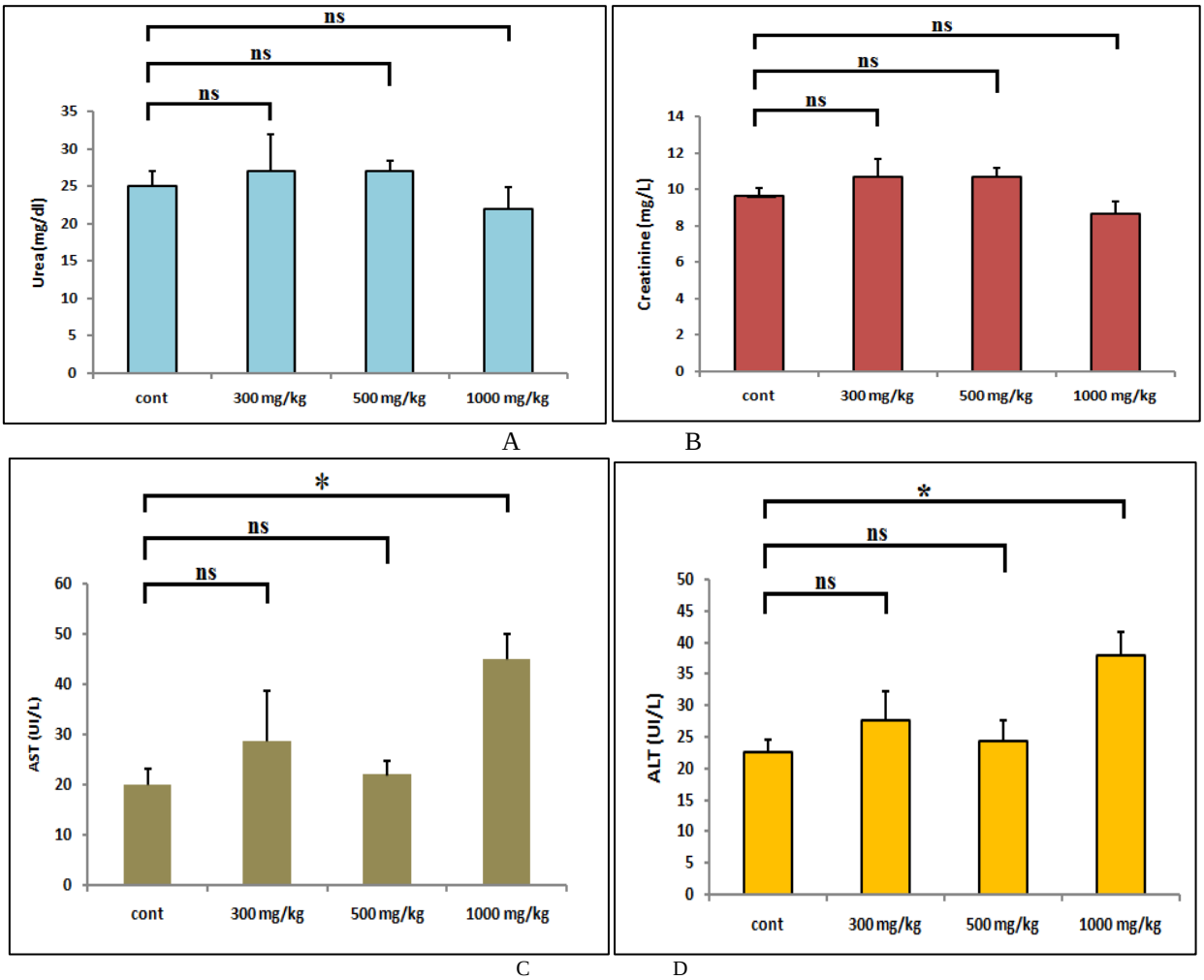

Figure 3: Effects of the administration of the remedy on the biochemical parameters of the rats (A: Effects of the remedy on the urea; B: Effects of the remedy on the creatinine; C: Effects of the remedy on the AST; D: Effects of the remedy on the ALT)

*Data are expressed as mean ± S.E.M at risk α = 5%. N= 6 rats per group. The data are analyzed by one-way analysis of variance (ANOVA) followed by Dument's test *p<0.05: significant difference compared to the control group; **ns:** difference not significant*

