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EFFECT OF *VERNONIA COLORATA* (WILLD.) DRAKE LEAVES ON PARACETAMOL-INDUCED HEPATOTOXICITY IN WISTAR RATS.

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ABSTRACT

The liver is a vital organ playing an important role in maintaining homeostasis, detoxifying and eliminating toxic substances. However, drugs and pathogens cause liver damage and disease. The aim of this work was to evaluate the hepatoprotective and/or hepatorestorative effects of the aqueous extract of the leaves of *Vernonia colorata* (AEVC) on the hepatotoxicity induced by paracetamol in rats. Thirty female and male rats of 8-week-old were randomized and divided by sex into six lots of five rats. During three weeks, rats of these different lots received respectively NaCl (0.9%), paracetamol, paracetamol and Legalon® (silymarin), paracetamol and AEVC at three different doses: 50, 100, and 250 mg/kg bw. We found that paracetamol (1 g/kg bw) induced a highly significant increase ($p < 0.001$) in plasma levels of biochemical parameters (alanine aminotransferase, alkaline phosphatase, total and direct bilirubin) in male and female rats, compared to neutral control (0,9% NaCl). Additionally, it also caused a significant decrease in relative liver weights in female rats. AEVC inhibited all these deleterious effects of paracetamol on liver. These beneficial effects of AEVC on liver were comparable to that of Legalon® (70 mg/kg bw). We suggested that AEVC has hepatoprotective and/or hepatorestorative properties via a silymarin-like mechanism action: stabilization of cell membrane and upregulation of intracellular glutathione. This last idea remains to be confirmed by histological studies.

KEYWORDS: *Vernonia colorata*, hepatoprotective/hepatorestorative, paracetamol, Wistar rat.

INTRODUCTION

The liver is an important organ in intermediate metabolism, in detoxification and elimination of toxic substances.[1] It plays an important role in maintaining homeostasis, nutrition and growth of the organism.[2]

However, under the effect of environmental factors, pollutants, drugs, medicines and other xenobiotics, it can be "damaged", weakened and its physiological functions significantly altered. This is the case with hepatitis or cirrhosis.[3] In general, liver disease is one of the main causes of death in the world: it is responsible for about 2 million deaths per year. Taking medication is one of the causes of these liver diseases.[4, 5, 6] According to Kirchain and Allen[7], 40,000 to 45,000 people suffer from drug-induced liver injury each year. Paracetamol, an over-the-counter analgesic and antipyretic, is the most commonly used clinically important drug associated with liver damage.[8] It induces hepatotoxicity that remains the most common cause of acute liver failure in many countries[6] Access to material for modern treatments of liver disorders remains relatively low in our regions, and these treatments often deal with deleterious side effects.[9] Thus, the use of medicinal plants remains common for their hepatoprotective effects. Therefore, herbal formulations should be developed with advanced pharmacological experiences.[10]

Vernonia colorata, from the Asteraceae family, is used in traditional medicine in Africa for the treatment of diabetes, skin rashes and acute hepatitis.[11] It has already been shown that its use would not pose a risk of toxicity.[12]

The present study aims to evaluate, in rats, the hepatoprotective (or hepato-repairing) effects of the aqueous extract of the leaves of *Vernonia colorata* during intoxication induced by paracetamol.

MATERIAL AND METHODS

Plants

The fresh leaves of *Vernonia colorata* were harvested at Zogona (12° 22' 42.05" N; 1° 29' 59.87" W), Ouagadougou city, in October 2018 between 7 a.m. and 11 a.m. The authentication of the species was carried out by the team of the Herbarium of the University Joseph KI-ZERBO (Burkina Faso). A sample of the plant was kept there under the identification number, 17964.

Animals

The animals used were female and male Wistar rats from the animal facility of University Joseph KI-ZERBO. These animals were subjected to standard animal husbandry conditions. They had free access to drinking water and food. Animal handling and treatment was done in accordance with ethical standards for laboratory animal research. The experimental protocol approval number is CE-UJKZ/2020-04.

