Evaluation Of Hepatoprotective Activity Of Aqueous Extract Of *Indian Costus Roots* On Paracetamol Induced Hepatotoxicity In Albino Mice

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Abstract: *Indian Costus* (Zingiberaceae) is widely employed in various traditional medicines. The present study is aimed at evaluating the hepatoprotective effect of aqueous extract of *indian costus* roots by paracetamol-induced liver damage in mice. The experimental mice were separated into five groups (n = 7 mice): group-A which kept as normal control group; group-B was treated with paracetamol (14 mg/kg b.w.) as therapeutic doses once a day intraperitoneal (IP) for four days ; group-C was treated with paracetamol (85 mg/kg b.w.) as overdoses once a day by IP administration for four days; ; groups-D and E received orally (10 µl) 10% *Indian costus* roots extract every day for eight days. On the fifth day , the animals of groups -D and E were treated with paracetamol (14 and 85 mg/kg b.w.) once a day for four days followed by IP administration. At the end of the experimental period, blood was obtained from each maus for the determination of serum levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Biochemical analysis of the serum obtained showed a significant increase in the levels of AST, ALT and ALP in mice administered with14 mg/kg b.w. and 85 mg/kg b.w of paracetamol. Pre-treatment of the animals with the extract caused a decrease in the levels of these enzymes. In parallel, the histopathological assessments of the liver sections of mice also proved that extract markedly minimized the PA toxicity and maintained the liver tissues.This study has shown that the aqueous extract of *indian costus* roots *a* possesses slight hepatoprotective property.

Keywords: *Indian Costus*, Zingiberaceae, Hepatoprotective activity, Paracetamol

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1. Introduction:

The liver plays a key role in most metabolic processes and excretion especially detoxification. It performs a vital function in detoxifying a wide range of toxic chemicals, both those produced internally and those coming from the environment.

Paracetamol also known as acetaminophen (N-acetyl-para-amino-phenol(APAP) is a widely used medication which has a good safety profile used to treat mild to moderate pains and aches, reduce fever (Lesko and Mitchell 1999; Cranswick and Coghlan 2000). . Paracetamolis a phenacetin metabolite (Prescott, 1980) . Paracetamol has beneficial therapeutic effects for the body when administered in proper therapeutic doses (Hazai et al .,2005) but generate liver necrosis and malfunction at elevated dose (Dahlin et al .,1984, Boyd; Bereczky, (1966). The therapeutic dose of paracetamol in adults is 1 g (14 mg/kg body weight) and the maximum dose in 24 hours is 4 g (Ferner et al .,2011). The National Poisons Information Service (NPIS) in the UK defines the paracetamol overdose as a dose greater than the licensed daily dose and more than or equal to 5g (75 mg/kg body weight) in 24 hours for treat pain or fever or excessive amounts of paracetamol ingested over a period of less than 1 hour; usually in the context of self-harm. An overdose causes severe nephrotoxicity and hepatotoxicity (Vermeulen et al ., 1992). The purpose of our study was to determine the hepatotoxic effect of therapeutic and toxicity dosing of paracetamol in addition investigation the effect of the water extracts of *Indian costus* on paracetamol induced hepatic injury in mice. Costusspeciosus (Family: Costaceae) is animportant medicinal plant widely used for the treatment of various diseases (Rajasekharan et al .,1996). The family Costaceae (Zingiberaceae) is divided into 52genera and includes about 1,300 species (Lijuan et al .,2011; Singh et al .,2011).

C. speciosusisamong the most effective traditional medicinal plant (Tushar et al ., 2010). *Indian Costus* has been mentioned in Prophets medicine for treatment of anemia, asthma, bronchitis, leprosy, jaundice, and skin and urinary diseases (Sivarajan, and Balachandran, 1994).

The rhizomes and roots of Costusspeciosus are characterized as bitter, astringent, purgative, anthelmintic, antioxidant,antitumor improves digestion and stimulant (Nahak and Kantasahu 2011; Deni, 2008) . Juice of the rhizomeis used to relive from headache and for cooling (Bhuyan and Zaman, 2008). The rhizome of C.speciosus has hepatoprotective properties(Bhuyan and Zaman, 2008) . Research has indicated that the MeOH extract of Costusspeciosus rhizomes provided significant hepatoprotective activity against Paracetamol-induced liver damage in mice (Srivastava et al ., 2013; Baiaan,2018; Hazai et al .,2001) . In this study, the hepatoprotective potential of *Indian costus* rhizomes was estimated against Paracetamol-induced acute hepatic damage. . Additionally, the histopathology of the liver was observed microscopically for any PA-induced toxic changes.

