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Hepato-Protective Effect of *Curcuma longa* against Paracetamol-Induced Chronic Hepatotoxicity in Swiss Mice

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Abstract

*Curcuma longa*L. (*Zingiberaceae*), a natural spice, has been usually used in Algeria to treat gastrointestinal and liver disorders. This study aims to evaluate protective and anti-inflammatory properties of aqueous extract of *C. Longa*L.rhizome against hepatic damages induced by Paracetamol. The mice were divided into four groups (n=11), the hepatotoxicity was induced in mice by oral administration of acetaminophenat the last seven weeks. The aqueous extract was also administered daily for 14 weeks with subjected of Paracetamol, the negative control group, and treated group with turmeric extract. Histopathological study of the liver and several serum markers as serum albumin, gamma GT, blood glucose and transaminases (ALT and AST) were analyzed. The results of biochemical parameters revealed increasing levels in ALT (108.54U/L), AST (256.07U/L), and serum albumin (31.2g/L) in treated intoxicated group compared to Paracetamol intoxicated group. Thus, the results demonstrated decreasing in levels of glycemia (0.3 g/L) and gamma GT (134.20 U/L).Moreover, the liver sections revealed macroscopically significant lesions, (hepatic necrosis) bloating and hydropic lesions, vacuolization and steatosis in intoxicated mice. On the other hand, these lesions are less important in the treated group with only turmeric. Animals were observed for any symptoms of toxicity after administration of extract to ensure its safety. This results show that the extract of *C. longa* has hepatoprotective potential that could be partly attributed to developed as drugs for the treatment of liver diseases.

Keywords: *Curcuma longa*; Paracetamol; mice; hepatotoxicity; hepato-protective effect.

1. Introduction

Paracetamol, known as acetaminophen or APAP, is one of the most commonly used oralanalgesics and antipyreticsdescribed in the 1960 in the USA (Yoon *et al*., 2016). It has an excellent safety profile when administered in proper therapeutic doses, but excessive use causes Paracetamol poisoning and generates liver damages(Woolley and Woolley, 2017).In the United States and the United Kingdom, Paracetamol is the most common cause of acute liver failure (Ryder and Beckingham, 2001; Ferri, 2016). The toxic dose of paracetamol is highly variable; in general the recommended maximum daily dose for healthy adults is 4 grams(Freifeld*et al.*, 2014; Twycross *et al.*, 2017). In the first 24 hours following overdose, usually 7g per day (Ferri, 2016; Woolley and Woolley, 2017) people have few or nonspecific symptoms, like abdominal pain or nausea, yellowish skin, blood clotting problems, and confusion occurs (Podolsky*et al*., 2016; Yoon*et al*., 2016). Additional complication may include pancreatitis, low blood sugar and lactic acidosis paracetamol toxicity can cause death without treatment after two weeks of exposure(Freifeld*et al.*, 2014; Yoon*et al.*, 2016).There are risk factors which can increase this toxicity, like alcoholism, malnutrition and certain other medications (Ferri, 2016). Paracetamol poisoning is the most common cause of acute liver failure that results not from paracetamol itself, but from one of its metabolites, N acetyl–*p*-benzoquinone imine (NAPQI) (Webb *et al.*,2016) which decreases the liver's glutathione and directly damages hepatocytes(Prout*et al.*, 2014).

Curcuma longa(*Zingiberaceae*)is a rhizomatous perennial herb; the common name of this species is turmeric (Kurkum in Algeria), original from to South Asia especially India and Malaysia(Akram*et al.*, 2010; Amel, 2015). It is commonly known as a spice and pigment for preparing culinary dishes (Delaveau, 1987; Ghosh *et al.*, 2011).This plant species is well-known in India for its therapeutic properties; its rhizome juice is used are taken orally for the treatment of many diseases such as liver problems, gastrointestinal disorders, asthma, bronchitis, gonorrhea and urinary disorders, or as antihelmintic(Perry *et al.*, 2010). In addition; its essential oils including tumerone, atlantone, and zingiberone are used in the treatment of carminative, stomachic and tonic (Yadav *et al.*, 2017). The anticancer, antidiabetic, antioxidant, anti-inflammatory, and antimicrobial activities of aqueousextracts obtained from of the rhizome of *C. longa* were previously reported (Amel, 2015).The turmeric is used in combination with other plants or spices in the food and medical fields. Hence, a mixture of the juice of this plant with dried root of *Pandanus odoratissimus* Linn. f.and water, taken early in the morning orally for 1-week can be used to remedy urinary disorders (Mishra *et al.*, 2015).