Table III: Hematological parameters evaluation

Parameters	Doses			
	Group 4 Control	Group 1 300mg /kg bw	Group 2 500mg /kg bw	Group 3 1000mg /kg bw
WBC (10^3/mm^3)	5.56 ±1.96	11.63±3.65	6.79 ± 3.20	7.63 ± 4.80
RBC (10^6/mm^3)	5.96 ±0.99	6.73±1.10	6.13 ± 1.60	6.31±1.32
HGB (g /dL)	10.96 ± 1.65	12.33±2.37	11.5 ± 3.11	12.10 ±2.08
HCT (%)	32.16 ± 5.34	35.5 ± 5.57	32.29 ±8.40	35.4± 5.72
MCV (fl)	54.06 ± 0.35	52.9 ± 0.52	53.8 ± 0.62	56.63± 4.35
MCH en Pg	18.36 ± 0.32	18.20±0.62	18.66 ± 0.26	19.26 ± 1.55
MCHC (%)	34.10 ± 0.7	34.5 ± 1.55	34.8 ± 0.75	34.10 ± 0.5
PLT (10^9/L)	674 ± 37	665±21	687.33 ± 28.3	651.66±30.71

Red Blood Cells (RBC), White Blood cells (WBC), Hematocrit (HCT), Hemoglobin (HGB), platelets (PLT), Mean corpuscular volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).
*Data are expressed as mean ± S.E.M at risk α = 5%. N=6 rats per group. Data are analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's test. * p < 0.05: significant difference compared to the control group.*

• Histopathological Examination of Organs

Figures 4, 5 and 6 showed photomicrographs of histological sections of heart, liver and kidney respectively. These pictures showed normal structure and did not reveal any morphological abnormality that could be due to the remedy.

In both the control and treated rats, heart sections showed normal cardiomyocytes without alteration and any hemorrhagic region in extracellular space.

Liver section of treated rats showed cells binuclear without any distortion similar to the control group. The hepatocytes were clearly visible and morphologically identical on each picture. No lyses, necrosis, or hemorrhagic regions were observed.

Kidneys sections didn't present morphological modifications for the treated groups. Nephron cells with nucleoti were clearly visible with no degradation. Architecture of glomerular was normal and identical to the control group.

