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"HEPATOPROTECTIVE EFFECTS OF THE AQUEOUS EXTRACT OF CLOVE *(SYZYGIUMAROMATICUM)* AGAINST PARACETAMOL-INDUCED HEPATOTOXICITY AND OXIDATIVE STRESS IN RATS"

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ABSTRACT

Hepatoprotective activity of aqueous extract of clove (Syzygium*aromaticum*) in albino rats was observed against controversial hepatotoxicity effects of paracetamol(PCM) induced liver toxicity in rats. Paracetamol was administered to induce hepatic damage in Wistar albino rats. 100 and 200 mg/kg doses of clove extract and were used as treatment groups. In present study, the effects of clove extract [100 mg per kg B.W. and 200 mg/kg.B.W.] as alone. On PCM-induced hepatotoxicity were examined. Rats were divided haphazardly into six groups containing 6 rats each. The control group received distal water (D.W.). Other groups were treated with PCM (600mg/kg) alone, (600mg/kg PCM + 100mg/kg clove extract), and (600mg/kg PCM + 200mg/kg extract) respectively for 4 weeks. The blood samples were analyzed for biochemical labels of hepatic injury and tissue samples were subjected for assessing of liver antioxidants and histopathological studies. Analysis of the treated rats with PCM (600 mg/kg) showed Paracetamol induced male rat hepatotoxicity represented by significant decline in the serum total albumin ($P< 0.05$). Conversely, the study declared significantly increment ($P< 0.001$), bilirubin, ALT, AST and ALP as shown in group2 (induction group) in compared with group1 (control group). While, simultaneous administration of clove aqueous extract (100mg/kg and 200mg/kg) with paracetamol, was displayed significantly attenuated the adverse changes in the serum total albumin, bilirubine, ALT IU/L, AST, and ALP. The histopathological examination in the liver of rats also encouraging that clove extract markedly diminished the toxicity of PCM and keeps the histoarchitecture of the liver tissue to near normal. Hence, the results postulate that clove extract acts as a potent hepatoprotective agent against PCM induced hepatotoxicity in rats.

KEYWORDS: Hepatotoxicity, *Syzygiumaromaticum*, Wister rats**,** Paracetamol**,** Serum liver enzymes.

INTRODUCTION

The liver is a key organ in the body and is the First official for the metabolism of internal and external agents. It plays vital role in drug disappearing and detoxification and liver damage may be caused by xenobiotics, alcohol consumption, malnutrition, infection, anemia and medications (Mroueh et al., 2004). Furthermore, the liver has been expected to be especially susceptible to the noxious effects of miscellaneous use and until now PCM hepatotoxicity influences are still expostulative.

Various types of drugs such as acetaminophen, chloroquine and isoniazid are inducers of hepatotoxicity in world. More of the hepatotoxic chemicals damage liver cells basically by inducing lipid peroxidation and other oxidative damages (Hiraganahalli, et al., 2012). Paracetamol (PCM) is acetaminophen or N-acetyl-p-aminophenol (APAP), but a high dose can lead to undesirable side effects, such as hepatotoxicity with oxidative stress as one of the possible mechanisms mediating the event. It was first discovered in 1889 and is a widely used no prescriptive analgesic and antipyretic agent (Brown, 1968). Paracetamol (PCM) is widely used as analgesic and antipyretic drug, but at high dose it leads to undesirable side effect s, such as hepatotoxicity.Paracetamol metabolism depends on the age and sex, after reception a therapeutic dose, paracetamol tends to sulfation and glucuronidation and outputs non-toxic metabolites with urine.