Preparation of extracts

The leaves of *Vernonia colorata* were dried in the laboratory under artificial ventilation in the shelter sun and dust. Five hundred grams (500 g) of *Vernonia colorata* leaf powder were macerated in a stainless-steel beaker at 30°C in 3000 mL of distilled water for 24 hours. The macerated obtained was filtered through a fine-mesh nylon cloth. The residue is taken up in a volume of 1000 mL of distilled water, mixed and filtered again. The filtrates obtained were then centrifuged at 2000 rpm for 10 min. The supernatants were collected and lyophilized to obtain the aqueous extract of *Vernonia colorata* (AEVC).

Substances

Paracetamol (Entrance, Pharmaceuticals & Research Centre, Ghana) and Légalon® (Laboratoire Mylan Medical SAS) have been used for the induction and treatment of hepatotoxicity, respectively. The diagnostic kit used for the estimation of AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), alkaline phosphatases (ALP) and bilirubins, is from Ranbaxy Diagnostics Ltd. A standard gavage tube was used for substance administration. A ketamine/xylazine solution, 1 mL of ketamine (50 mg/mL) to 0.7 mL of xylazine (20 mg/mL), was used for anesthesia.

Hepatoprotection study

The hepatoprotection test was performed according to the protocols described by Muriel et *al.*, Lin and Karin and Madhu et *al.* [13, 14, 15] Thirty (30) male rats and thirty (30) female rats of 8 weeks weighing between 200 and 250 g were divided into six (6) lots of five (5) rats, and by sex. The different lots were treated orally as follows.

- a neutral control lot, treated with NaCl (0.9%);
- a negative control lot, treated with paracetamol (1 g/kg bw (body weight));
- a positive control lot, treated with paracetamol (1 g/kg bw) and Legalon® (70 mg/kg bw);
- three test lots, treated with paracetamol (1 g/kg bw) then with AEVC at the respective doses of 50, 100 and 250 mg/kg bw.

Administration volumes were adjusted to 0.5 mL/100 g bw.

The treatment with paracetamol was done twice a week (Mondays and Thursdays), and those with Legalon and AEVC, four times a week (Tuesdays, Wednesdays, Fridays and Saturdays); all over a period of three (3) weeks (see Table 1).

Table 1: Substance Administration Schedule

	Lots					
Days	Neutral C.	Negative C.	Positive C.	Test 1	Test 2	Test 3
Monday	Solvent	Paracetamol	Paracetamol	Paracetamol	Paracetamol	Paracetamol
Tuesday	Solvent	Solvent	Legalon	AEVC50	AEVC100	AEVC250
Wednesday	Solvent	Solvent	Legalon	AEVC50	AEVC100	AEVC250
Thursday	Solvent	Paracetamol	Paracetamol	Paracetamol	Paracetamol	Paracetamol
Friday	Solvent	Solvent	Legalon	AEVC50	AEVC100	AEVC250
Saturday	Solvent	Solvent	Legalon	AEVC50	AEVC100	AEVC250
Sunday						

C. = Control ; Solvent = NaCl à 0,9%

At the end of the experiment, the rats were anesthetized by intraperitoneal injection of a ketamine/xylazine solution as prepared above, at the rate of 1 ml per 100 g of body weight. The blood was then taken by cardiac puncture for biochemical analyses, before the animals were sacrificed for liver observations and weighing.

Statistical analysis

The data was entered with the Excel 2016 software package. The GraphPad Prism 5.03 software (GraphPad Software Inc, San Diego, USA) was used for descriptive analysis (means, standard errors of the men-SEM), statistical tests and graphical representations. To compare the means, One-way analysis of variance (ANOVA I) was used, followed by Tukey-Kramer post-hoc test, with GraphPad in SAT software. The difference between the values was considered statistically significant, very significant and highly significant if, respectively, $p < 0.05$; $p < 0.0l$; and $p < 0.00l$.

RESULTS

Biochemical parameters

In both males and females' rats, paracetamol administration increased ($p < 0.001$) the blood levels of biochemical parameters (transaminases, alkaline phosphatases, and bilirubins), compared to the neutral control. And these effects were inhibited by Legalon (see Fig. 1 to Fig. 5). That, excepted aspartate transferase (AST) related results for female rat (see Fig 2B). AEVC results are detailed below.

