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Full Length Research Paper

Effect of pineapple (*Ananas comosus*) on haematological and biochemical parameters in albinos Wistar rats intoxicated with Doliprane®

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The aim of this study is to evaluate the liver's protection of *Ananas comosus* on albinos Wistar rats intoxicated with Doliprane® which contained 20% of paracetamol. Four batches of 30 were used. The negative control group was not treated with Doliprane® and *A. comosus*. Batches no. 2 and no. 3 were treated for six weeks with Doliprane® (2 g per kg of body weight for each day). Each rat of batch 2, has received by cramming, 0.06 ml/kg of body weight for each day of the stalk against 0.12 ml per kg of body weight for each day for batch no. 3. The batch no. 4 has been addicted to Doliprane® (2 g / kg of body weight for each day) and was not treated with *A. comosus.* A haematological study plus determination of the serum level of glucose, triglycerides and cholesterol was carried out. Doliprane® induced a hypochromic anemia in the rats of batch 4. This anemia has been corrected with the stalk of *A. comosus* in the rats of batches no. 2 and no. 3. The serum level of glucose and triglycerides increased in the rats of batch no. 4 ($p < 0.0001$), but that of cholesterol decreased ($p < 0.0001$). The stalk of *A. comosus* protects the liver against haematological and biochemical aberrations induced by Doliprane®.

Key words: *Ananas comosus* (pineapple), hepato-protection, Doliprane®, toxicity.

INTRODUCTION

The lesions associated with acute doses of some drugs such as paracetamol constitute an important part and incidence, probably underestimated on the liver diseases (Gruchalla, 2000). Their detection and early etiological diagnosis are in the field of clinical practice and have a daily predict particular importance. Approximately 10% of all cases of acute hepatitis are caused by drug effects, as 40% of those occurring are after fifty years old (Sgro et al., 2003).

Women are nearly three times more frequently affected than men, and in cases of acute hepatitis, mortality or the likelihood of the need for a transplant is close to 20%. Half of fulminant hepatitis cases are as a result of drugs and according to the European transplant registry, it accounts for 6% indications of liver transplantation (Cullen and Mechanistic, 2005). The higher proportions in Scandinavian countries (5, 4%) and England (5%) were due to self-medication.

The liver, being the ''gate way'' as well as the centre of metabolism of drugs and other xenobiotics in the body, is perpetually predisposed to the effect of drugs and chemicals taken in consciously or accidentally. Hence in Africa, hepatotoxicity still ranks high as a threat to livelihood as many of the people, especially the illiterate ones, routinely engage in self-medication with drugs like paracetamol, Doliprane®, vitamin C, etc. This study therefore seeks to evaluate the cytoprotective effects of pineapple (*Ananas comosus*) in Doliprane®-induced liver

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Abbreviations: MCV, mean blood volume; **LD_{50},** half maximal lethal dose; **ATP,** adenosine triphosphate; **TGMH,** mean corpuscular hemoglobin.

damage in albinos Wistar rats.

MATERIALS AND METHODS

Animals

The study was conducted on male albinos Wistar rats weighing 130 - 150 g and treated with *A. comosus.*

Plants

A. comosus, a variety bread sugar, is produced in the Farm of Application of Agriculture College of Sekou, Department of the Atlantic.

Ananas comosus extract

The stalk of fresh pineapple was transformed to obtain the extract, using the technique of Park et al. (2001) in the Laboratory of Pharmacognosy and Essential Oils in Cotonou.

Preparation of Doliprane® for the induction of liver toxicity

Following the search for LD_{50} (4 g/kg PV), using Maurer's technic (2001), 2 g of Doliprane® PV per kg per day was administered to induce hepatotoxicity in the rats. This dose was dissolved in 2 ml of water and administered individually and orally to Wistar rats for 6 weeks.

Groups of animals

Batch 1 (30 rats): These animals served as negative control, receiving neither the Doliprane® nor *A. comosus* extract.

Batch 2 (30 rats): These were orally given Doliprane® (2 g per kg body weight) and treated with 0.06 ml / kg PV/ day of *A. comosus* extract for 42 days.

