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Original Research Article

Hepatoprotective Effect of Leaves of *Passiflora foetida* Linn. (Passifloraceae) Against Paracetamol-Induced Liver Damage in Wistar Albino Rats

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Abstract: Liver disease is one of the most common public health problems in the word. In this study, we investigate the possible hepatoprotective effect of the aqueous extract of *Passiflora foetida* (AEPF) using paracetamol-induced liver damage albino rats as animal model. The rats were divided in groups consisting of six animals each and treated for seven consecutive days as following: group I was the normal control and received distilled water, group II was the negative control treated with distilled water; group III served as the positive control and pretreated with 100 mg/kg of silymarin; groups IV, V and VI were pretreated with the AEPF doses of 400, 600 and 800 mg/kg respectively. At the seventh day, the rats from group II to VI were given a dose of 2 g/kg of paracetamol (PCM) and 24 hours after this treatment, blood samples and pieces of liver were collected for biochemical and histopathological analysis. Results showed that in group II, PCM increased significantly the activity of liver enzymes (ALT, AST, and PAL), the serum level of total and direct bilirubin and decreased significantly the serum level of total cholesterol, triglyceride and total protein in rats. Also, the liver tissue of the PCM-treated group exhibited severe acute centrilobular associated to periportal necrosis and haemorrhagic lesions in comparison to normal control rats. However, the biochemical and histopathological adverse effects of PCM were suppressed or reduced significantly by the pretreatment of rats with silymarin and AEPF dose dependently. These data indicated that *Passiflora foetida* possessed hepatoprotective effects similar to those of silymarin probably due to its saponins, phenols and flavonoids contents.

Keywords: *Passiflora foetida*, hepatoprotective effect, paracetamol, silymarin, liver.

INTRODUCTION

The liver is a soft, friable organ that is the largest gland in the human body, accounting for around 2% to 3% of the average body weight (Abdel-Misih & Bloomston, 2014; Rajguru & Mitesh, 2017). This organ is involved in several vital function and has a very important role. It participates in the process of digestion by the bile secretion and transforms the discontinuous supply of nutrients absorbed by the digestive tract into a continuous flow of nutrients (Messaoudi, 2017). Liver is also responsible for eliminating of certain metabolic wastes such as ammoniac, bilirubin and most xenobiotics. Hence, the liver is exposed to various chemical or infectious attacks which sometimes have serious repercussions on the body, such as hepatitis, cholestasis, cirrhosis, fibrosis and steatosis (Marrone *et al*., 2017). Liver diseases affect more than fifty millions persons in the word (Thompson *et al.*, 2017) and drugs are a major cause of liver damage (Aashish *et al*., 2012). The therapy applied is based on antibiotics

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which prevent or reduce the signs of hepatic encephalopathy, by the decrease of NH3 and volatile fatty acids production, and decrease in the conversion of methionine into mercaptans by the intestinal flora (Fromonot, 2001). However, this therapy sometimes induce harmful side effects, worsening liver disease consequences (Sahu, 2007). The development of new therapies to solve the problem of liver disease becomes a necessity. This therapy needs to be safer, less expensive and easily available globally. However, modern medicine sometimes proves to be too expensive and inaccessible to the majority of populations in developing countries in general and particularly in sub-Saharan African. According to a World Bank report, 41% of the population of sub-Saharan Africa live below the international poverty line (World Bank, 2018). Therefore, low-income populations living in this part of the continent and especially those in rural areas use traditional medicine to treat disease related to liver dysfunction. Indeed, traditional medicine has remained for centuries the most accessible and cheapest health system and medicinal plants contribute significantly to the life of rural populations and to social balance in Africa (Schmelzer and Gurib-Fakim, 2008). Because of their enormous potential, the search for new effective and less aggressive bioactive substances from these plants becomes a priority.

Passiflora foetida Linn. (Passifloraceae), one of the 304 high pharmaceutical plants among the 5000 species which has been identified in Ivory Coast (Adjanohoun & Aké-Assi, 1970) appears promising for its nutrient content, phytochemical composition and its easy accessibility. This plant was found to have estrogenic, analgesic, antibacterial, antioxidant and antidiarrhoeal properties (Bendini *et al*., 2006 ; Mohanasundari *et al*., 2007; Baby *et al*., 2010; Rahman *et al*., 2011; Sathish *et al*., 2011 ; Bleu *et al*., 2012).

The aim of this study was to evaluate the hepatoprotective effects of the aqueous extract of the leaves of *Passiflora foetida* in Wistar albino rats.

MATERIAL AND METHOD

Plant Material

The fresh leaves of *P. foetida* were collected from Toumodi, a department in the Center of Ivory Coast. The plant was identified in the National Center of Floristic where a voucher specimen was deposited under the number 746B.

Animals

Three to four month old males and female Wistar albino rats (*Rattus norgevicus*, Muridae) weighing between 160 and 220g obtained from the vivarium of the Superior Normal School of Abidjan were used for the experiments. The rats were kept in a room at ambient temperature (28±2°C) with a photoperiod of 12 hours light / dark and a 50-60% hygrometry. They were free allowed to water and commercial food (15% protein, 5.3% fat) supplied by IVOGRAIN industry (Abidjan, Ivory Coast). All the animals were acclimatized to the laboratory conditions for two weeks prior the experiments beginning. Experiments were conducted according to the EU Directive 2010 /63/ EU for animal experiments.