2. Materials and methods

2.1. Plant material

The *Indian costus* roots were collected from numerous herb stores in Albeida-Libya. They were washed, dried under shade and then powered with a mechanical grinder.

2.2. Extraction of Plant Material

Dried *indiancostus* roots (10g) was soaked in boiling water (100 ml) for 2 hour, allowed to cool and filtered using Whatman filter paper.Then, stored at 4°C. The final concentration of the aqueous extract was 10 %.

2.3. Experimental animals

Healthy albino male mice weighing between 20-25 g were selected for the acute toxicity and hepatoprotective, they were procured from the small animal house of faculty of veterinary medicine-omar al-mukhtar university-Elbeida-Libya. They were provided with food and tap water. The experimental mice were separated into five groups (n = 7 mice): group-A which kept as normal control group; group-B was treated with paracetamol (14 mg/kg b.w.) as therapeutic doses once a day intraperitoneal (IP) (Steinebrunner et al., 2014) for four days ; group-C was treated with paracetamol (85 mg/kg b.w.) as overdoses once a day by IP administration for four days; groups-D and E received orally (10 µl) 10% *Indian costus* roots extract every day for eight days. On the fifth day , the animals of groups -D and E were treated with paracetamol (14 and 85 mg/kg b.w.) once a day for four days followed by IP administration . On the ninth day, the mice were sacrificed and the collected blood samples were sent to the laboratory for biochemical analysis.

2.4. Biochemical analysis

Centrifugation of the blood samples was carried out at 4000 rpm at 4°C for 15 min to get serum, which was kept at -80 °C for assessing serum enzymes levels. The concentration of the serum enzyme like alanineaminotransferase (ALT) was measured by Hitachi-902 fully automated Chemistry analyzer by Roche diagnostics (Bergmeyer et al., 1986b), aspartateam-inotransferase (AST) by Hitachi-902 fully au-tomated Chemistry analyzer by Roche diagnos-tics (Bergmeyer et al., 1986a) and alkaline phosphatase (ALP) levels was measured by Hitachi-902 fully automated Chemistry analyzer by Roche diagnostics (Shaw et al., 1983). After collection of blood, the liver was immediately removed and rinsed in ice cold normal saline, blotted with filter paper and weighed.

2.5 Histopathological Studies

Small pieces of liver tissues in each group were collected in 10% neutral buffered formalin for proper fixation. The specimens were embedded in paraffin to prepare for sectioning (4-5 µm) than subjected to hematoxylin and eosin (H & E).These sectionswere examined photo microscopically forsteatosis,necrosis and fatty changes of hepaticcells (Koyloff G1971)

2.6 Statistical analysis:

The data are expressed as Mean ± SEM. The results of the study was analyzed using one-way ANOVAfollowed by Tukey test (SPSS version19). To discriminate among means the *F-test* was applied. The level of significance was $P \leq 0.05$, $P \leq 0.001$ and $P \leq 0.0001$.

3. Results:

3.1: ***Effect of oral administration of aqueous extract of indian costus roots on serum enzyme AST, ALT and ALP levels (U/L) in albino mice after IP injection of paracetamol therapeutic doses compared to normal control.***

Our results indicated significant effect in levels of AST, ALT and ALP enzyme in mice after IP treated with paracetamol therapeutic doses when compared to normal group. Oral administration of aqueous extract of indiancostus roots caused a significant reduction of AST,ALT and ALP levels (U/L) , almost comparable to the normal control group (Table 1).

Table 1: Effect of oral administration of aqueous extract of *indiancostus* roots on serum enzyme AST, ALT and ALP levels (U/L) in albino mice after IP injection of paracetamol therapeutic doses compared to normal control

Enzyme	Control group	paracetamol therapeutic doses group	paracetamol therapeutic doses +*Indian costus*
AST(U/L)	333..28 ± 5.3	433.75 ± 10.9 **	342.85 ± 9.1**
ALT(U/L)	81.95 ± 2.5	98.38 ± 2.5 *	88.42 ± 3.0*
ALP(U/L)	116.57 ± 1.4	155.18 ± 2.0 **	129.14 ± 3.1**

All vaues are presented as Mean ± SEM, n =7, *P≤ 0.05,** *P* < 0.001

3.2 :*Effect of oral administration of aqueous extract of indian costus roots on serum enzyme AST, ALT and ALP levels (U/L) in albino mice after IP injection of paracetamol overdoses compared to normal control*

The results of hepatoprotective activity of aqueous extract of *indian costus* roots in albino mice are summarized in Table 2. The hepatic enzymes AST, ALT and ALP in serum were significantly increased (*P* < 0.05) after IP treated with paracetamol overdoses when compared with control. Aqueous extract of *indian costus* roots treated animals showed significant decrease (*P* < 0.001) in the levels of AST, ALT and ALP enzymes when compared with control group (Table 2).