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The bright yellow color of turmeric originates mainly from polyphenolic pigments known as curcumin(diferuloylmethane) (Lim *et al*., 2001). Curcumin, the principal curcuminoid found in the rhizome of turmeric, is generally considered its most active compound. Several published studies showed that curcumin has various therapeutic properties, like anticancer effect involving in inhibiting the growth of several different types of cancer, antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective and neuroprotective activities (Lim *et al.*, 2001). The curcumin also exhibited the ability to block NF-κB and the mutagenic response in *Helicobacter pylori*-infected epithelial cells (Sarkar *et al.*, 2016).

The present study was undertaken to assess the hepatoprotective effect of aqueous extract of *C. longa* rhizomes administrated with paracetamol overdoses induced liver damage in mice, represented by blood transaminases (TGO and TGP), albumin, total protein and sugar levels, confirmed by the histological study of mice's liver. Treatment of mice with only the aqueous extract of *C. Longa*at concentration of 2 mg/kg per day is examined for the first time.

2. Materials and Methods

2.1. Chemicals

All chemicals and reagents were used of analytical grade. Assay kit for serum aspartate aminotransferase and alanine aminotransferase were taken from Dialab, Austria, and Paracetamol (PCM) from Sigma-Aldrich USA.

2.2. Plant Material and Plant Extract Preparation

Turmeric dried rhizomes was purchased from alocal market, Algeria. Species identification was performed by botanical Professors of the University of Mostaganem (Algeria).The rhizomes were cleaned, dried, and homogenized in distilled water at a ratio of 1:10 of plant to water and left macerated for 6 hours at 25°C with occasional shaking and stirring. The mixture was then filtered and concentrated, and the resulting liquid was orally administrated to animals with final concentration as 2mg/kg per day.

2.3. Animals

Forty four healthy adult mice, aged of 30 ± 2 days and weighing from 35−40 g, were obtained from Pasteur Institute of Algeria. They were maintained at room temperature (25.5°C) with a 12 h light/dark cycle, and have been given a commercial pellet diet 18 g/day/mouse (obtained from Algerian National Food Office of Cattle) and fresh drinking mineral water.

2.4. Hepatoprotective Assay

The *in vivo* hepatoprotective activity of aqueous extract of turmeric rhizome was determined using the Paracetamol-induced hepatotoxicity test in mice, as previously described by Jarsiah *et al.* (2018) with minor modifications.

The mice were randomly divided into four experimental groups, containing eleven mice in each group. The first group received mineral water and served as negative control group (C). Group II serving as Paracetamol intoxicated group(P) received100mg/kg at the first seven weeks increased to 200mg/kg at the last seven weeks. Group III serving as Paracetamol-treated group received paracetamol poisoning doses same as Paracetamol intoxicated group and treated by turmeric extract (P+T) at a dose of 2 mg/kg which showed no signs of toxicity in mice. Finally, Group IV serving as treated turmeric group(T) received 2mg/kg. These doses were administrated orally by force-feeding.

The period of experimentation was 14 weeks under standards laboratory conditions. The animals received the corresponding dose of the respective test solution daily for 14 consecutive weeks. 72 h after the end of experimentation, mice were scarified by decapitation (chloral 3%).

The blood was collected in sterilized centrifuged tubes which were then centrifuged to get serum for biochemical studies. The animals were then sacrificed by cervical dislocation and the liver was removed and dissected out for histopathological studies.

2.5. Determination of Liver Function and Biochemical Parameters

The collected serum was tested according to the standard liver enzymes determination assays comprising glucose, albumin, gamma GT, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were measured using automat COBAS 6000 analyzer.

2.6. Histopathology

The liver tissue was dissected out and fixed in the 10% neutral buffered formaldehyde for 24 hours, dehydrated in gradual ethanol (50–100%), cleared in xylene, and surrounded by paraffin wax. The sections, which were 4-5µm thick, were then prepared using rotary a Leica microtome thickness (Leica RM 2125 RTS, Singapore) and stained with hematoxylin and eosin dye 1% for 40 seconds for microscopic observation of histopathological changes in the liver. Next, the liver sections were scored and evaluated according to the severity of the hepatic injury including fatty changes, cell necrosis, lymphocytes, hyaline and ballooning degeneration as described by Jarsiah *et al.* (2018) with slight modifications.

2.7. Statistical Analysis

Statistical analysis data obtained are presented as mean ± standard error of mean (SEM). The data were performed using one-way analysis of variance (ANOVA) followed by LSD test with $\alpha = 0.05$. This treatment was carried out using the SPSS v. 23.0 program (IBM Corp., Armonk, New York, USA).