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzoquinoneimine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion (Boyd and Bereczky, 1966). Where About 4% of a therapeutic dose is metabolised by the cytochromes P450, chiefly CYP2E1, to a potentially toxic intermediate metabolite N-acetyl-p-benzoquinone imine

(NAPQI). The highest concentration of CYP2El is located in centrilobular hepatocytes around the central vein and reflects the primary hepatic injury produced by paracetamol. Under normal conditions and therapeutic doses, NAPQI integrates with intracellular glutathione to become a non-toxic mercapturate derivative with urinary excretion. However, after taking a high dose, the normally minor CYP2E1 pathway becomes important. When the production of NAPQI surpass the capacity to detoxify it, the increase NAPQI binds to cellular components, causing mitochondrial damage and eventually the death of the hepatocyte. If a sufficientadequate dose is taken, hepatocyte death may be massive and produce acute liver failure (Basu, et al., 2014; Heard, et al., 2012).

Today, herbal medicine is becoming more and more popular as a safety and effective means of treatment for many different medical conditions. Herbs are often favored because they are natural and do not put noxious chemicals into the body. Herbs are available fresh, as capsules, powders, extracts, roots, etc (Dhinahar, et al., 2011).

However, usually that the use of aromatic plants hardly be higher in popular medicine, which had previously been documented their characteristics which include: anti-bacteria, fungi and yeasts (Karkosh, 2012). Most of those properties of these plants have able to control microorganisms related to skin diseases,. In addition to tooth decay and food rotting (Chaieb et al., 2007).

In any case, during the past few years there was observed an exponential growth in the field of herbal medicament that has acquired wide popularity medicines due to natural sources and the lack of side effects. Currently it has been evaluated natural diverse products to its protection of hepatic different patterns of hepatotoxicity (Grover , et al., 2002; Madkour, et al., 2013).

Moreover, clove is one of the most valuable spices used for centuries as a preservative for food and many medical purposes. The origins of this type of original spices in Indonesia, either at the present time are grown in different regions of the world (Cortés-Rojas et al., 2014). Where half of the world production of cloves used in Indonesia to kretek cigarettes by one part of the clove mixture with two parts of tobacco. Where the their benefit were obtained through the flower and bud as an anti-viral and antibacterial, libido exciting, high blood pressure, carminative, it's main prefer component is eugenol (de Paoli et al., 2007; Politeo et al., 2010; Koba et al., 2011).

Furthermore, Clove buds products more than 15 to 20% of a volatile oil that is accountable for the Distinctive smell and flavor. The main components of clove bud oil distilled (60-90%) are phenylpropanoids, including in the first place eugenol (4-allyl-2-methoxyphenol) and carvacrol, thymol and cinnamaldehyde and carvacrol, thymol and cinnamaldehyde (Chomchalow, 1996).

Clove is described as a repellent for fever, disinfectant and sterilizer for the stomach, heals sores and pain of the head and protects from epidemics and helps digestion and calms dental pain and reduces inflammation and allergy alerts the heart and stomach. And it is extracted from the seeds of clove oil pilot called eugenol, which has a special dental pain therapeutic properties, and can also extract the oil from the clove tree and its leaves market by distillation (Nosrati, et al., 2011; Djilani, 2012).

Furthermore, Clove is one of the wealthiest resources of phenolic compounds and it has big potential for pharmaceutical, cosmetic, food and agricultural applications [Diego, et al. 2014]. Since flavonoidsd are capable to decline xenobiotic-induced hepatotoxicity in animals and repel the damaging influences of oxidative stress, collaborating with natural systems like endogenous protective antioxidant enzymes, clove appears antioxidant peculiarities and its extracts could be used as food antioxidants [Kadarian, et al., 2002; Diego, et al., 2014].

However, commonly use of liver function tests in order to monitor for liver disease, to observe the development of hit disease and monitor the effects of these properties of potentially hepatotoxicity drugs (Muriel, et al., 1992).

Increment the levels of AST and ALT are an indicator of cellular leakage and functional disorder of the liver cell membrane(AbolfathiMohajeri, et al., 2012). ALP is membrane cohesion and its changing is likely to be affecting the permeability of the membrane and disturbances in the transfer of outputs (Mehana, et al., 2012). On the other hand, albumin and bilirubin values are connected with the excretive and synthetic functions of hepatic cells (Muriel, et al., 1992).