Transaminases

Alanine aminotransferase (ALT)

In male rats, the effect of paracetamol was inhibited by the extracts at doses of 100 and 250 mg/kg bw (highly significant inhibition, $p < 0.001$). These inhibitions were significantly greater than that induced by Legalon ($p < 0.001$). See Figure 1A. In female rats, all used doses of extract induced a highly significant inhibition ($p < 0.001$) of the paracetamol effect; and this inhibition was comparable to that of Legalon (Fig 1B).

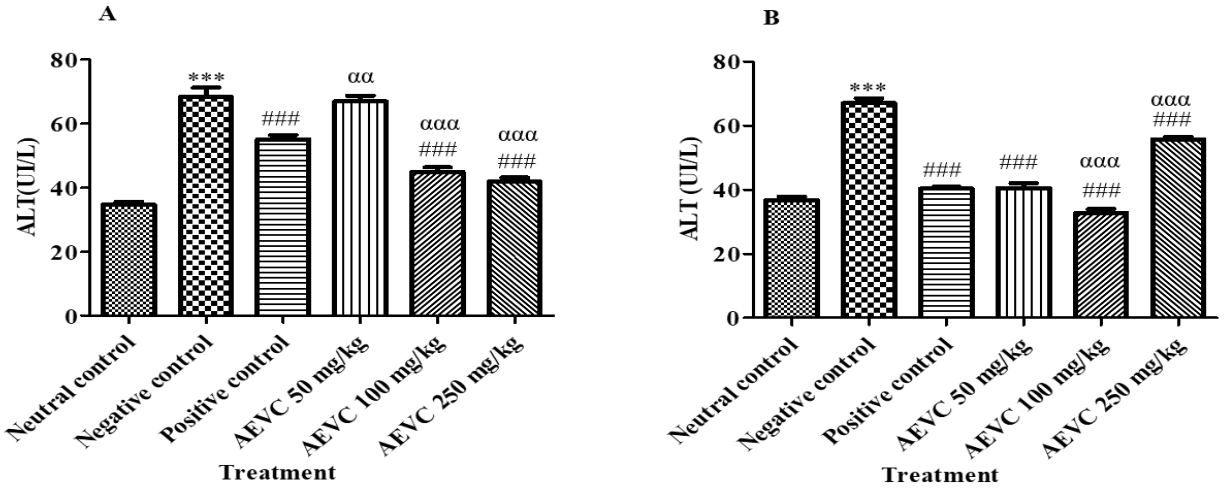

Figure 1: Effect of aqueous extract of *Vernonia colorata* on ALT levels in rats.

A: Male; B: Female. Values are expressed as mean ± standard error; n = 5.
Significant difference from negative control: # ($p < 0.05$); ## ($p<0.01$); ### ($p<0.001$);
Significant difference from neutral control: * ($p < 0.05$); ** ($p<0.0l$); *** ($p < 0.00l$);
Significant difference from the positive control: α ($p < 0.05$); αα ($p < 0.01$); ααα ($p < 0.001$)

Aspartate transferase (AST)

In male rats, AEVC, at 250 mg/kg bw induced a highly significant inhibition ($p < 0.001$) of the paracetamol effect. This inhibition was not found at the lower doses of AEVC ($p > 0.05$). See Fig 2A. In female rats, for all the treatments used, no significant variation in AST levels was observed, compared to the neutral control (Figure 2B)