Preparation of the extract

The aqueous extract of *P. foetida* was prepared basing on previous studies (Bleu *et al*., 2012). The leaves of this plant were collected and dried at an ambient temperature (28±2°C) without exposure to sun light and crushed to obtain a powder using an electric grinder (Restsch SK/100C, Germany). Fifty grammes (50g) of this powder was macerated in 1500 ml of distilled water for 24 hours in a magnetic agitator (Janke & Kunkel RH, Germany) and filtered using Whatman paper number 1. The solution obtained was evaporated in an air circulating oven (MEMMERT UF55, Germany) at 50°C until total dryness. The aqueous extract was recovered and stored at 4°C in a refrigerator for the different tests.

Phytochemical screening

The determination of the secondary metabolites of the aqueous extract of *P. foetida* was carried out using qualitative standard tests (Ouattara *et al*., 2021). The phytochemical compounds tested were: sterols, polyterpenes, polyphenols, flavonoids, tannins, quinones, alkaloids and saponosides.

Toxicological study

The oral acute toxicity of the plant extract was determined basing on the OECD guidelines 423 for the testing of chemicals (OECD, 2001). The limit test was used in this experiment. Six female rats were divided into two groups consisting of 3 animals each. Group I served as control and was administered orally distilled water and the group II was the test group and received an oral single dose of 5000 mg/kg b.w., using an intragastric sound. The maximum volume of liquid administered to each rat did not exceed 1 ml/100g b.w. After the treatment, the animals were observed individually at least once during the first 30 minutes, periodically for 24 hours and daily for a total of 14 days. Observations included clinical signs of mortality or toxicity such as respiratory, eyes, somatomotor activity, tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma (OECD, 2001; Mishra *et al*., 2021; Ouattara *et al*., 2021). Furthermore, the rats were weighed before and every two days after the dosing.

Evaluation of the hepatoprotective activity

Experimental design

This study was conducted using a modified method described by some authors (Yahya *et al*., 2013; Okokon *et al*., 2017; Tafere *et al*., 2020). Thirty-six (36) rats of both sex were divided into six groups consisting of 6 animal each (3 males, 3 females) and treated orally for 7 consecutive days as following:

Groupe I: normal control group received distilled water;
Groupe II: negative control group received distilled water;
Groupe III: positive control group pretreated with 100 mg/kg b.w of sylimarin;
Group IV: extract test group pretreated with 400 mg/kg b.w of AEPF;
Group V: extract test group pretreated with 600 mg/kg b.w of AEPF;
Group VI: extract test group pretreated with 800 mg/kg b.w of AEPF.

The extract doses was chosen basing on previous subchronic toxicity study of AEPF which showed that the maximal doses of 800 mg/kg b.w did not induce any behavioural abnormality and change of biochemical markers of liver and kidney in rats (Bleu *et al*., 2011). All the above treatments were given once daily using an intragastric sound. At the seventh day, 3 hours after administration of these treatments, a unique dose of 2 g/kg b.w of paracetamol was given orally to rats of groups II to VI.

Biochemical analysis

At the eighth day, the rats were weighed and sacrificed by rapid decapitation and blood sample were collected separately in tubes. The serum of each rat obtained after centrifugation of the blood at 3500 revolution per minute for 5 minutes was used for determination of biochemical markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, total protein, albumin, total cholesterol and triglyceride by standard methods using an automatic analyser (Hitachi 902, Japan) (Tafere *et al.*, 2020).

Histopathological examination

After the blood collection, the liver was dissected out and weighed. A piece of each organ was fixed in 10% buffered formalin for 48 hours. Then the liver tissue was gradually dehydrated in ethanol (50° - 70° - 80° - 96° - 100°), embedded in paraffin at 58°C and sections of 4-5 µm in thickness were cut by a microtome and stained with haematoxylin-eosin (Yahya *et al*., 2013; Abdullah *et al*., 2017). The histological structure of liver was observed under light microscope (optika XDS-2, Italy) and photomicrographs were taken.

Statistical analysis

The statistical study of the results was conducted using *Statistica* 7.1 software. Values are expressed as mean ± standard error on the mean (SEM). One-way analysis of variance (ANOVA) was performed to assess the significance of the differences observed between control and treated groups by the *post hoc* Tukey test for multiple comparisons. If value of $p<0.05$, the difference was considered statistically significant.

RESULTS

Chemical Composition

The result of the phytochemical screening of the aqueous extract of *P. foetida* showed that this extract contains sterols, polyterpenes, polyphenols, flavonoids and saponosides. However, tanins, quinones and alkaloids were absent in this extract (Table 1).

Table 1: phytochemical compounds of the aqueous extract of *P. foetida*

Chemical compounds	Tests used	Aqueous extract
Sterols and polyterpenes	Liebermann test	+
polyphenols	$FeCl_3$ test	+
flavonoids	Cyanidin test	+
tanins	Stiasny test	-
quinones	Borntraeger test	-
alkaloids	Bouchardat and Dragendorff test	-
saponosids	Foam test	+

***(-)**: absence; (+): presence.*

Acute toxicity

All the rats treated with the dose of 5000 mg/kg b.w did not present any sign of toxicity nor lethality when compared to the control group within the 14 days of observation. In addition, any significant change in the body weight of the treated group was recorded.