Table 2: Effect of oral administration of aqueous extract of *indian costus* roots on serum enzyme AST, ALT and ALP levels (U/L) in albino mice after IP injection of paracetamol overdoses compared to normal control

Enzyme	Control group	paracetamol over doses group	paracetamol over doses +*Indian costus*
AST(U/L)	333..28 ± 5.3	446.67 ± 10.4 **	396.55 ± 7.4**
ALT(U/L)	81.95 ± 2.5	102.12 ± 4.5 *	91.22 ± 1.5*
ALP(U/L)	116.57 ± 1.4	167.22 ± 1.6 **	144.15 ± 2.1**

All vaues are presented as Mean ± SEM, n =7, *P ≤ 0.001,** P ≤ 0.0001.

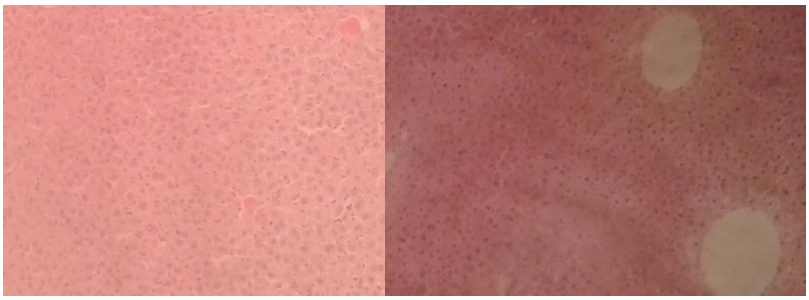
3.3: Histopathology

The Histopathological studies of the liver sections of normal control group showed no histological changes in sinusoids and hepatocytes and normal central vein (Figure 1A,B)..

The paracetamol therapeutic doses group showed hepatic cell necrosis along with dark appearance of the cytoplasm and increased size of the sinusoids with deterioration of central vein (Figure 2A).

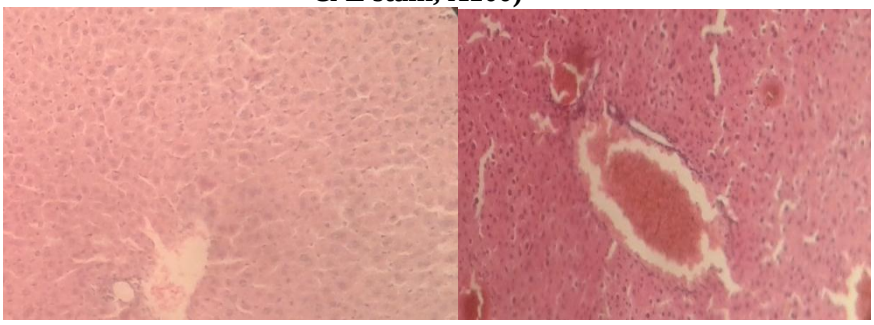
Severe hepatotoxicity was observed in paracetamol overdoses group by severe necrosis with disappearance of nuclei,dark appearance of the cytoplasm, inflammatory changes with signs of vascular congestion and a degenerative phenomenon , increase areas of red blood cells into the interstitial and amidst the spaces between hepatocytes (Figure 2B).

Pre-treatment with aqueous extract of *indian costus* roots showed no hepatocyte necrosis ,normal hepatic cells with portal vein and portal artery in the paracetamol therapeutic doses group (Figure 3A), while mild degree of hepatocyte necorsis,mild inflammation with little increase in size of hepatocytes observed in paracetamol overdoses group (Figure 3B).