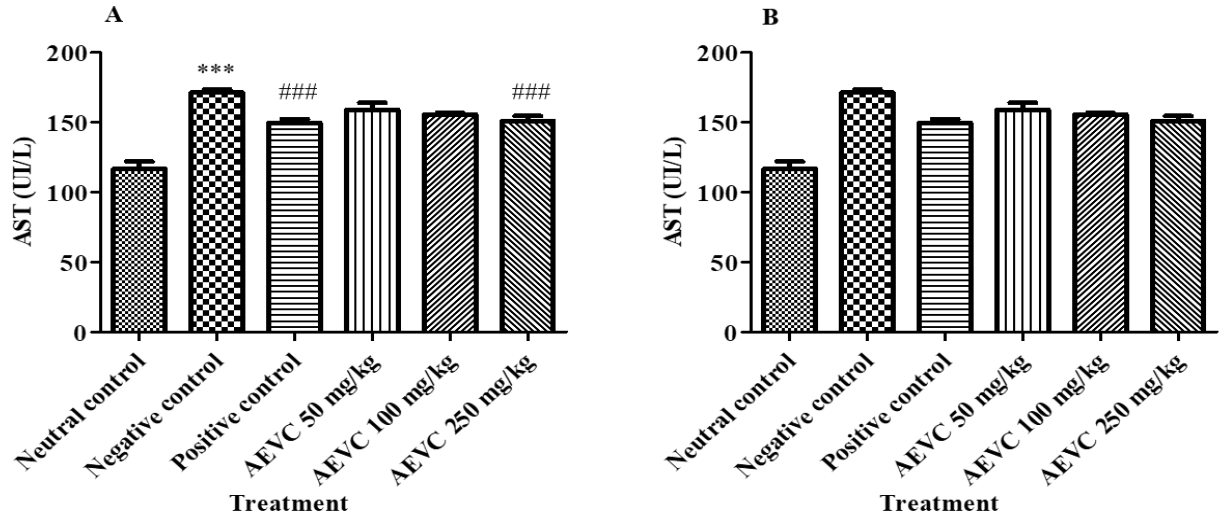

Figure 2: Effect of aqueous extract of *Vernonia colorata* on AST levels in rats.

A: Male; B: Female. Values are expressed as mean ± standard error; n = 5.
Significant difference from negative control: # ($p < 0.05$); ## ($p<0.01$); ### ($p < 0.001$);
Significant difference from neutral control: * ($p < 0.05$); ** ($p<0.0l$); *** ($p < 0.00l$);

Alkaline phosphatase

In both males and females' animals, all used doses of AEVC induced a highly significant inhibition ($p < 0.001$) of the effect of paracetamol. These inhibitions were comparable to Legalon effect (Fig 3A and 3B).

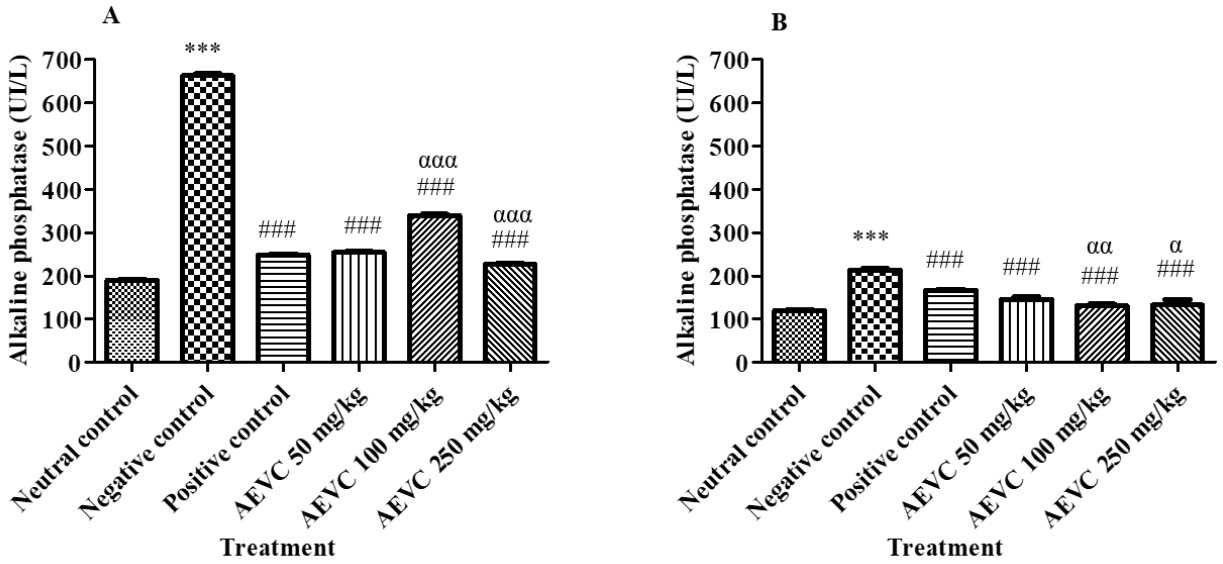

Figure 3: Effect of the aqueous extract of *Vernonia colorata* on the level of alkaline phosphatase.