Hepatoprotective activity

Effect on the liver and body weight

The effect of treatments on the body weight and the relative weight of the liver are presented in the Table 2. At the end of the experiment, the body weight of all the treated groups did not change significantly when compared to the normal control group. However, paracetamol at the unique dose of 2 g/kg b.w induced a highly significant increase ($p<0.01$) of the relative weight of the liver which was significantly reduced ($p<0.05$) in rats by the pretreatment with silymarin at 100 mg/kg b.w and AEPF at doses of 400, 600 and 800 mg/kg b.w in comparison to PCM treated-group.

Table 2: Effects of the treatments on the liver and body weight of the rats

Treatments	Body weight (g)		Liver weight (g/100g b.w)
	Initial	Final	
Normal control (distilled water)	195.83±23.71	206.00±25.39	2.34±0.10
Negative control (PCM 2 g/kg)	193.83±23.43^{ns}	204.16±22.69 ^{ns}	3.49±0.27***
Positive control (Sil 100mg/kg + PCM 2g/kg)	194.33±21.22 ^{ns}	205.00±21.22 ^{ns}	3.12±0.10$^{\#}$
AEPF (400 mg/kg) + PCM (2 g/kg)	192.83±27.20 ^{ns}	202.83±27.37 ^{ns}	3.17±0.19$^{\#}$
AEPF (600 mg/kg) + PCM (2 g/kg)	198.16±25.65 ^{ns}	209.16±26.82 ^{ns}	3.04±0.24$^{\#}$
AEPF (800 mg/kg) + PCM (2 g/kg)	191.83±22.95 ^{ns}	198.16±33.38 ^{ns}	3.20±0.13$^{\#}$

Values are expressed as mean ± ESM (n=6). $^{***}p<0.001$ vs normal control group; $^{\#}p<0.05$ vs PCM treated group (negative control); sil: silymarin; ns: not significant.

Effects on the biochemical parameters

The administration of paracetamol at the unique dose of 2 g/kg b.w caused a highly significant increase ($p<0.01$) of the activities of liver enzymes ALT, AST and ALP when compared to the normal control group. Total and direct bilirubin serum level was also high significantly augmented ($p<0.01$) by the PCM treatment. However, the increase effects of these biomarkers induced by PCM were significantly reduced or suppressed by pretreatments of rats with silymarin (100 mg/kg) and AEPF (400, 600 and 800 mg/kg) excepted the activities of transaminases (ALT and AST) at the AEPF dose of 600 mg/kg b.w.

Furthermore, PCM induced a significant reduction ($p<0.05$) of total cholesterol, triglyceride and total protein level which was increased significantly ($p<0.05$) by the pretreatment of rats with silymarin and AEPF at different doses in comparison to PCM group (Table 3).

Effects on the liver histology

The histological examination of liver of the normal control group showed normal architecture of the hepatic tissue characterized by a well organization of hepatic cells in the centrilobular and periportal area (Figure 1A). However, the administration of PCM to rats caused a disorganization of the hepatic tissue which was found to exhibit mainly an acute severe centrilobular necrosis associated to varyng severity of periportal necrosis and the presence of haemorrhagic lesions (Figure 1B).

The pretreatment of rats with silymarin prior to administration of PCM caused a disappearance of the severe centrilobular necrosis with persistence of discrete haemorrhagic lesions and periportal necrosis (Figure 1C). The pretreatment with AEPF at the dose of 400 mg/kg b.w induced a total disappearance of the hepatic tissue abnormalities caused by PCM and appearance of hepatocellular apoptosis figures (Figure 1D).

The liver tissue of animals pretreated with the AEPF dose of 600 mg/kg b.w showed a discrete centrilobular and periportal necrosis (Figure 1E) and these abnormalities was aggravated by the pretreatment at the dose of 800 mg/kg b.w with in addition the presence of apoptosis figures (Figure 1F). All the histopathological effects obtained by the different treatments on the architecture of the liver tissue are presented in the table 4.

Table 3: Effects of silymarin and AEPF on the serum level of biochemical parameters in PCM-induced liver damage Wistar rats

Treatments	Normal control (distilled water)	Negative control (distilled water + PCM 2g/kg)	Positive control (Silymarin 100g/kg + PCM 2g/kg)	AEPF 400 mg/kg + PCM 2g/kg	EAPF 600 mg/kg + PCM 2g/kg	EAPF 800 mg/kg + PCM 2g/kg
ALT (IU/L)	67.33±11.39	165.00±18.75***	101.00±17.64$^{\#\#\#}$	112.16±21.95$^{\#\#}$	187.50±30.25^{ns}	143.00±14.31$^{\#}$
AST (IU/L)	243.33±15.76	801.16±153.55***	502.33±111.60$^{\#\#}$	477.16±142.53$^{\#\#}$	867.33±120.24^{ns}	143.00±14.31$^{\#}$
ALP (IU/L)	562.16±47.49	645.00±49.53*	576.66±30.98$^{\#}$	521.33±21.63$^{\#\#\#}$	493.66±21.52$^{\#\#\#}$	543.83±46.61$^{\#\#}$
TB (mg/L)	5.83±1.16	8.16±0.75**	5.33±1.03$^{\#\#\#}$	6.16±1.47$^{\#}$	4.83±1.47$^{\#\#\#}$	6.00±1.26$^{\#\#}$
DB (mg/L)	0.58±0.09	0.87±0.10***	0.53±0.16$^{\#\#}$	0.30±0.18$^{\#\#\#}$	0.42±0.04$^{\#\#\#}$	0.41±0.29$^{\#\#}$
TC (g/L)	0.92±0.04	0.72±0.12**	0.99±0.20$^{\#}$	0.87±0.09$^{\#}$	1.01±0.17$^{\#\#}$	0.87±0.11^{ns}
TRG (g/L)	1.02±0.18	0.81±0.06*	0.99±0.15$^{\#}$	0.83±0.09 ^{ns}	0.82±0.07 ^{ns}	1.10±0.29$^{\#}$
TP (g/L)	73.00±1.78	70.83±0.75*	72.33±1.03$^{\#}$	72.83±1.16$^{\#\#}$	72.66±1.63$^{\#}$	71.83±0.75$^{\#}$
ALB (g/L)	38.66±1.50	38.83±1.94 ^{ns}	41.00±1.41^{ns}	39.33±1.96 ^{ns}	39.33±2.06 ^{ns}	39.00±1.89 ^{ns}