Batch 3 (30 rats): These were orally given Doliprane® (2 g per kg body weight) and treated with 0.12 ml / kg PV / day of *A. comosus* extract for 42 days.

Batch 4 (30 rats): These were orally given Doliprane® (2 g per kg body weight per day) for 42 days and have not received treatment with extract of *A. comosus*.

Study of chemical properties of *A. comosus*

The properties which are attributed as flavonoids biological effects of the extract of *Ananas comosus* are sought and separated by thin layer chromatography, carried out by the methods of Park et al. (2001).

Blood sample and assay haematological parameters

Starting from the tail vein, 2 ml of blood were collected in vacuum tubes with anticoagulant. The blood collected in tubes with anticoagulant (sodium citrate) was used in establishing the haemogram.

The analysis was performed haematologically with two milliliters of blood on EDTA (8.5%). The counting of leukocytes ($x10^3$/ml blood), red (10^6/ml blood), platelets (10^3/de blood) and hematocrit (%), hemoglobin (g / dl of blood), the average blood volume (MCV) (µm3), the average concentration in blood hemoglobin (CGMH in (mg/100 ml of red blood cells)) and grade average blood hemoglobin (TGMH in (pg / red blood cells)) were made using the S Coulter more. Counting the number of red blood leukocyte and balance were determined on the stained smears in May-Grünwald-Giemsa.

Biochemicals parameters

Biochemical assays were performed using commercial kits that were ready for use. Spinreact kits were used for the determination of glucose, but Biosystems kits were used for the determinations of triglycerides and cholesterol.

Statistical methods

After treatment, analysis of variance was carried out using the Software Statistica 6.0 (1998). If there are significant differences, the student t test Newman-Keuls was used.

RESULTS

Haematological study

The haematologic parameters have addressed the following parameters: haematocrit, erythrocyte numbers, haemoglobin, white blood cells numeration, leukocyte formula, average blood volume (MCV), mean blood concentration haemoglobin (CGMH) content and average blood haemoglobin (TGMH). The values of haematocrit Wistar rats ranged from 41.12 ± 0.11 in animals of batch 1 to 21.90 for those in batch no. 4 through intermediate values in rats of batches no. 2 and no. 3 ($p < 0.0001$).

A numeration of the erythrocyte introduced important changes and this varies from 7.66 ± 0.04 in animals of batch no. 1 to 4.54 ± 0.02 in those of batch no. 4 ($p < 0.0001$). The number of red blood cells was significantly higher in rats of batches no. 2 and no. 3 when compared to that of batch no. 4 ($p < 0.0001$). The maximum haemoglobin concentration was observed in rats of batch 1 (12.62 ± 0.01), while the lowest was among those of batch no. 4 (7.6 ± 0.009) ($p < 0.0001$). Intermediate values were found in rats of batches no. 2 and no. 3 ($p < 0.0001$).

The lowest value of 5.25 ± 0.02 for leukocytes was found in rats of batch no. 4 against 6 ± 0.01, 6 ± 0.02 and 6.60 ± 0.02 for animals of batches no. 3, 2 and no. 1 ($p < 0.0001$), respectively. The lower values of neutrophils, lymphocytes and platelets were found in rats of batch no. 4 ($p < 0.0001$).

MCV values were significantly lower for rats of batch no. 4 (48.31 ± 0.37) when compared respectively to those of batches no. 1, no. 2 and no. 3 ($p < 0.0001$). The average concentrations in blood hemoglobin (CGMH) have been fluctuating with the lowest value of 24.55 ± 0.19 in rats of batch no. 4 ($p < 0.0001$). The average content in blood haemoglobin varied between batches, while the highest value of 16.67 ± 0.008 was achieved in rats of

Table 1. Haematological parameters of Wistar rats intoxicated with Doliprane® and treated with stem of *A. comosus.*