A: Males; B: Females. Values are expressed as mean ± standard error; n = 5.
Significant difference from negative control: # ($p < 0.05$); ## ($p<0.01$); ### ($p < 0.001$);
Significant difference from neutral control: * ($p < 0.05$); ** ($p<0.0l$); *** ($p < 0.00l$);
Significant difference from the positive control: α ($p < 0.05$); αα ($p < 0.01$); ααα ($p < 0.001$)

Total bilirubin

In all animals, the extract (all doses) had a highly significant inhibitory effect ($p < 0.001$) on paracetamol effect (Figures 4A and 4B). This AEVC effect was comparable to that of Legalon in males' rats. But un females' rats, at 50 and 100 mg/kg bw, it was weaker than Legalon effect ($p < 0.05$ and $p < 0.01$, respectively). See Fig 4B.

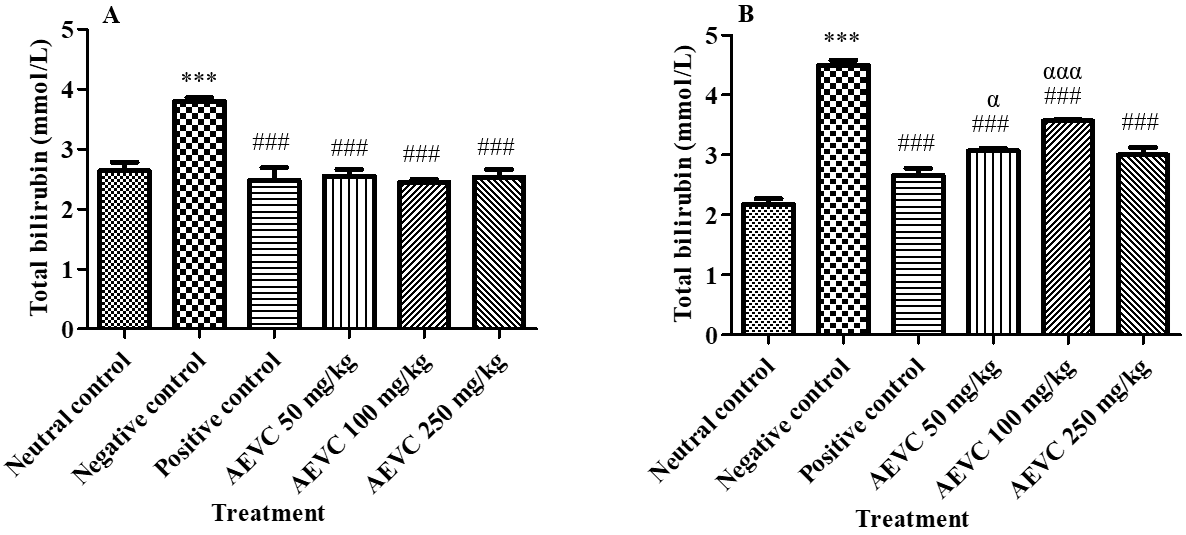

Figure 4: Effect of the aqueous extract of *Vernonia colorata* on the level of total bilirubin.

A: Male; B: Female. Values are expressed as mean ± standard error; n = 5.
Significant difference from negative control: # ($p < 0.05$); ## ($p<0.01$); ### ($p < 0.001$);
Significant difference from neutral control: * ($p < 0.05$); ** ($p<0.0l$); *** ($p < 0.00l$);
Significant difference from the positive control: α ($p < 0.05$); αα ($p < 0.01$); ααα ($p < 0.001$)

Direct bilirubin

In males' rats, AEVC (all doses) had no significant effect on that of paracetamol (Figure 5A). But in females' rats, all doses of AEVC induced a highly significant inhibition ($p < 0.00l$) of the effect of paracetamol. This AEVC inhibitory effect was comparable to Legalon one (Fig. 5B).