Values are expressed as mean ± ESM (n=6). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs normal control group; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs PCM treated group (negative control); ns: not significant, TB: total bilirubin, DB: direct bilirubin, TC: total cholesterol, TRG: triglyceride, TP: total protein, ALB: albumin.

Table 4: Histopathological effects of the treatments on the hepatic tissue of the rats

Treatments	Centrilobular necrosis	Periportal necrosis	Haemorrhage	Apoptosis
Normal control (distilled water)	-	-	-	-
Negative control (PCM)	+++	+++	++	-
Positive control (silymarin +PCM)	-	+	+	-
AEPF 400 mg/kg + PCM	-	-	-	+
AEPF 600 mg/kg + PCM	+	+	-	-
AEPF 800 mg/kg + PCM	++	++	-	+

The severity of the effects on the hepatic tissue depended to the treatment. -: no effect; +: discrete effect; ++: moderate effect; +++: severe effect.

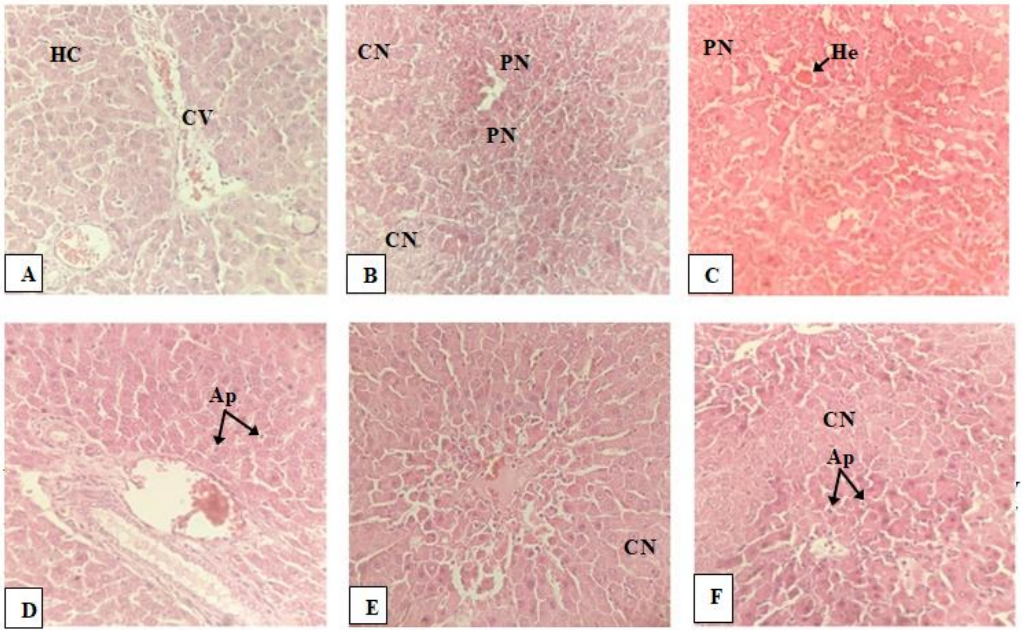

Figure 1: Photomicrograph of the histopathological changes induced on the liver; A: Normal control group showing normal architecture of the hepatic tissue; B: PCM group with severe centrilobular necrosis associate to periportal necrosis and presence of haemorrhage; C: Silymarin + PCM group revealing a disappearance of centrilobular necrosis but a persistence of discrete periportal necrosis and haemorrhage; D: AEPF 400 mg/kg + PCM group showing a total disappearance of toxic changes induced by PCM with a presence of apoptosis figures; E: AEPF 600 mg/kg + PCM group with a presence of discrete centrilobular and periportal necrosis; F: AEPF 800 mg/kg + PCM group which induced a moderate centrilobular and periportal necrosis and apoptosis figures. Ap: apoptosis; CN: centrilobular necrosis; CV: centrilobular vein; He: haemorrhage; HC: hepatic cells; PN: periportal necrosis. Magnification: x400; Stained: Haematoxylin-eosin

DISCUSSION

The phytochemical screening of *P. foetida* revealed that the AEPF obtained from leaves collected in the Center of Ivory Coast (Toumodi) contains sterols, polyterpens, flavonoids, and saponosids. This constitution of AEPF was different from that obtained previously from leaves collected in the south of the country which contained alkaloids but not sterols and polyterpens (Bleu *et al*., 2012). According to Liu *et al.*, (2016), environmental factors may influence the type and content of active substances of a plant. This result should be explained by the difference in environmental conditions of these regions, since the southern of the country is characterised by high humidity with semi-deciduous forest, low altitude and mean annual temperature while the center is consisted to savannah with low humidity and elevated annual mean temperature.