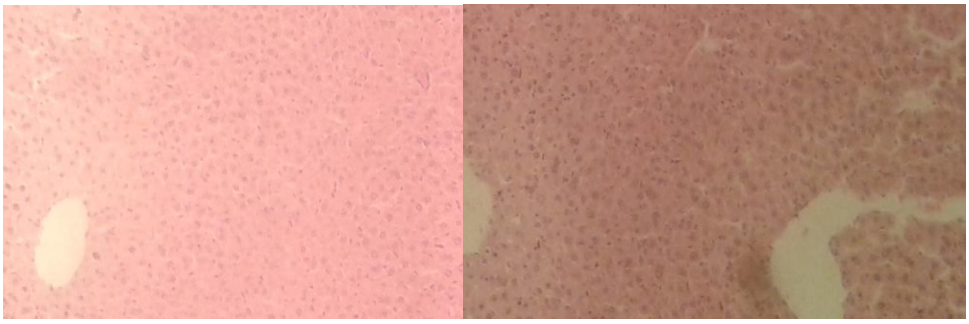
A B

Figure (1): A transverse liver section of mouse from control group showing no visible lesion (H & E stain, X100)



A B

Figure (2): A: a transverse liver section of therapeutic dose group of Paracetamol, only showing changes in the hepatocytes, such as increased size and dark appearance of the cytoplasm, increased size of the sinusoids(H & E stain, X100). B: a transverse liver section of over dose group of Paracetamol .only showing more abundant changes in the hepatocytes, such as increased size and dark appearance of the cytoplasm, inflammatory changes with signs of vascular congestion and a degenerative phenomenon and increase areas of red blood cells into the interstitial and amidst the spaces between hepatocytes (H & E stain, X100).



A B

Figure (3): A: a transverse liver section of therapeutic dose group of PA with orally 10µl of aqueous extract of indian costus roots showing most liver section with nearly normal feature (H & E stain, X100). B: a transverse liver section of over dose group of PA with orally 10µl of aqueous extract of *indiancostus* roots ,showing some changes in structure of liver, such as some filtered red blood cells and little increase in size of hepatocytes (H & E stain, X100).

4: Discussion

After taken orally, paracetamol is fast absorbed from the small intestine, while absorption from the stomach is negligible (Prescott, 1980). The peak plasma concentration of paracetamol was reached after 20 minutes to 1.5 hours (Forrest et al ., 1982). Paracetamol is metabolized firstly in the liver, mainly by glucuronidation and sulfation, then the drug excreted in the urine, at least 90 % of the administered dose is excreted within 24 hours. Only 2–5% of the drug are excreted unchanged in the urine (Forrest et al ., 1982). Accounts for 50–70 % of paracetamol through the glucuronidation by UDP-glucuronosyltransferase (UGT1A1 and UGT1A6) metabolis while 25–35 % of the drug is converted to sulfate by sulfation enzymes SULT1A1, SULT1A3, and SULT1E1 (McGill and Jaeschke., 2013).

Paracetamol (acetaminophen) is a commonly and widely used analgesic and antipyretic agentat therapeutic doses (Hazai et al., 2001). As anti-inflammatory drugs (NSAIDs), paracetamol probably acts through the cyclooxygenase (COX) by inhibiting COX-1 and COX-2 enzymes (Graham et al ., 2013), which leads to inhibition the synthesis of prostaglandin. Prostaglandins are responsible for eliciting pain sensations (Ricciotti and FitzGerald., 2011). This occurs only when the concentration of arachidonic acid and peroxides is low (Graham et al ., 2013). The antipyretic actions of acetaminophen are likely attributed to direct action on heat-regulating centers in the brain, resulting in peripheral vasodilation, sweating, and loss of body heat (Bannwarth and Pehourcq., 2003). The exact mechanism of action of this drug is not fully understood at this time, but future research may contribute to deeper knowledge.

An additional, paracetamol causes severe nephrotoxicity and hepatotoxicity in overdose (Vermeulen et al., 1992). Cohen and Khairallah., 1997 reported that protects against PA hepatotoxicity by promoting the activity of antioxidant enzyme and suppressing peroxidation of lipid. Vaishwanar and Kowale., 1979 suggested that the assessment of clinical and experimental liver damage associated with enzyme activities such as GOT, GPT, ALP,total bilirubin and total protein. The present study investigated the protective effect of aqueous extract of *indiancostus* roots on paracetamol-induced hepatotoxicty in mice.The hepatotoxicty of paracetamol resulted from its metabolites, especially N-acetyl-p-benzoquinoneimine (NAPQI). NAPQI decreased the natural antioxidant, glutathione in the liver and as a result damages the hepatocytes, which finally leads to liver failure (Kulkarni, 2011). In the current study increased serum levels of ALT, AST and ALP in paracetamol therapeutic and over doses groups are the most important indicators of liver toxicity by paracetamol. This results are consistent with the study of Kang., 2013. The decrease observed in the increased levels of the enzymes AST, ALT and ALP following the administration of paracetamol may be attributed to the protective effect of the aqueous extract of *indian costus* roots .which proved its ability to save the hepatocytes from the damage that lead to secretion of ALT, AST, and ALP to the blood stream (Baiaan,.2018). On the other hand, the possible mechanism responsible for the protection of the paracetamol induced liver damage by the extract of *indian costus* roots because of its phytochemicals, because a number of scientific reports indicates the role of certain ,phenolics flavonoids, triterpenoids and steroids inhepatoprotection against hepatotoxins (Srivastava et al., 2011; Devi and Urooj., 2010).