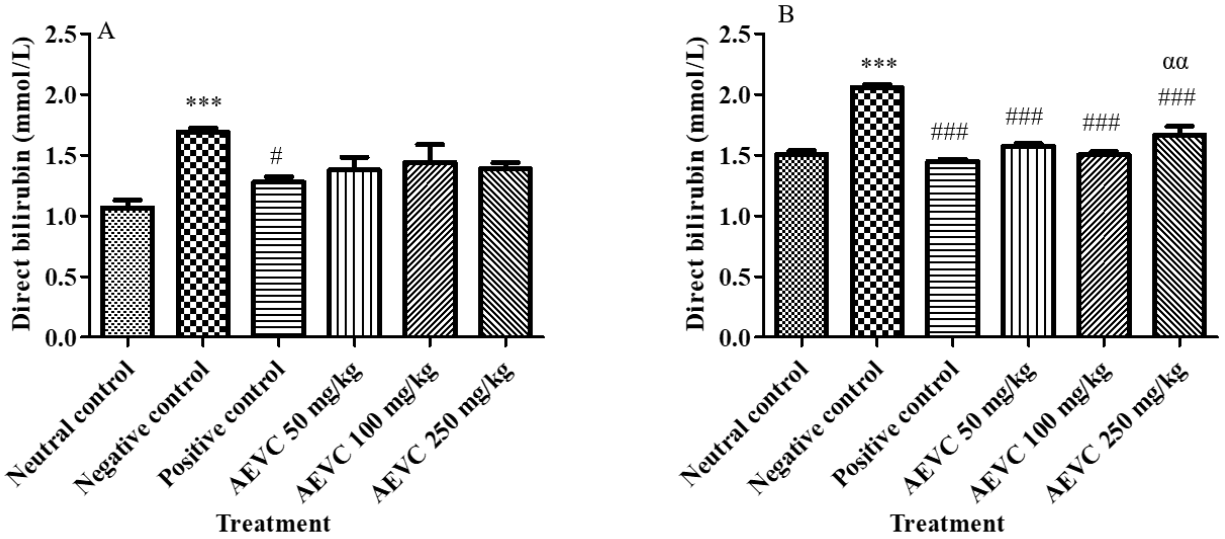

Figure 5: Effect of the aqueous extract of *Vernonia colorata* on the level of direct bilirubin.

A: Male; B: female. Values are expressed as mean ± standard error; n = 5.
Significant difference from negative control: # ($p < 0.05$); ## ($p<0.01$); ### ($p < 0.001$);
Significant difference from neutral control: * ($p < 0.05$); ** ($p<0.0l$); *** ($p < 0.00l$);
Significant difference from the positive control: α ($p < 0.05$); αα ($p < 0.01$); ααα ($p < 0.001$).

Relative weight of liver

In males' rats, for all treatments, it was not observed significant variation of relative weight of liver. But in females, paracetamol induced a highly significant increase of relative liver weight, and this effect was significantly inhibited by Legalon. AEVC, like the Legalon, had an inhibitory effect on paracetamol one at 50 mg/kg bw ($p < 0.01$) and at 250 mg/kg bw ($p < 0.05$). See Table 2.

Tableau 2I: Evolution of the relative weights of liver of male and female rats

	Sexes	
Treatments	**Male**	**Female**
Neutral control	3.12 ± 0.19	2.24 ± 0.17
Negative control	3.02 ± 0.12	$3.20 \pm 0.11^{***}$
Positive control	2.88 ± 0.08	$2.68 \pm 0.04^{\#\#}$
AEVC 50	2.85 ± 0.08	$2.71 \pm 0.04^{\#\#}$
AEVC 100	2.63 ± 0.05	2.97 ± 0.04
AEVC 250	2.57 ± 0.06	$2.73 \pm 0.04^{\#}$

Values are expressed as mean ± standard error; n = 5.
Significant difference from negative control: # ($p < 0.05$); ## ($p<0.01$); ### ($p < 0.001$);
*Significant difference from neutral control: * ($p < 0.05$); ** ($p<0.0l$); *** ($p < 0.00l$);*