The goal of this deliberating was carried out to examine the anti activity of clove extraction against heptatotoxicity with regard to its effects on liver cells; histopathology, routine liver function tests.

MATERIAL AND METHODS

Animals

Male Wister albino rats (Rattusnorvegicous) were obtained from animal center of Thi-Qar university \college of science/ Iraq, weighing 250 ± 10 g, age (6-80) months, The animals were kept in standard conditions (23 ± 2 °C, 12 h light / dark cycle). Standard diet and water were given ad libitum.

Drug and plant extract

Paracetamol drug (SDI Co, Iraq) were dissolved in normal saline before use. While Clove dried flower buds were purchased from the local market, Iraq.

Kits for Biochemical Analysis

Kits for Biochemical Analysis Diagnostic commercial kits for biochemical analyses were purchased from (cat no. 80014, France, and (cat no .al 146 and cat no. AS 147, united kingdom).

Experimental Design and assembling of rats

Paracetamol (PCM) hepatotoxicity was induced in three groups of rats. Where, the animals were divided randomly into six groups (6 each), the first group was given normal saline (the vehicle) 0.5ml / animal /daily /I.P.,for 4wks, to serve as (negative) control group. The second group (induction) or positive group was given paracetamol (PCM) 600mg/kg/ orally (P.O), alone, three times weekly for 8 weeks. The third and fourth groups were received clove extract (100mg/kg/ day and 200mg/kg/ day) alone respectively for 8 weeks as a single daily (P.O.), while the animals in fifth and six groups were received (600mg/kg PCM + 100mg/kg B.W. clove extract), and (600mg/kg PCM + 200mg/kg clove extract) respectively, to the end of the experiment.

At the end of the treatment period, all animal were killed by neck dislocation after light anesthesia with diethyl ether. Blood samples were taken by cardiac puncture. The collected blood samples were permitted to clot. Sera were removed by centrifugation at 3000 rpm for 5 min. and stored at -20°C until analyzed.

Biochemical assay

Liver function tests were carried out by an enzymatic assay (Drotman and Lawhorn, 1978). Where Serum blood samples were analyzed for the activities of Aspartate Transaminase (AST), Alanine transaminase (ALT), alkaline phosphatase (ALP) and for concentration of albumin and bilirubin. using a spectrophotometric autoanalyzer (Olympus AU-2700).

Histopathological examination

Necropsy was conducted to identify gross lesion, after anesthetizing, the rats were dissected. The livers were removed and cleaned. Liver tissue samples were fixed in 10% formalin for24 and then washed –dehydrated-cleared and the organ was embedded in paraffin(Izbicki, 2002), sections about 4µm diameter was cutting and stained with hematoxylin-Eosin (H and E) for microscopic examination(Andrew, et al., 2008).. The histological changes were assessed by observation of lesions microscopically.

Statistical analysis

Statistical Package (SPSS, version 16) was done using student T-test for the analysis of the data. Furthermore, to determine the significance among groups. Where Data were expressed between means (Mean±Standard deviations (Mean±SD). Namely,the results in all above were accepted as statistical significant when the (p) value less than ($p< 0.05$).