In histopathological studiesof the liver tissue of mice received distilled water (normal control group) showed apparently normal hepatic tissue.

Paracetamol therapeutic doses group showed hepatic cell necrosis along with dark appearance of the cytoplasm and showed sever inflammation with focal necrosis with

disappearance of nucleiin. Paracetamol overdoses group showing more abundant changes in the hepatocytes, such as increased size and dark appearance of the cytoplasm, inflammatory changes with signs of vascular congestion and a degenerative phenomenon and increase areas of red blood cells into the interstitial and amidst the spaces between hepatocytes

Liver tissues of paracetamol.therapeutic doses mice pretreated with aqueous extract of indian costus roots (10%) showed almost normal hepatocytes. It is concluded that aqueous extract of *indian costus* roots was able to reverse the hepatotoxicity caused by paracetamol. This was in agreement with the previous study, which reported the protective potential of *Costusspeciosus* rhizomes extract on CCl4-induced hepatotoxicity in rats (Verma and Khosa., 2009). The hepatoprotective effect of *Costusspeciosus* could be credited to the existence of steroidal saponins and different glycosides (Devi and Urooj., 2010; Verma and Khosa.,2009) .pretreated with aqueous extract of *indian costus* roots (10%) on paracetamol overdoses group showed mild degree of hepatocyte necorsis, mild inflammationwith little increase in size of hepatocytes observed which maybe augment the extract doses in these group was required.

In conclusion, the results of this study demonstrate that the aqueous extract of *indian costus* roots has hepatoprotective action against paracetamol induced hepatic damage in mice. Our results show that the hepatoprotective effects of *Indian costus* may be due to its antioxidant and free radical scavenging properties. Further investigation to determine the exact phytoconstituents that is responsible for its hepatoprotective effect is recommended for further studies.

الملخص : القسط الهندي من النباتات التي تستخدم كثيرا في الطب الشعبي في علاج الكثير من الامراض مثل السرطانات وامراض الدم والامراض التنفسيه وقد كان الهدف من هذا البحث هو دراسة تاثير مستخلص جذور القسط الهندي علي السميه الكبديه المستحدثه في الفأران بواسطة عقار الباراسيتامول واعتمدت الطريقه علي تقسيم الفأران لخمس مجموعات هي ا ،ب ،ج ،د ،هـ حيث كانت المجموعه أ هي المجموعه الضابطه وحقنت المجموعه ب بالجرعه العلاجيه لعقار الباراسيتامول وهي 14 ميكروجرام لكل كيلو جرام من وزن الجسم بينما حقنت المجموعه ج بالجرعه السميه وهي 85 ميكروجرام لكل كيلو جرام من وزن الجسم داخل الغشاء البروتيني لمدة اربع ايام وجرعت المجموعتين د و هـ بالمستخلص المائي لجذور القسط الهندي عن طريق الفم لمدة اربع ايام ثم حقنت في اليوم الخامس بعقار الباراسيتامول بالجرعات العلاجيه للمجموعه د والجرعه السميه للمجموعه هـ لمدة اربع ايام ثم ذبحت الفأران وتم ارسال عينات الدم الي المختبرلفحص انزيمات الكبد وهي انزيم ناقلة الالانين وانزيم ناقلة الاسبرتات وانزيم الفوسفاتاز القلوي واخذت عينات من الكبد لاجراء الفحص النسيجي وتبين من النتائج ان المستخلص المائي لجذور القسط الهندي سبب في انخفاض الاثار السميه علي الكبد التي سببها عقار الباراسيتامول في المجموعات د و هـ مقارنتاً بالمجموعتين ب و ج التي لم تجرع بمستخلص القسط الهندي مايشير الي ان القسط الهندي يمكن ان يستخدم كعلاج للسميه الكبديه الناتجه عن تعاطي الادويه ويحتاج لاجراء المزيد من الابحاث

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