3. Results and Discussion

3.1. Determination of liver function and biochemical parameters

Oral administration of paracetamol (100 and 200 mg/kg) for 2 weeks indicated anelevation in serum liver enzymes such as alanine transaminase[Fig. 1-a],aspartate transaminase[Fig.1-b], gamma GT[Fig. 1-c], blood glucose level[Fig. 1.d], and albumin [Fig. 1.e] compared with the control group. All of these results were ameliorated by coadministration of turmeric aqueous extract. Therefore, These findings may be in concord with many other studies, thus the obtained results were similar with that acquired by of Jarsiah*et al.* (2017) after intraperitoneal injection of 70.150 and 300 mg/kg of APAP to albino Wistar rats, the

overuse of acetoaminophen in mice and rats can caused severe and extensive necrosis cells (liver damages), and increased serum ALT/AST levels in rats. The ameliorative effect of curcumin and its derivates against liver injury induced by several drugs, such as paracetamol (Girish *et al*., 2009) has been reported. The results attained by Somchit*et al.*(2005), suggested that ethanolic extract of *C. longa* (100 mg kg^{-1}) has a potent hepatoprotective effect against paracetamol-induced liver damages in rats at 600 mg kg^{-1}expressed by lowered serum liver enzyme activities. Also, Salama *et al.*(2013) reported the hepatoprotective effect of ethanolic extract of this species at 2 and 5 g/kg on thioacetamide induced livercirrhosis in rats with low levels of liver biochemistry.