RESULTS

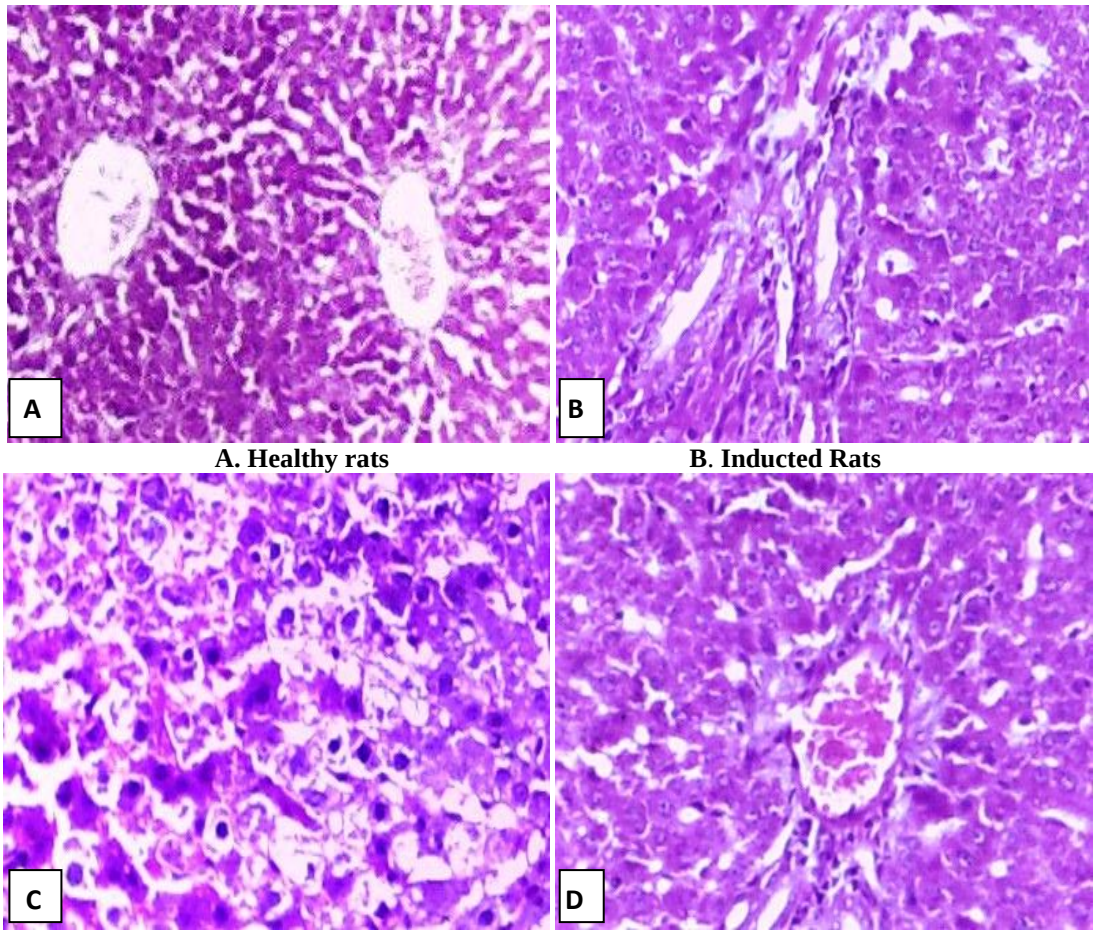
As shown in table1, the results of hepatoprotective activity of clove aqueous extract and The hepatotoxicity of paracetamol(PCM) in rats, demonstrated that the effects whether via clove aqueous extract or PCM on serum levels of albumin, bilirubin and liver enzymes (ALT, AST and ALP) in rats. Where paracetamol hepatotoxicity was correlated with significant decrease in the serum albumin (mg/dl) $P< 0.05$. Conversely, this study was appeared significantly increased ($P< 0.001$) in bilirubin (mg/dl), ALT, AST and ALP as appear in group2 (induction group) in comparison with group1 (control group). But when using of extract alone in healthy rats (group3 and 4) caused little insignificant changes in serum total bilirubin, albumin, ALT, AST and ALP. Anyhow, immediate administrations of extract (100mg/kg and 200mg/kg) with paracetamol (600mglkg) displayed significant decreased the adverse changes in the serum total bilirubin, ALT, AST and ALP ($P< 0.01$). While, Concomitant oral administration of extract (100mg/kg and 200mg/kg) with paracetamol (group 5 and 6) showed significant increment ($P<0.05$) in the mean values of serum total albumin. In Compared with paracetamol -treated rats. However, it didn't repeat them to the normal limits.