The toxicological analysis did not show any lethality nor abnormality in the comportment of the mice when treated with the oral unique dose of 5000 mg/kg b.w, indicating that the oral LD_{50} of this extract was greater than 5000 mg/kg b.w.

The liver is the primary target for drug toxicity, as it is the primary site for the metabolism and elimination of chemicals. Therefore, hepatotoxicity induced by drugs such as paracetamol is common (Sultana *et al.*, 2016).

In this study, the hepatoprotective effects of AEPF was evaluated in the context of hepatotoxicity induced in rats by PCM. Thus, the administration of PCM at the dose of 2 g/kg b.w induced in rats a highly significant increase ($p<0.001$) in the relative weight of the liver compared to control group. However,

pretreatment of animals with silymarin (100 mg/kg b.w) and AEPF (400, 600 and 800 mg/kg b.w) reduced significantly ($p<0.05$) the increase induced by PCM. The same results were obtained by Yahya *et al*. (2013) with the methanolic extract of *Bauhinia purpurea* (Leguminosae) and by Taj *et al*. (2019) with the ethanolic extract of *Stokeyia indica* (Cystoseiraceae) in rats. PCM causes a hepatic congestion linked to an accumulation of red blood cells in the endocytic vacuoles and sinusoids, which leads to an increase of the liver weight in relation to the blood flow inside this organ associated with an increase in the level of hepatic haemoglobin (Hinson *et al*., 2010). Silymarin and AEPF would reduce the relative weight of the liver by inhibiting the congestant activity of PCM. The weight of the liver is therefore a reliable indicator for evaluating the manifestations of hepatotoxicity in rodents.

Furthermore, the effects of silymarin and AEPF on hepatic enzymes and other serum biochemical parameters in PCM intoxicated rats were investigated. When administered to rats, PCM caused a highly significant increase ($p<0.001$) of transaminases ALT and AST and then a significant increase ($p<0.05$) of PAL activities in comparison to normal control group. This effects on the hepatic enzymes was reversed by silymarin and by AEPF (400, 600 and 800 mg/kg b.w) which induced highly significant reductions of the effects of PCM. This result confirmed the effect of *P. foetida* in intoxicated rats obtained by Ramasamy *et al*. (2009). These authors demonstrated that the ethanolic extract of fruits of this plant reduced significantly the hepatic enzymes augmentation induced by carbon tetrachloride. The effects of AEPF on the increase in hepatic enzymes activities induced by PCM are also similar to those of the ethanolic extract of stems of *Homalium lelestui* (Flacourtiaceae) (Okokon *et al*., 2017) and the aqueous extract of *Croton macrostachyus* (Euphorbiaceae) (Tafere *et al*., 2020) on the effects of the same drug in mice and rats respectively.

PCM causes well-known dose-dependent cytolytic hepatitis. The toxicity of PCM is dependent on its metabolism mediated by cytochrome P450 in a hepatic derivative, the N-acetyl-*p*-benzoquinone imine (NAPQI). This is produced in large quantities, following the ingestion of large dose of PCM, due to saturation of metabolic pathways of PCM inactivation. After depletion of cellular glutathione stores, NAPQI binds covalently to intracellular proteins and results in centrilobular hepatocytes necrosis (Mégarbane et al., 2007). This is followed by a release of liver enzymes into the blood followed by a very large increase in their blood level (Hinson *et al*., 2010; Subramanian *et al*., 2013; Abirami *et al*., 2015; Rotundo & Pyrsopoulos, 2020).

ALT and AST are enzymes which catalyse respectively the transfer of amine function of alanine and aspartate to α-ketoglutarate to generate pyruvate and glutamate which are important contributors to the citric acid cycle (Giannini *et al*., 2005). ALT is a specific marker of the liver making it an important indicator of drug-induced hepatotoxicity (Dufour *et al*., 2000). As for PAL, its level rises in the event of hepatic cells necrosis but also in the event of bile ducts obstruction (Girish and Pradhan, 2012).

The reduction in the effect of PCM on the hepatic enzymes activity by silymarin is linked to the hepatoprotective properties of this substance. Indeed, according some authors, silymarin suppressed hepatic lesion caused by alcohol or certain drugs such as PCM by stabilizing the external membrane of hepatocytes, by potentiating the antioxidant defence of hepatic cells by scavenging free radicals, by maintaining the pulse of glutathione and superoxide dismutase, then stimulating regeneration and multiplication of liver cells (Charrié *et al*., 2017). Thus, AEPF could promote a reduction in the activities of ALT, AST and PAL and restore liver function by acting through the same mechanisms as silymarin since the antioxidant properties of *P. foetida* leaf extracts was demonstrated by several authors (Ajan & Patil, 2019; Chiavaroli *et al*., 2020). Furthermore, the effect of this extract was dose dependent since it was much greater on the hepatic enzymes at the dose of 400 mg/kg than at doses of 600 and 800 mg/kg b.w.