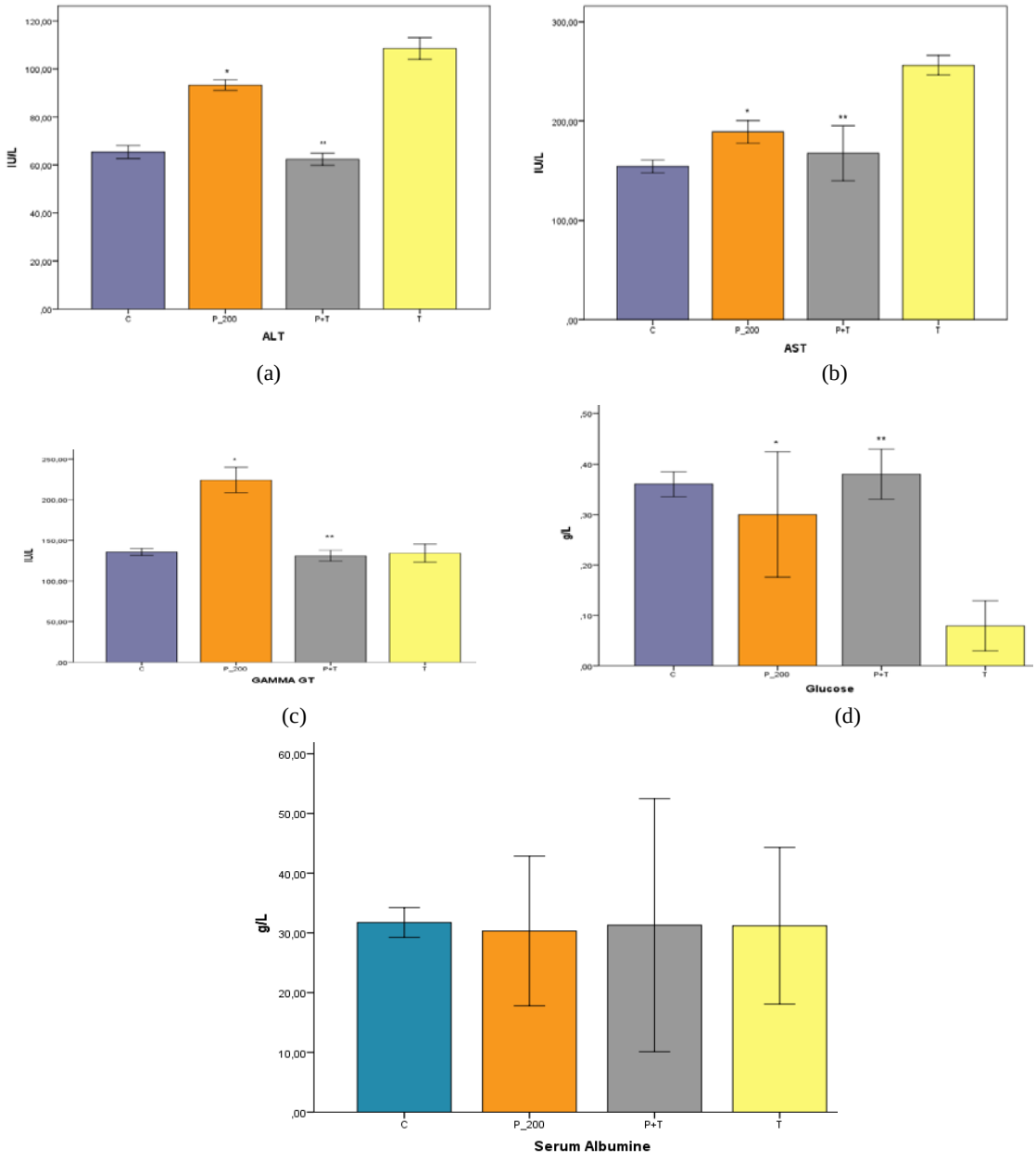

Figure 1. biochemical parameters levels; (a) ALT, (b) AST, (c) Gamma GT, (d) Glucose, and (e) albumin in serum mice of different experimental groups tested for 14 weeks.

The most frequently used preclinical species for drugs hepatotoxicity are rats and mice. The severity of the overall liver injury is very similar between mice and humans (McGill *et al.*, 2012). However the injury process progresses much faster in mice than in humans, with peak transaminases (ALT and AST) values, as indicator of liver cell death, between 12 - 24 h in the mouse (McGill *etal.*, 2013) and 36-48 h in humans after overdose (Lee, 2013). The mouse model of APAP hepatotoxicity is superior to other animal's model and most closely resembles the human pathophysiology in terms of liver injury and recovery. In the present study, the results showed an increased serum ALT/AST and GT levels in mice, which are proved byRay *et al.* (1996). An elevation of glucose level in serum of intoxicated group was observed compared to control mouse that may be caused by oxidative stress and NAPQ effect. The administration of phytosome curcumin effectively suppressed paracetamol-induced liver injury evidenced by a reduction of lipid peroxidation level, and elevated of enzymatic antioxidant

activities of superoxide dismutase, catalase, glutathione peroxidase in mice liver tissue (Tung *et al.*, 2017).

The Results showed that curcumin exerts remarkable protective, anti-inflammatory and therapeutic effects of oxidative associated liver diseases against hepatotoxicity induced by paracetamol (**Watkins and Seeff, 2006**). The ameliorative effect of curcumin and its derivatives against liver injury induced by several drugs, such as paracetamol has been reported(**Nabavi*et al.*, 2014**)

In the present study, the results were confirmed by other research, which conclude that the antioxidant ability of curcumin and its derivatives is shown to be the main protective mechanism against drug induced liver damages (**Negi *et al.*, 2008**). Oetari*et al.* (1996) demonstrated that curcumin is a strong inhibitor of cytochrome P450; it can also normalize antioxidant enzymes and amelioratedacetoaminophen induced liver damage.

3.2. Histopathology

The histological observations of control mice did not show any histological alterations in the hepatocytes.The liver sections showed normal structure with no damage in the central vein and no change in sinusoids and hepatocytes architecture [Fig.2,3,and 4]. In toxic control group, the liver sections showed hepatocytes necrosis with strict damage associated with central vein due to paracetamol .

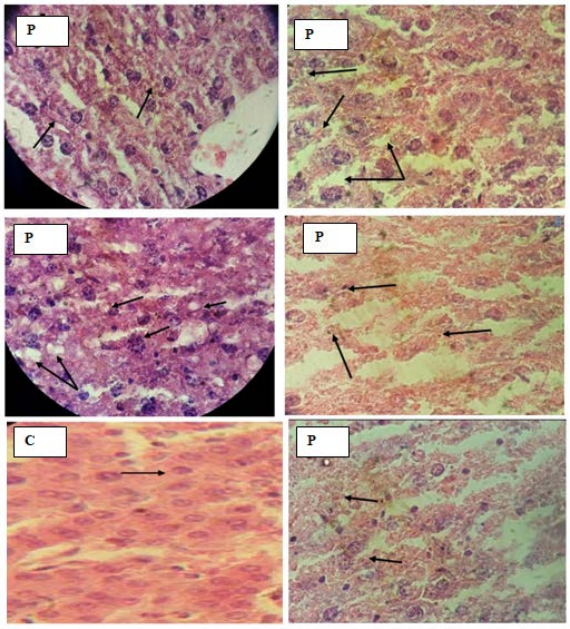

Figure 2. Microscopic study of liver tissue performed by staining (H&E) in mice, (P) intoxicated group with two doses of paracetamol (100 mg/kg, 200 mg/kg) compared with control group (C) (G×100). The black arrows indicate hepatocytes necrosis and strict and severe damage in intoxicated mice tissue, normal structure with no damage in control mice tissue.