Histopathologicalexamination of the liver sections in current study gets from each group. photomicrograph of the liver section obtained from groups treated either with normal saline (vehicle) or PCM administration and rats that were treated at same time with clove extract (two doses). The control group declares normal cellular architecture with identified hepatocytes structure, sinusoidal spaces and central veins, with normal radial arrangements around central vein (CV) (Figure 1a). In the meantime, grievous histopathological changes were clearly noticed in PCM-intoxicated rats revealing centrilobular hepatic necrosis, with occasional plotted areas of moderate necrosis. Pronounced grade of fatty changes and marked centrilobular hepatic infiltration of lymphocytes and nuetrophils were obviously observed, congestion in portal vein (PV) with fibrous tissue proliferation, extended from portal area and forming portal bridging fibrous septae (Fig. 1b), besides, showing ballooning degeneration of hepatocytes associated with vacuolar changes (Fig.1c).When clove extract was treated simultaneously withPCM administration, significant decreases of hepatocellular changes were observed. In addition, showing closer to normal liver structure (Figure 1 c). Identical observation was seen when clove extract alone was administered to the rats.

Table1: Effect of administration of low dose (10mg/kg/day) and high dose (20 mg/kg/day) of clove extract on Paracetamol induced hepatotoxicity in rats. Values are expressed as Mean ± SD.

Groups	Bilirubin mg/dl	Albumin g/dl	ALT U/L	AST U/L	ALP U/L
G1	0.61±0.01	5.02±0.77	55.29±4.32	69.81±8.44	148.91±13.70
G2	1.51±0.11 *	3.1±0.66 *	181.90±14.50*	198.60±17.80*	238.23±18.02 *
G3	0.59±0.01NS	4.88±0.05 NS	54.47±4.62 NS	72.61±7.43 NS	145.95±12.02NS
G4	0.66±0.05 #	5.04±0.10 #	56.85±7.32#	68.62±13.12#	144.92±14.25#
G5	1.01±0.04 #	4.51±0.09 #	126.36±7.32 #	141.63±11.60#	201.31±13.14 #
G6	0.99±0.11 #	4.59± 08 #	121±9.01 #	128±6.61 #	198.05± 13.33#

* Significant change (P< 0.05).compared to control vehicle group.
#: Significant change (P< 0.05) compared to induction group.NS: Non-significant



A. Healthy rats **B. Inducted Rats**

C. Inducted Rats **D. PCM rats treated with Extract**

Figure 1: a- Histopathology of healthy rat liver declaringnormal histology of hepatic structure. B–c. Inducted rats liver showing intensity fatty changes, sinusoidal feathery degeneration and necrosis, congestion in portal vein (PV) with fibrous tissue proliferation, ballooning and sever degeneration of hepatocytes d. healthy rat liver treated with clove extract plus PCM, showing mild fatty change, mild sinusoidal dilation and congestion viz closer to normal hepaticstructure. (H&E 200X).

DISCUSSION

Liver regulates many important functions including metabolism, and is an important organ for detoxification of the liver, which can cause damage to the liver during the metabolic interaction. Pathogenesis of this damage includes all types of cells in the liver by death and renewal processes and progress to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma (Giannelli et al., 2003). Well, in experimental studies, Paracetamol is widely used as a hepatotoxin in high dose (overdose). The effectiveness of any preventive drug to the liver relied mainly on its ability reducing injury effects and the preservation of the natural function of the liver, which is trouble by hepatotoxin (Dutta, et al., 2013). However, the hepatotoxicity is caused by the reaction metabolite of Paracetamoli.e N-acetyl-p-benzoquinoneimine (NAPQI), which causes oxidative stress and glutathione exhausting. (Boyd andBereczky. 1966). Paracetamol toxicity is due to the composing of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome [Dahlin, 1984] or depletion of hepatic glutathione is a condition

for paracetamol induced hepatotoxicity [Moron, 1979, Gupt, 2006]. Nonetheless, the association of paracetamol abusiveness and hepatotoxicity is getting significance because of its usage as a suicidal agent either alone or in combination with other analgesic mixtures. A dose of 15—50 gm/day is dangerous and may result in death of a person due to hepatonecrosis (Prescott, et al., 1971). Nonetheless, usually, AST and ALP are existing in high concentration in liver. These enzymes are discharge from the cells and their levels in the blood increment. Because of hepatocyte necrosis or anomalies membrane permeability (Shah, 2002).