Treatment	GR (10^6 /mm^3 of blood)	GB (10^3 /mm^3 of blood	Hb (g/dl)	Hte (%)	VGM (%)
Batch no. 1	7.66±0.04 a	6.60±0.02 a	12.62±0.01 a	41.12±0.11 a	53.75±0.38 a
Batch no. 2	6.45±0.01 b	6.04±0.02 b	10.19±0.01 b	34.83±0.14 c	54.04±0.27 a
Batch n° 3	6.86±0.01 c	6±0.01 b	10.19±0.02 b	36.47±0.13 b	53.16±0.21 a
Batch n° 4	4.54±0.02 d	5.25±0.02 c	7.56±0.009 c	21.90±0.12 d	48.31±0.37 b
Probability	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***
cv%	2.23	2	0.88	2.06	3.35

***Highly significant difference; GR: Red blood cells (10^6 / mm^3 of blood); GB: white blood cells (10^3 / mm^3 of blood); Hte: hematocrit (%); VGM: Volume globularia average (fL); Hb: haemoglobin (g / dl).

Table 2. Haematological parameters of Wistar rats intoxicated with Doliprane® and treated with stem of *A. comosus.*

Treatment	CGMH (10^3/mm^3 of blood)	TGMH (pg/ blood cell)	L (%)	N (%)	P (10^3/mm^3 of blood)
Batch n° 1	30.70±0.08 b	16.48±0.10 a	39.87±0.92 a	67.70±0.24 a	1071.03±0.24 a
Batch no. 2	29.26±0.13 c	15.80±0.04 b	37.83±0.63 b	62.63±0.35 b	961.50±0.36 c
Batch no. 3	27.95±0.14 d	14.85±0.05 c	37.63±0.08 b	59.93±0.13 c	982.0±2.50 b
Batch n° 4	34.55±0.19 a	16.67±0.08 a	25.97±0.25 c	51.90±0.27 d	850.47±0.09 d
Probability	<0,0001***	<0,0001***	<0,0001***	<0,0001***	<0,0001***
cv%	2.53	2.52	8.89	2.37	0.72

***Highly significant difference; L: lymphocytes (%); N: neutrophils (%); P: platelets (10^3/mm^3 de sang); CGMH: Midlle concentration globular haemoglobin (g/l); TGMH: Midlle rate globular haemoglobin (pg/ blood cell).

Table 3. Concentrations of glucose, triglycerides and cholesterol on the Wistar rats.

Batches	Biochemical parameters		
	Glucose (g/L)	Triglycerides (g /L)	Cholesterol (g/L)
Batch no. 1 (negative)	0.99 ± 0 .10c	0.50 ± 0.01d	0.63 ± 0.01a
Batch no. 2 (low treatment)	0.99 ± 0.00b	0.58 ± 0.02c	0.45 ± 0.02c
Batch no. 3 (high treatment)	0.99 ± 0.00b	0.61 ± 0.02b	0.48 ± 0.00b
Batch no. 4 (non treated)	1.28 ± 0.13a	0.71 ± 0.00a	0.32 ± 0 .02d
C V	7.82	3.05	3.74
P	***< 0.0001	***<0.0001	***<0.0001

***Highly significant difference.

batch no. 4 (p <0001). The haematologic parameters are expressed in Tables 1 and 2.

Biochemical parameters

The levels of glucose, triglycerides and cholesterol on rats addicted with Doliprane® and treated with *A. comosus* were investigated. The rate of glucose increased on rats of batch no. 4 ($p < 0.0001$). However, significant difference was noticed with the rats of batches no. 2 and no. 3. The levels of triglycerides increased in animals of batch no. 4 compared to those of batch no. 1 ($p < 0.0001$), but the difference is very significant between the rate of triglycerides of batches no. 2 and 3 ($p < 0.0001$). However, the cholesterol level decreased more in rats of batch no. 4 than those of batch 1 ($p < 0.0001$). The same situation was noticed with the batches that received the treatment based on *A. comosus* ($p < 0.0001$) (Table 3).