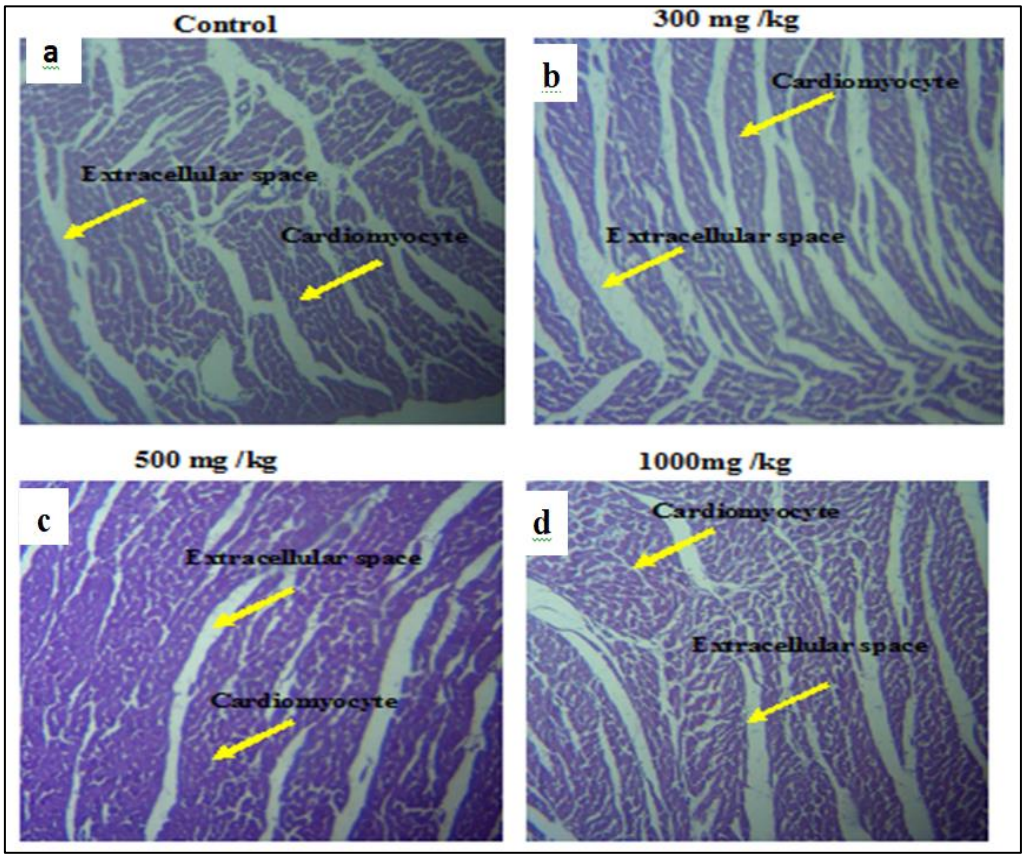

Figure 5: Heart histological sections of rats exposed for 28 days of treatment (H&E-stained, X100) a) Normal control b) 300 mg/kg bw of remedy c) 500 mg/kg bw of remedy d) 1000 mg/kg bw of remedy

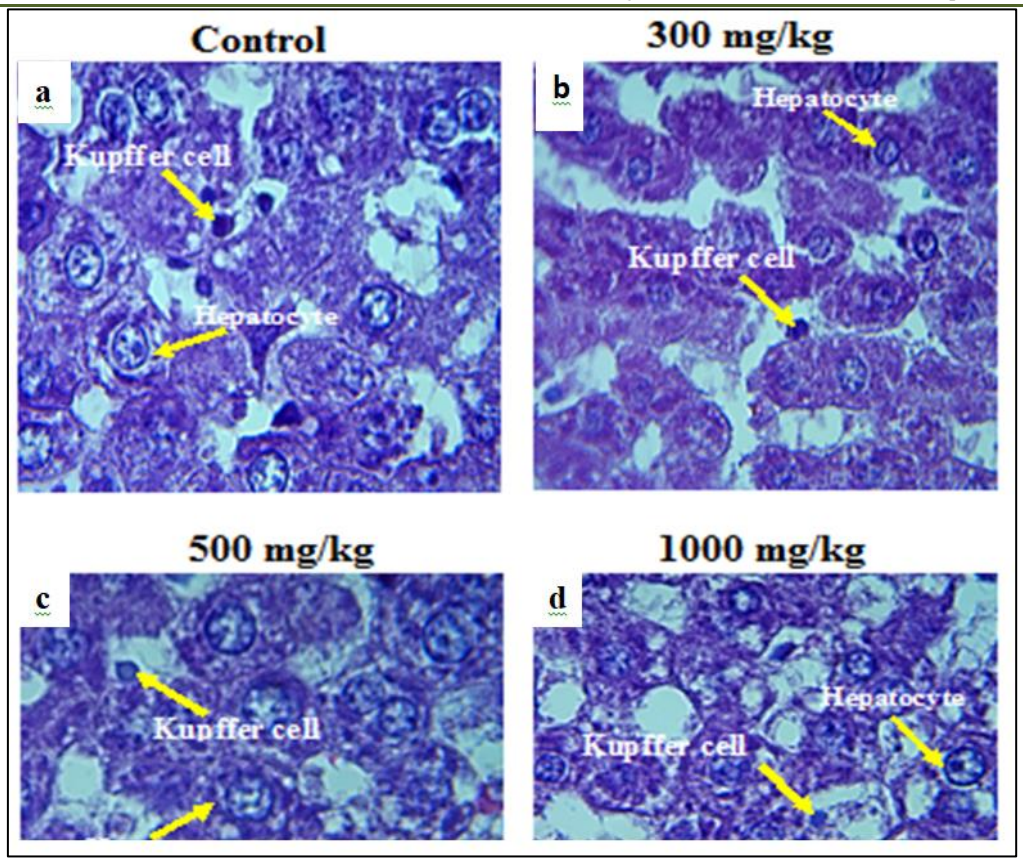

Figure 6: Liver histological sections of rats exposed for 28 days treatment (H&E-stained, X 200) a) Normal control b) 300 mg/kg bw of remedy c) 500 mg/kg bw of remedy d) 1000 mg/kg bw of remedy