The results of the biochemical analysis of liver markers were corroborated by those of the histopathological analysis of this organ. In effect, the histological section of liver of rats treated with PCM indicated the presence of severe acute centrilobular necrosis associated to periportal necrosis and haemorrhagic lesions, indicating the hepatotoxicity of this drug at a dose of 2 g/kg b.w which would explain the increase of the blood levels of ALT, AST and PAL in rats. However, this hepatotoxicity was very significantly reduced by pretreatment of the animals with silymarin and AEPF at 400 mg/kg b.w showing the disappearance of the lesion induced by PCM except for the persistence of haemorrhage in the case of silymarin. Similar histopathological data have also been demonstrated by Yahya *et al*. (2013) and by Mondal *et al*., (2020) respectively when studying the hepatoprotective effects of methanolic extract of *Bauhinia purpurea* (Leguminosae) leaf and the ethyl acetate extract of *Mollatus repandus* (Euphorbiaceae) stem in rats.

The combination of the results of the biochemical analysis of liver enzymes and the histopathological examination of this organ showed that AEPF exerted hepatoprotective effects in rats at the dose of 400 mg/kg b.w.

The biochemical essay of total and direct bilirubin, total cholesterol, triglyceride, total protein and albumin tended to confirm hepatoprotective properties

revealed by analysis of liver enzymes. In fact, PCM caused a highly significant ($p<0.01$) increase in the serum levels of total and direct bilirubin in rats. But this augmentation was suppressed by pretreatments with silymarin and AEPF ($p<0.05$). Bilirubin is a fat soluble macromolecule resulting from the destruction of the red blood cells, the catabolism of the heme and the destruction in the bone morrow of the red blood cells precursors before their release in the peripheral blood. It is formed mainly in the spleen from where it is transported by the blood to the liver before being stored in the hepatocytes and conjugated with glucuronic acid to become water soluble and excreted in the bile. High above normal bilirubin levels suggest underlying liver disease (Thapa & Walia, 2007; Ku & Lazim, 2017). Therefore, bilirubin is used as an indicator to assess the secretory function of hepatocytes (Al-Harbi et *al.*, 2014). In this experiment, the increase in the serum level of total and direct bilirubin could be explained by the weakening of the hepatocyte membrane followed by the release of these biomarkers into the blood caused by PCM. The reduction of bilirubin in the serum of rats by *P. foetida* confirmed the hepatoprotective effects of this plant.

Lipids and proteins are considered as the essential constituents of the cell membrane. This study revealed that PCM induced a significant ($p<0.05$) decrease of total cholesterol, triglyceride and total protein but this effect was reversed significantly ($p<0.05$) by silymarin and AEPF. The disorganization of cell membrane and the denaturation of lipids and proteins or their metabolism caused by the PCM would be at the origin of the decrease of these parameters. The effects of silymarin and AEPF would result from their antioxidant activities by reducing the formation of free radicals and protecting the cell membrane, confirming the hepatoprotective role of AEPF.

The hepatoprotective properties of AEPF could be linked to the presence in this extract of phenols, saponins, and flavonoids such as apigenin and naringenin which are known for their antioxidant and hepatoprotective effects (Patel *et al*., 2011; Sanchez-Marzo *et al*., 2019; Ajane and Patil., 2019; Chiavaroli *et al*., 2020).

CONCLUSION

This study demonstrated that the aqueous extract of *P. foetida* possessed hepatoprotective effects against Paracetamol similar to those of silymarin. These effects resulted in reduction or suppression of the biochemical parameters changes and of the alteration of the histological architecture of the liver induced by paracetamol. The presence in this extract of phenols, saponins and flavonoids (apigenin and naringenin), substances well known for their antioxidant and hepatoprotective activities could explain the effectiveness of this plant against liver damage.

REFERENCES

- Aashish, P., Tarun S., & Pallavi, B. (2012). Drug-induced hepatotoxicity. *Journal of Applied Pharmaceutical Sciences*, 2(5), 233-243.
- Abdel-Misih, S. R. Z., & Bloomston, M. (2010). Liver anatomy. *Surgical Clinics of North America*, 90(4), 643-653.
- Abdisa, Z., & Kenea, F. (2020). Phytochemical screening, antibacterial and antioxidant activity studies on the crude root extract of *Clematis hirsute*. *Cogent Chemistry*, 6, 1. DOI: 10.1080/23312009.2020.1862389.
- Abdullah, Khan A. M., Ahmad, W., Ahmad, M., & Nisar, M. (2017). Hepatoprotective effect of solvent extracts of *Viola canescens* Wall. Ex. Rob. against CCl4 induced toxicity through antioxidant and membrane stabilizing activity. *BMC complementary and alternative medicine*, 17, 10. DOI 10.1186/s12906-016-1537-7.
- Abirami, A., Nagarani, G., & Siddhuraju, P. (2015). Hepatoprotective effect of leaf extracts from *Citrus hystrix* and *C. maxima* against paracetamol-induced liver injury in rats. *Food Sci*ence and *Human Wellness*, 4(1), 35-41.
- Adjanohoun, E., & Aké-Assi, L. (1970). Pharmaceutical plants from Ivory Coast. Agreement n°701437, approved by the Ministry of Planning, University of Poitier, France, 343p.
- Ajane, G., & Patil, A. S. (2019). Evaluation of Antioxidant Potential of *Passiflora foetida* Extract and Quantitative Evaluation of its Phytochemical Content-A Possible Natural Antioxidant. *Pharmaceutical Chemistry Journal*, 6, 14-24.
- Al-Harbi, N., Imam, F., Nadeem, A. Al-Harbi, M. Iqbal, M., & Ahmad, S. (2014). Carbon tetrachloride-induced hepatotoxicity in rat is reversed by treatment with riboflavin. *International Immunopharmacology*, 21, 383-388.
- Baby, E., Balasubramaniam, A., Manivannan, R., Joce, J., & Senthilkumar, N. (2010). Antibacterial activity of methanolic root extract of *P. foetida* Linn. *Journal of Pharmaceutical Sciences and Research*, 2(1), 38-40.
- Bendini, L., Cerretani, L., Pizzolante, L., Toschi, T. G., Guzzo, F., Ceoldo, S., Marconi, A. M., Andreetta, F., & Levi, M. (2006). Phenol content related to antioxidant and antimicrobial activities of *Passiflora foetida sp.* extracts. *European Food Research and Technology*, 223(1), 102-109.
- Bleu, G. M., Kouakou, K., Toure, A., & Traore, F. (2011). Étude de la toxicité aiguë et subchronique de l'extrait aqueux de *Passiflora foetida* Linn. (Passifloraceae) chez les rats et les souris. *International Journal of Chemical and Bioogical Sciences*, 5(5), 1777-1789.
- Bleu, G. M., Kouakou, K., Zahoui, O. S., Touré, A., & Traoré, F. (2012). Oral acute toxicity and estrogenic effects of the extracts of *Passiflora*