DISCUSSION

Haematological study

The number of red blood cells obtained in animals of batch no. 4 is very low compared to the results of Boukerche et al. (2004). These authors have found $8.45.10^6$ / mm^3 as the number of red blood cells in healthy Wistar rats. The Doliprane® influenced the number of red blood cells causing anemia in the intoxicated Wistar rats. Indeed, paracetamol induces serious liver dysfunction

and abnormal formation of blood cells including red blood cells, leukocytes and platelets in rats intoxicated (Kurtovic and Riordan, 2003). The numbers of red blood cells in rats, not intoxicated with Doliprane®, are stable and vary little from one subject to another. Even if this stability seems to be a characteristic of healthy Wistar rat, the number of red blood cells obtained in rats of batch no. 1 in this study is slightly higher than the value of $7.1.10^6$ / mm^3, proposed by Lahouel et al. (2004).

In the rats intoxicated with Doliprane® and treated with *Ananas comosus*, there is a progressive cell regeneration remarkable in rats of lot no. 3. A similar result was observed by Lahouel et al. (2004) in Wistar rats intoxicated with 'paracetamol' and treated with propolis. Similar results were obtained by Vialia (1998) regarding hemoglobin and hematocrit. About the white blood cells and platelets, changes have been observed particularly in rats of batch no. 4 in which the number was lower. These low values are linked to the toxic action of 'paracetamol' which can induce leukopenia and thrombocytopenia in cases of severe liver dysfunction already demonstrated by the study of Moling et al. (2006). The significant decrease of haemoglobin in rats of batch no. 4, combined with a significant decrease of MCV and a concomitant decline of CGMH and TGMH, indicate a trend towards hypochromia.

This decrease of blood cells was significantly corrected in rats of lots no. 2 and no. 3 following the action in favor of *A. comosus*. Indeed, this fruit contains flavonoids compounds identified by the phytochemical screening of pineapple in this study. As a result, the pineapple has a potential anti-oxidant and its high content of vitamin C would also contribute to a little (over 30%) of its antioxidant potential (Szeto et al., 2002). This property of *A. comosus* promotes its favorable action on the liver in regulating hematopoiesis.

The decrease in hemoglobin has been proven by Lahouel et al. (2004) in rats addicted to paracetamol. Paracetamol could inhibit the synthesis of heme in red blood cells and cause signs of anemia described by Bottomley and Muller-Eberhard (1998). These signs are offset by the beneficial effect of *A. comosus*. However, red blood cells remain poor heme, hence the decline in CGMH, already indicated by the study's results in rats of batch no. 4. These erythrocytes also increase in the blood before they complete maturation so MCV decreases.

Concentration of glucose, triglycerides and cholesterol in rats intoxicated with Doliprane® and treated with the extract of the stem of *A. comosus*

The biochemical parameters are of paramount need to assess many diseases. They can evaluate the possible effect of a toxic agent on the physiological functions of the organism (Smaoui et al., 2000). In addition, the liver is the main organ involved in the metabolism of glucose (McGeown, 2003). The results of this study indicate an increased level of glucose in rats of batch no. 4. This could be explained by the nature, metabolism and dose of Doliprane®. The increase of glucose in rats of batch no. 4 could objectify impairment of liver metabolism. This result is confirmed by the work of Knobeloch et al. (2000), because the cytotoxicity of Doliprane® creates an ionic imbalance and the need for ATP can be explained by higher blood glucose levels.

In this study, the number of red blood cells significantly decreased in rats intoxicated with Doliprane® and untreated by *A. comosus*. Before the attack, which is linked to the toxicity of paracetamol, the red blood cell develops its own defense mechanism that requires ATP provided mainly by the erythrocyte glucose. It is therefore believed that achieving the integrity of red blood cells and release of glucose are linked to the toxicity of 'paracetamol'. Then, the red blood cells have lost their main source of energy and are unable to activate their defense system. However, the blood glucose level of batches no. 2 and no. 3 had been reduced, but has not reached that of the rats of batch no. 1. The gradual restoration of glucose metabolism is related to the favorable action in the liver of *A. comosus* on rats addicted to Doliprane®. Indeed, after screening, this fruit contains phytochemical elements established in this study like tannins. Tannins are astringent substances and are used for healing and anti-bleeding because they have a strong affinity for proteins they make hastily. As polyphenols, they are recognized for their effective anti-oxidant and anti-degenerative (Porter, 1989). The results also reveal a clear increase in the concentration of triglycerides in rats of batch no. 4. This can be explained by weathering of lipids metabolism. In all attacks by xenobiotics, the metabolism of fat liver is amended to the production of defense systems and to neoglucogenese (McGeown, 2003).