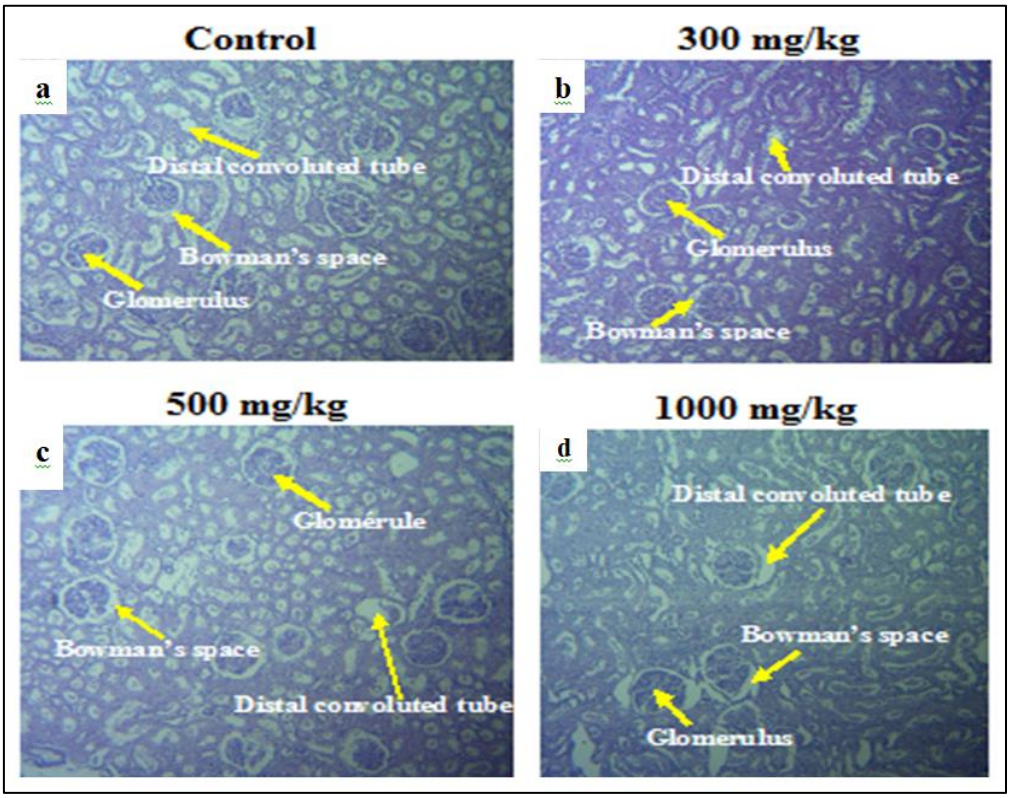

Figure 7: kidney histological sections of rats exposed for 28 days treatment (H&E-stained, X100) a) Normal control b) 300 mg/kg bw of remedy c) 500 mg/kg bw of remedy d) 1000 mg/kg bw of remedy

DISCUSSION

Acute toxicity test showed that the remedy administered orally in a single high dose did not cause death or behavioral changes; the result showed that from day 7 onwards, the remedy administered at 3000 mg/kg significantly increased the weight of the rats compared to the controls.

The LD50 is estimated to be greater than 3000 mg/Kg bw. According to the Global Harmonized System of Classification (GHS) and the toxicity scale, the herbal medicine (HMP) "DAOUTRA EPIGASTRO", has a toxicity index equivalent to 5, (Charles *et al.*, 2016; Koua, 2020). These results are similar to those obtained by Manda *et al.,* (2017) who showed that "Nature", a traditional remedy used in the treatment of malaria, has an LD50 greater than 5000 mg/Kg bw when administered orally. This low acute toxicity of the remedy could have as causes the individual or associative effects of the plants composing the remedy "DAOUTRA EPIGASTRO". Indeed, Benny *et al.,* (2021) showed that *Zingiber officinale* administered orally has an LD50 greater than 2000mg/kg, thus having a toxicity index equivalent to 5. Similarly, *Xylopia aethiopica* with an LD50 higher than 5000mg/kg, can be classified as non-toxic according to the Globally Harmonised System of Classification (GHS) (Mindédé *et al.,* 2022). These results contributed to demonstrate that some HMP prescribed by the traditional therapist are safety and could be used by populations. Indeed, under the conditions of traditional use, the traditional practitioner advises adult patients weighing approximately 60 kg, one (1) tea glass of "DAOUTRA EPIGASTRO" twice daily corresponding to 9 mg/kg bw. This dosage was very inferior to 3000 mg/kg bw, and that demonstrated that this herbal product could not induce significant disorders in patients.