foetida Linn. (Passifloraceae) leaves in Wistar albino rats. *Annals of Biological Research*, 3(9), 4609-4616.

- Charrié, J. C., Chastel, B., Cieur, C., Combe, P., Damak, M., Hedayat, K., & Saigne-Soulard, C. (2017). Medicinal plants: Integrative clinical phytotherapy and endobiogenic medicine. Ed. Lavoisier Tec & Doc, Paris, France, 704 p.
- Chiavaroli, A., Di Simone, S. C., Sinan. K.I., Ciferri, M. C., Flores, A. G., Zengin, G., Etienne. O. K., Ak, G., Fawzi, M. M., Jugreet, S., Cziáky, Z., Jekő, J., Recinella, L., Brunetti, L., Leone, S., Angelini, P., Venanzoni, R., Menghini, L., Ferrante, C., & Orlando, G. (2020). Pharmacological Properties and Chemical Profiles of *Passiflora foetida* L. Extracts: Novel Insights for Pharmaceuticals and Nutraceuticals. *Processes, 8*, 1034.
- Dufour, D. R., Lott, J. A., Nolte, F. S., Gretch, D. R., Koff, R. S., & Seeff, L. B. (2000). Diagnosis and monitoring of hepaticinjury II. Recommandation for use of laboratory tests in screening, diagnosis and monitoring. *Clinical Chemistry,* 46, 2000, 2050-2068.
- Fromonot, C. (2001). Peculiarities of hepatic pathology in the cat. Veterinary doctorate from the Faculty of Medicine of Créteil. National Veterinary School of Alfort, France, 62p.
- Giannini, G. E., Testa, R., & Savarino, V. (2005). Liver enzyme alteration: a guide for clinicians. *Canadian Medical Association Journal (CMAJ)*, 172(3), 367-79.
- Girish, C., & Pradhan, SC. (2012). Hepatoprotective activities of picroliv, curcumin, and ellagic acid compared to silymarin on carbon tetrachloride-induced liver toxicity in mice. *Journal of Pharmacology and Pharmacotherapeutics*, 3, 149-155.
- Hinson, A. J., Dean, W. R., & Laura, P. J. (2010). Mechanisms of Acetaminophen-Induced Liver Necrosis. *Handbook of Experimental Pharmacology*, 196, 369–405.
- Ku, L. C., & Lazim, N. S. M. (2017). Direct photometry non-invasive bilirubin device *International Research Journal of engineering Technology (irjet)*, 4, 401- 404..
- Liu, W., Yin, D., Li, N., Hou, X., Wang, D., & Li, D. (2016). Influence of Environmental Factors on the Active Substance Production and Antioxidant Activity in *Potentilla fruticosa* L. and Its Quality Assessment. *Scientific Report*, 6, 28591.
- Marrone, G. F. G., Vaccaro, M., Biolato, L., Miele, A., Liguori, C., Araneo, F. R., Ponziani, N., Mores, A., & Gasbarrinit, A. (2017). Drug-induced liver injury, the diagnosisis not easy but always to keep in mind. *European Review of Medical and Pharmacological Sciences*, 21, 122-134.
- Messaoudi, D. (2017). Hepatoprotective effect and antioxidant properties of *Santolina chamaecyparissus*. Doctoral thesis, Ferhat Abbas Sétif 1 University, Algeria, 101 p.
- Mishra, V., Soren, A. D., & Yadav, A. K. (2021). Toxicological evaluation of betulinic acid and ursolic, common constituents of *Houttuynia cordata* used as an anthelmintic by the Naga tribe in North-east India. *Future Journal of Pharmaceutical Sciences*, 7, 39.
- Mondal, M., Hossain, M., Hasan, R., Tarun, T.I., Islam, A. F., Choudhuri, M. S. K., Islam, T. M., & Mubarak, M. S. (2020). Hepatoprotective and Antioxidant Capacity of *Mallotus repandus* Ethyl Acetate Stem Extract against D-Galactosamine-Induced Hepatotoxicity in Rats. *ACS Omega*, 5(12), 6523-6531.
- OECD. (2021). Guidelines for the testing of chemicals, revised draft guidelines 423; acute oral toxicity-acute toxic class method, revised document, 14p.
- Okokon, J. E., Simeon, J. O., & Umoh, E. E. (2017). Hepatoprotective activity of the extract of *Homalium letestui* stem against paracetamol-induced liver injury. *Avicenne Journal of phytomedicine*, 7(1), 26-36.
- Ouattara, A., Kpan, W.B., Ouattara, D., Kone, M. W., & Komoe, K. (2021). The acute toxicity of *Sargassum fuiltans* (Børgesen) Børgesen and *Sargassum natans* (Børgesen) Børgesen on some rats of wistar stock. *Journal of Animal and Plant Sciences*, 47 (3), 8542-8551.
- Patel, S. S., Soni, H., Mishra, K., & Singhai, A. K. (2011). Recent updates on the genus Passiflora: A review. *International Journal of Research in Phytochemistry and Pharmacology*, 1(1), 1-16.
- Rahman, A., Hossain, A., Hasan, S., & Hossain, M. (2011). Antinociceptive, antidiarrhoeal and cytotoxic activities of *Passiflora foetida. Pharmacologyonline*, 1, 228-236.
- Rajguru, J., & Mitesh, R. D. A. (2017). Study on the morphological variations of the human liver and its clinical implications. *National Journal Integrated Research in Medicine*, 8(5), 75-78.
- Ramasamy, A., Balasundaram, J., Sundararaman, J., Rajappan, M., & Raju S. K. (2009). Effect of fruits of *Passiflora foetida* Linn on CCl4-induced hepatic injury in rats. *Journal of Pharmaceutical Research*, 2(3), 413-415.
- Rotundo, L., & Pyrsopoulos, N. (2020). Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. *World Journal of Hepatology*, 12(4), 125-136.
- Sahu, S. (2007). Hepatotoxicity. From Genomics to *in vitro* and *in vivo* Models. John Wiley & Sons Ltd, pp 191-635.
- Sanchez-Marzo, N., Pérez-Sánchez, A., Ruiz-Torres, V., Martínez-Tébar, A., Castillo, J., Herranz-López, M., & Barrajón-Catalán, E. (2019). Antioxidant and Photoprotective Activity of Apigenin and its Potassium Salt Derivative in