However, this study appeared that the activities of serum of AST and ALT are cytotolic indicator enzymes inverting hepato-cellular necrosis, meaningly they are discharged into the blood after cell membrane injuring. Hence, we used the activities of AST, ALT and ALP in the circulation as markers of hepatic damage.

However, Giving of clove extract at doses of 100 and 200 mg/kg, p.o., daily resulted in a significant sluggish of moderate rise of serum enzyme markers in paracetamol- induced group, in comparable to group II. In which, proposing that the Clove extract as other plant extracts may prevent hepatic injury associated with overdose of PCM administration. Where, found there are significant elevations in serum of (AST, ALT and ALP) levels in PCM rats compared with healthy animals (control group).This may relate to immoderate accumulation of amino acids in the serum of animals as a consequence of the moving of amino acids from protein stores (Abolfathi, et al., 2012). These findings are agreement with the results of a previous study in which paracetamol was administered to rats (Sener, et al., 2003). Furthermore, the bilirubin serum level took the same manner. On the other word, a decline of serum bilirubin in this study confirms the hepatoprotective activity of clove extract. Conversely, the serum level of hepatocellular albumin marker was significantly declined in group II that treated with a paracetamol alone. That is verifying the depleting of the liver function .in compared to group I (control) (Table1). These scores were in line with the results of previous studies that weregivenparacetamol to rats (Sener, et al., 2003). It is authenticated that the cloves extract to have a preventive influence on plasma membrane of hepatocytes (Diego, et al. 2014). On the other word, hepatic injury induced by paracetamolin which caused a decrease in albumin. In addition, hepatic transaminases such as AST and ALT It is still considered the gleaming standards for assessing the hepatic injury (Michaut,. et al., 2014). Moreover, leakage of liver enzymes into blood when injury to the liver due to increased permeability, injury and necrosis of the hepatic cells.Therefore, AST, ALT and ALP levels are elevating in liver toxicity (Dutta, et al., 2013). Moreover, (Kumar et al., 2004), was appeared that is One of the hallmark features of hepatic damage is emergent leakage of cellular enzymes into plasma. Besides, the range and sort of liver injury can be entered based on the existence or absence of specific enzymes in the blood surge. However, in this study, hepatoprotective effect of clove plant extract is confirmed by the amelioration ALT, AST and ALP levels. Where simultaneously treatment with this extract inhibiting PCM induced AST and ALT altitudes. Also emphasised that there are Previous studies have documented elevations of transaminases after APAP treatment (Asha et al., 2004). The increment is time dependent with importance elevation noted after two days proposing severe hepatocellular injury caused by oozing of these enzymes into circulation that is normally cytoplasmic in position (Chung et al., 2001).

The biochemical results were also proved by histological manifestations. The changes at most comprise hepatocellular necrosis, fatty accumulation, inflammatory cells infiltration and other histological observations which were also in line with the scores of other workers (Agbaje, et all., 2009;Matsura, et al., 2006). However, histopathological outcomes further added more verification to the hepatoprotective influence of clove extract. Hence, the hepatoprotective impacts of clove extract could be ascribed to its interference with many pro- and inflammatory mediators which are getting before hepatotoxicity.

CONCLUSION

The administration of cloves simultaneously with paracetamol for end of experimental (4wks) led to protects of hepatic cells by inhibition of oxidative effort to paracetamol in rats. So it showed significantly responding to changes in the functional test. These results confirm paracetamol toxicity to the hepatic cells, simultaneously has implications strong protection of hepatic cells against hepatotoxicity in rats by its ability to antioxidants. However, it is needed more study about the mechanism by which clove extract makes its anti-oxidants effects on human being which are still unknowable. Though, considering our results in this animal model, it is recommended that the clove plant extract maybe used in the future as hepatoprotactive drug from different liver injuries.

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