Contrary to the concentrations of the previous biochemical parameters, cholesterol levels fell significantly in rats of batch no. 4. The pronounced drop of cholesterol is not in accordance with the results of Boukerche et al. (2004). The alteration of lipid metabolism in the liver would have induced liver failure relayed by hypocholesteremia following the cytolysis in the liver and hepatocyte necrosis previously reported by Dougnon et al. (2006) .The presence of bromelain in *A. comosus* and notified by Maurer (2001), could also contribute to the hepatoprotection of this fruit.

Conclusion

This study has shown an increase in blood glucose and triglycerides levels, followed by a drop in cholesterol level, in Wistar albino rats treated with Doliprane®, indicating that the drug was acutely toxic. This was confirmed by

hematological parameters that revealed a hypochromic anemia in the intoxicated rats. These different biochemical and haematological changes were, however, markedly ameliorated upon administration of pineapple (*A. comosus*) extract.

REFERENCES

Bottomley SS, Muller-Eberhard V (1998). Pathophysiology of the heme synthesis. Pemin. Hematol. 25: 282-303.

Boukerche S, Aouacheri W, Saka S (2004). Les effets toxiques des nitrates: étude biologique chez l'homme et chez l'animal. Ann. Biol. Clin. 65(4): 385-391.

Cullen J, Mechanistic M (2005). Classification of liver injury. Toxicol. Pathol. 33: 6-8.

Dougnon TJ, Kpodékon TM, Lalèyè A, Adjagba M, Darboux R (2006). Effets de *Ananas comosus*, variété pain de sucre" sur les lésions hépatiques dues à l'intoxication au paracétamol chez le rat Wistar. Société de Biologie Clinique; 10: 47-50.

Gruchalla RS (2000). Clinical assessment of drug-induced disease. Lancet, 356: 1505-1511.

Knobeloch L, Palna B, Hogan A, Postele J, Anderson H (2000). Blue baies and nitrate contaminated well water. Environ. Heal Persp. 108: 675-678.

Kurtovic J, Riordan SM (2003). Paracetamol-induced hepatotoxicity at recommended dosage. J. Int. Med. 253: 240-243.

Lahouel M, Ouadi O, Khiari N (2004). Effets hépatique et hématologique du paracétamol. Saidal info. 7 : 60-66.

Maurer HR (2001). Bromelain: biochemistry, pharmacology and medical use. Cell Mol. Life Sci. 58(9): 1234-1235.

McGeown JG (2003). Physiologie. L'essentiel. *Editions Maloine,* Paris. pp. 226-229.

Moling O, Cairon E, Rimenti G (2006). Severe hepatotoxicity after therapeutic doses of acetaminophen. Clin. Ther. 28: 755-60.

Porter LJ (1989). Tannins in Methods in Plant Biochemistry (plant phenolics), éds. PM Dey and JB. Harborne. Academic Press; London, pp. 389-419.

Sgro C, Clinard F, Ouazir K (2003). Incidence of drug-induced hepatic injuries: a French population-based study. Hepatol. 36: 451-455.

Smaoui M, Yhorbel F, Boujelbene M, Makni-Ayadi F, El Féki A (2000). Impact de l'exposition chronique au gaz d'échappement d'origine automobile sur certains biomarqueurs touchant la fonction hormonale sexuelle mâle, la fonction rénale et l'hémogramme chez le rat. Pollut. Atmosphérique, 167(2): 439-449.

Szeto Y, Tomlinson TB, Benzie IF (2002). Total antioxydant and ascorbic acid content of fresh fruits and vegetables: implications for diertary planning and food preservation. J. Nutr. 87: 55-59.

Vialia A (1998). Eléments de toxicologie. Editions médicales internationales. Paris. pp. 25-28.