Human Keratinocytes and Absorption in Caco-2 Cell Monolayers. *International Journal of Molecular Sciences*, 20(9), 2148-2160.

- Sathish, R., Sahu, A., & Natarajan, K. (2011). Antiulcer and antioxidant activity of ethanolic extract of *Passiflora foetida*. *Indian Journal of Pharmacology*, 43(3), 336-339.
- Schmelzer, G. H., & Gurib-Fakim, A. (2008). Ressources végétales de l'Afrique tropicale 11(1). Plantes médicinales 1. [Traduction de: Plant Resources of Tropical Africa 11(1). Medicinal plants 1. 2008]. Fondation PROTA, Wageningen, Pays-Bas / Backhuys Publishers, Leiden, Pays-Bas / CTA, Wageningen, Pays Bas, 869 p.
- Subramanian, M., Balakrishnan, S., Chinnaiyan, S. K., & Kumar, S. V. (2013). Hepatoprotective effect of leaves of *Morinda tinctoria* Roxb. against paracetamol induced liver damage in rats. *Drug invention today*, 5(3), 223-228.
- Sultana, N., Sadia, C., & Hossain, M. (2016). Effects of ashwagandha (*withania somnifera*) root extract on architecture of liver tissue against gentamicin induced hepatotoxic rats. *International Journal of Pharmacy and Biological sciences*, 7(3), 2016, 78 – 82.
- Tafere, G. G., Tuem, B. K., Gebre, K. A., & Balasubramaniam, R. (2020). In vitro antioxidant and in vivo hepatoprotective activity of root bark extract and solvent fractions of *croton macrostachyus* Hochst. Ex Del. (Euphorbiaceae) on paracetamol-induced liver damage in mice. *Journal of experimental pharmacology*, 12, 301-311.
- Taj, D., Tariq, A., Sultana, V., Ara, J., Ahmad, V. U., & Ehtesamul-Haque, S. (2019). Protective role of *Stokeyia indica* in liver dysfunction and associated complication in acetaminophen intoxicated rats. *Clinical Phytosciences*, 5, 28.
- Thapa, B. R., & Walia, A. (2007). Liver function tests and their interpretation. *Indian Journal of Pediatrics*, 74, 663-671.
- Thompson, M., Yogini, J., Ilya, W., & Wi, L. (2017). Hepatotoxicity: treatment, causes and applications of medicinal plants as therapeutic agents. *Journal of Phytopharmacology*, 6(3), 186-193.
- World Bank. (2018). Poverty and Shared Prosperity 2018. Piecing together the poverty puzzle. www.worldbank.org.
- Yahya, F., Mamat, S. S., Kamarolzaman, M. F. F., Seyedan, A. A., Jakius, K. F., Mahmood, N. D., Shahril, M. S., Suhaili, Z., Mohtarrudin, N., Susanti, D., Somchit, M. N., The, L. K., Salleh, M. Z., & Zakaria, Z. A. (2013). Hepatoprotective Activity of Methanolic Extract of *Bauhinia purpurea* Leaves against Paracetamol-Induced Hepatic Damage in Rats. *Evidence-based complementary and alternative medicine*, article ID 636580, 10p. https: //doi.org/10.1155/2013/636580.

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