In group treated with turmeric aqueous extract, the liver sections showed minor to moderate diffuse granular degeneration and necrosis in liver cells, reduced damage and regeneration of hepatocytes, bile duct, branch of hepatic portal vein and minimal necrosis caused by paracetamol intoxication .

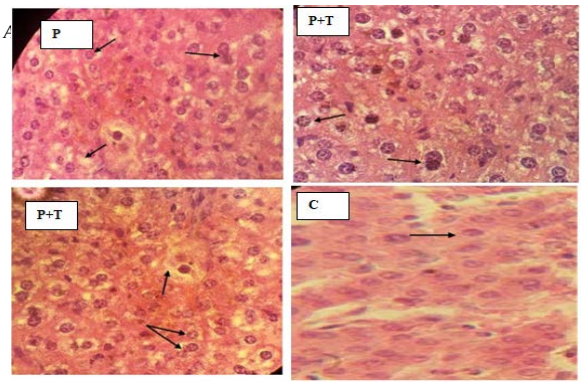

Figure 3. Microscopic study of liver tissue performed by staining (H&E) in mice, (P) intoxicated group with two doses of paracetamol (100 mg/kg, 200 mg/kg) and treated by curcumin (2 g/kg) compared with control group (P+T) (G×100). No specific alterations or damage in liver tissue. The black arrows indicate a best regeneration of liver tissue (cell's mitosis)

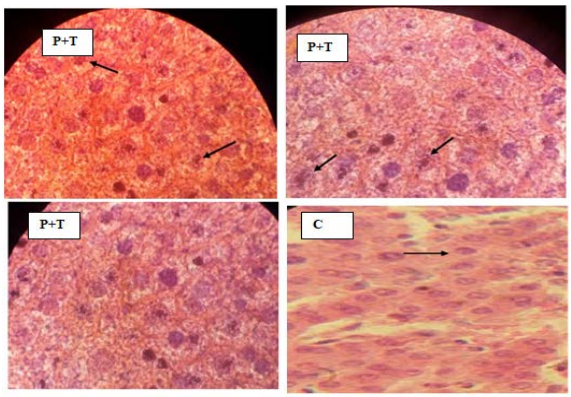

Figure 4. Microscopic study of liver tissue performed by staining (H&E) in mice, (P+T) treated control group with curcumin (2 g/kg) compared with control group (C) (G×100). The black arrows indicate normal structure of liver tissue compared with control.

Histopathology of the liver in control group and treated mice by *C.longa*at a dose of 2g/kg, mice showed no specific pathological changes. Effects of acetoaminophen began to appear from doses of 100mg/kg and 200mg/kg: presence of steatose and different lesions appear mild hyperemia oedema of the portal area and mild infiltration of inflammatory cells and apoptosis were observed, similar results with others studies (Jarsiah *et al.*, 2017), less important in treated group by curcumin.Our histopathological study of APAP toxicity shows obvious difference between the treated poisoned group and the poisoned one. Histopathology and liver biochemistry were significantly lower in the *C. longa* treated groups with a dose of 2mg/kg compared with controls. Similar results were obtained with ethanolic extract of *C.longa* rhizome by Salama *et al.* (2013). Many reports indicated that curcumin induced apoptosis and inhibited hepatocytes proliferation (Wang *et al*., 2012). The progression of liver damages caused by acetaminophen or paracetamol could be inhibited by the antioxidant activities of curcumin and the normal status of the liver could be preserved (Salama *et al.*, 2013). The model of cell death during paracetamol hepatotoxicity is controversial. Animal studies have concluded that the injury is caused by necrosis, an increasing number of reports suggests that apoptotic cell death plays a significant role.

4. Conclusion

Overall, these outcomes suggest the protective role of aqueous extract of *C. longa* with a weak concentration in the prevention of paracetamol-induced hepatic toxicity in micecause hepatotoxicity by forming reactive metabolites, which were accompanied with a decrease of chemical parameters in hepatic tissues. Changes in serum liver levels might contribute the molecular mechanisms of the tested plant associated with oxidative modifications in liver tissue. Consequently, the protective effect of *C. longa* against liver damage induced by Paracetamol could be explained by its antioxidant properties. However, clinical studies are necessitated to examine such an effect in humans.

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