DISCUSSION

Paracetamol-induced hepatotoxicity is an experimental model to assess the potency of hepatoprotective agents. Hepatic toxicity to paracetamol is linked to the formation of a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), produced by a minority metabolic pathway dependent on cytochrome P450 2E1 (CYP2E1). At toxic doses, the neutralization capacities of NAPQI by glutathione are overwhelmed, resulting in cytolysis and dose-dependent hepatocyte necrosis.[16] This leads to the release of transaminases (ALT and AST), alkaline phosphatase (ALP) and total bilirubin (BT) and direct bilirubin (BD) in the blood. Indeed, under normal physiological conditions, these substances are present in the liver. But in the event of liver cell necrosis, they are found in abnormally high levels in the blood. [17, 18] Similar results are found in the case of poor regulation of hepatocyte membrane permeability. The assay of these enzymes is essential for the evaluation of liver function. In case of liver disease, there is an increase of the blood levels of ALT and AST, of ALP, and of bilirubins.[19] Particularly, low ALT blood level is a specific indicator of liver integrity.[20] Similarly, increased blood ALPs reflect pathological impairment of bile flow[21], and

abnormally elevated plasma bilirubins indicate hepatobiliary disease and severe disruption of hepatocellular function.[20]

The toxic effects of paracetamol are inhibited by Légalon® whose active substance is silymarin, a polyphenolic flavonoid isolated from the fruit and seeds of *Silybum marianum*.[22] Silymarin has antioxidant potency with protective effects against toxicities induced by a wide variety of agents. This protection is achieved through inhibition of lipid peroxidation by trapping free radicals.[1, 23] It is linked to silymarin's ability to stabilize cell membranes and upregulate intracellular glutathione.[24, 25] The action of silymarin involves different biochemical events, such as increasing the synthesis of ribosomal RNA (rRNA) by stimulating the transcription of polymerase I and rRNA, blocking the absorption of toxins leading to cell membrane protection against osmotic stress and radical-induced damage.[24]
In the present study, through the blood levels of these enzymes, the induction of liver toxicity by paracetamol was shown, as well as the inhibition of that toxicity by silymarin (Legalon). This applies to both male and female rats, with lesser sensibilities for AST related results for female (Fig. 2B), for direct bilirubin related results (Fig. 5A) and relative weights of liver (Tab. II) for males' rats.

Like Legalon, AEVC induced significant to highly significant inhibition of paracetamol deleterious effects on liver. Additionally, the same differentiated sensitivities, according to the sex and parameters, were also found, suggesting a silymarin-like mechanism for AEVC action. AEVC would therefore have a protective and/or restoring effect on liver function against the harmful effects of paracetamol. This seems confirmed by similar results found by Iwalokun et *al.*[26] using the aqueous extract of plants of the same genus, *Vernonia amygdalina* in mice, and the alcoholic extract of *Vernonia cinerea* in rats.[27]

In a previous paper[12], we had shown the presence of terpene compounds, and non-flavonoid phenolics (saponosides, tannins and coumarins) in AEVC. These metabolites could explain the beneficial effect of AEVC on liver. This protective and/or restorative effect would be due to a synergist action of these secondary metabolites contained in the extract. This idea is in agreement with Al-Fatimi et *al.*, Talluri et *al.* and Akkol et *al.*[28, 29, 30], who showed that the plant hepatoprotective and antioxidant properties were supported by coumarins and other polyphenolics compounds. Like silymarin, AEVC stabilizes the plasma membrane, and thus prevents liver tissue damage. However, this remains to be confirmed by histological studies.

The results obtained also showed that paracetamol induced a highly significant increase in liver relative weight (in females' rats only). Similar effects (increased liver weight) were found in previous work performed on rats.[31] We found that AEVC induced a very significant inhibition of this paracetamol effect on liver relative weight. This result reinforces the hepatoprotective/hepatorestorative properties of AEVC.

CONCLUSION

The study confirmed the validity of the model used: paracetamol intoxication and interaction with Legalon, and AEVC. The reduction in hepatic damage due to paracetamol by AEVC is quantitatively comparable to that of Legalon (silymarin), and differentiated sensibilities according to sex and parameters suggested a silymarin-like mechanism for AEVC action. A histopathological study on the liver will provide better information on the properties highlighted.

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