Sub-acute toxicity assessment showed that after consumption of the remedy "DAOUTRA EPIGASTRO", the animals showed a stimulation of appetite for food. It is known that in addition to their therapeutic properties, medicinal plants can positively affect the nutritional status of animals. The increase in body mass of the rats is thought to be related to a probable effect of the remedy in stimulating their appetite. That may be a beneficial effect of the extract. The stimulation of appetite could be attributed to the individual action of the plants in the remedy or to their synergy. Indeed, one of the plants composing the remedy, *Nauclea latifolia* promotes the appetite for food and water (James *et al.,* 2014). The power of plants to promote appetite has also been highlighted by other authors such as Bintou (2010), Rhiouani *et al.,* (2008), Lakmichi *et al.,* (2011) and Manda *et al.,* (2017) in similar studies.

The results showed that the relative weights of (kidney and heart) in the treated groups were statistically identical to those in the control group. However, for the liver, a significant decrease was observed at all doses (300, 500 and 1000 mg/kg bw). This decrease would not be a toxic effect but rather beneficial for the patient. Indeed, a decrease in the relative weight of the liver could be consecutive to an elimination of fat (delipidation effects) in this organ by the remedy (Nguyen *et al.,* 2008; Ozturk *et al.,* 2009; Etame *et al.,* 2017).

The hematological and biochemical data were statistically identical to those of the control group, but AST and ALT values in treated rats were significantly different from those of the control group at 1000 mg/kg.

Transaminases are present in the liver, but also in muscle and the kidney, pancreas and other tissues. They are synthesized in the cytoplasm of cells in these organs and released into the circulation when these cells are damaged (Peirs, 2005). These enzymes increase in myopathy, rhabdomyolysis or myocardial infarction and AST, particularly in haemolysis.

ALT is more specific for liver damage, but AST is more sensitive (Goddard & Warnes, 1992).

The histological study revealed no evidence of hepatic cytolysis or cardiomyocytes degradation inherent in possible toxicity of the remedy at all doses compared to the control. However, elevated AST and ALT levels in animals treated at 1000 mg/kg may indicate liver dysfunction resulting from prolonged administration of the remedy at high doses.

Plants such as *Nauclea latifolia* and *Cassia sieberiana* used in the remedy could be at the origin of these changes in AST at 1000mg/kg, as they induced a significant modification of biochemical and hematological parameters according to several authors. Indeed, several studies show that *naucléa latifolia* stem extract causes an alteration of the secretory and excretory functions of the kidney (Arise *et al.,* 2012; James *et al.,* 2014). These authors also concluded that *naucléa latifolia* extract can also cause liver tissue destruction during prolonged administration in a dose-dependent manner. Studies on *cassia siberiana* root bark showed a significant elevation of AST and ALT from 20 mg/kg (Obidah *et al.,* 2009). A significant increase in serum urea and creatinine concentrations was also observed (Obidah *et al.,* 2009, Samira & Abdullahi, 2020).

Serum urea and creatinine are considered the main markers of nephrotoxicity, although serum urea is often considered a more reliable indicator of renal function than serum creatinine (Palani *et al.,* 2009). The determination of urea and creatinine revealed that administration of the remedy did not cause any changes, the values of these biochemical parameters remained within the normal range and did not vary from the

controls. Moreover, the histological study showed no alteration of renal structrure of the treated rat compared to the controls. The results are in agreement with those of Manda *et al.*, (2017), these authors showed that the remedy "Nature" had no effect on mean urea and creatinine levels.

CONCLUSION

"DAOUTRA EPIGASTRO" is a traditional medicine marketed for its anti-ulcer properties. The toxicological study of this polyherbal product showed that the remedy does not show any signs of acute toxicity by per os administration at the doses studied. The LD50 was estimated to be greater than 3000 mg / Kg. According to the Globally Harmonized System of Classification (GHS), "DAOUTRA EPIGASTRO" can be classified as a category 5 (low toxicity) of chemicals products.

Furthermore, when administered as a sub-acute toxicity, the animals showed a stimulation of the appetite for food after consumption of the remedy "DAOUTRA EPIGASTRO", the remedy did not induce any change in relative organ weights, except for the liver where a significant decrease at all doses (300, 500 and 1000 mg/kg bw) was noted compared to controls.

With regard to hematological and biochemical parameters, the remedy caused a significant increase in AST and ALT only at the single dose of 1000 mg/kg. Furthermore, it did not induce any significant change in renal function. This low toxicity of the remedy was confirmed by the histological study which did not reveal any structural alteration of the organs (Livers, Kidneys, Hearts) studied.

However, the remedy can have deleterious effects on the liver at high doses, around 1000 mg/kg when given for more than a month.

These results demonstrated that "DAOUTRA Epigastro" could be used at concentrations under 300 mg/kg bw without hematological and biochemical disorders after standardization testing of posology.

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