Original paper

Hepatoprotective Effect of Allopurinol against Paracetamol Induced Hepatotoxicity in Male Rats

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Abstract

Background: Liver is a vital organ regulating important metabolic functions. A number of chemical agents and drugs which are used on a routine basis cause cellular as well asmetabolic liver damage.
Objectives: This study was undertaken to assess the hepatoprotective effect of allopurinoll in paracetamol induced hepatotoxicity in male rats.
Material and method: A total of 18 adult male albino rats were randamized into 3 group Group1 was used as a control group ,group2: animals received an intraperitoneal injection of 300mg/kg paracetamol,group3 received 100mg/kg allopurinol orally(by oral gavage) 18 hrs before paracetamol administration.
all the animals were sacrificed after 12 hr from paracetamol dose. Blood samples were collected by cardiac puncture. Serum was separated and analyzed for various biochemical parameters (liver enzymes)liver was removed and kept in 10%formalin for histopathological study.
Results: Treatment of male rats with paracetamol led to significant ($p<0.05$) increase the activities of serum enzymes level GPT,GOT, ALP levels compared with the normal rats, In contrast prophylactic used ofallopurinol at100mg/kg orally treated rat prevented the liver damage as judged by the significant ($p<0.05$) decreased these enzymes levels, histopathologically allopurinol showed protective effect against paracetamol induce liver damge.
Conclusion: allopurinol could be beneficial for alleviating paracetamol toxicity. Further studies and parameter to measure oxidative strees are required, to explain these protective effects
Key words: allopurinol, paracetamol, hepatotoxicity, liver enzymes

Introduction

Liver is the key organ, which plays a vital role in regulating various physiological processes in the body. It is involved in various vital functions, such as metabolism, secretion, storage supply of nutrients and energy. It has great capacity fordetoxication and deposition of endogenous substances.[1,2] The liver is expected not only to perform physiological functions but also to protect against the hazards associated with harmful drugs and chemicals[3-5]. It is widely exposed to xenobiotics, hepatotoxins, and chemotherapeutic agents that lead to impairment of its functions. Most common causes of liver diseases are viral infections,[6,7] drugs,[8,9] toxic chemicals, excess consumption of alcohol and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells due to lipid peroxidation as well as other oxidative damages.[10,11] Liver diseases are regarded as one of the serious health disorders.[10]

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Allopurinol is a xanthine oxidase inhibitor used widely in treatment of gout, leishmaniasis, renal stones and compilations associated withradiation therapy .Allopurinol is widely used and generally welltolerated . However, in certain cases it may have toxic effects, such as vasculitis, toxic epidermalnecrolysis, eosinophilia, hepatitis, reduced renalfunction and bone marrow suppression, known as allopurinol hypersensitivity syndrome [12].

Xantine oxidase (XO) has been implicated as an important source of cytosolic O_2^- [13]The potent XO inhibitors allopurinol and its metabolite oxypurinol are also powerful scavengers of OH^- in vitro [(14)]. Allopurinol has a half-life of only 1 h, but it is rapidly converted to oxypurinol which has a half-life of 18-30 h. allopurinol and oxypurinol are not bound to serum proteins and are excreted mainly in the urine [(15).]

Liver diseases have become one of the major causes of morbidity and mortality all over world. From among, drug induced liver injury (DILI) is one of the most common causative factor that poses a majorclinical and regulatory challenge [16]. The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevationof liver enzymes to fulminant hepatic failure.

Paracetamol (PCM) also known as Acetaminophen, taken in overdose can cause severe hepatotoxicity and nephrotoxicity [17]. PCM is activated and converted by cytochrome P450 enzymes to toxic metabolite NAPQI (N-acetyl-p-benzoquinoneimine) that causes oxidative stress and glutathione (GSH) depletion [17,18]

In spite of tremendous advances in modem medicine, there are hardly any reliable drugs that protect the liver from damage and/or help in generation of hepatic cells.

Therapeutic doses of the analgesic drug acetaminophen (APAP) are readily detoxified by hepatic phase II drugmetabolizingsystems mediating glucuronidation and sulfation[19], with a small portion undergoing a cytochrome P-450-mediated bioactivation to the highly reactive electrophilic arylating intermediate N-acetyl-p-benzoquinoneimine (NAPQI) [20]. In rats and humans, NAPQI isdetoxified principally by conjugation with reduced glutathione (GSH) under spontaneous or glutathione Stransferase (GST)-mediated conditions to the 3-glutathione-S-yl-APAP conjugate[19].In the event of the intake of an overdose of APAP, the increased production of NAPQI rapidly overwhelms GST, eventually exhausts GSH, UDP-glucuronic acid and inorganic sulfate [21], inhibits GSH synthesis [21,22]and decreases cytosolic GST activity [23]. More importantly, this APAP metabolite is a major cause of hepatocellular damage, centrilobular hepatic necrosis and evenfatalities upon entering in adduct formation with liver macromolecules, especially proteins [24]. The hepatotoxicity of APAP is generally recognized to start with the formation of NAPQI and to be related tothe oxidative stress that develops as a result of the oxidative capacities of this reactive metabolic product.

Aim of the study: This study was undertaken to assess the hepatoprotective effect of allopurinoll in paracetamol induced hepatotoxicity in male rats.

Materials and Methods

Experimental animals

Eighteen white male rats weighing (250-300) gm were used in this study. These rats aged between (4-5) weeks, all animals were obtained from animal house of biology department /college of sciences /Kufauniversity / Iraq. Animals were kept in animal house at an ambient temperature of 25°C and 45 – 55% relative humidity, with 12 h each of dark and light cycles. Animals were fed pellet diet and water *ad-libitum*. The rats were divided randomly and equally into three groups of six rats.

First Group I: was the control group**Second Group II**: was given single dose of paracetamolintraperitoneally (300ml/kg)

Third Group III: was given 100mg/kg allopurinol orally (by using oral gavage) and then after 18 hrs given 300mg/kg paracetamolintraperitonialy

Biochemical parameters:

At the end of the experiment, the overnight fasted animals (the control and experimental animals)were sacrificed . Blood samples were collected by cardiac puncture, 5 ml,Ofblood samples were collected from heart and put in tubes without EDTA and centrifugation at 3000g for 15 minutes for obtained serum. Thebiochemical parameters included Alanine aminotransferase(ALT), Aspartate aminotransferase(AST), alkaline phosphate (ALP) protein and albumin.

Drugs:

paracetamol**:** was used in a dose of 300mg/kg.300mg/2ml paracetamolampoule, supplied by (OUBARI PHARMA)

allopurinol: was used in a dose of 100mg/kg.100mg tablet was suspended in distilled water and given oraly

Histopathological examination

Conventional techniques of paraffin-wax sectioning and haematoxylineosin staining were used for histological studies (Drury and Wallington, 1981).

Statistical Analysis

Data for hepatoprotective activity were expressed as Mean ± SEM from six rats in each group. Hepatoprotective activity were analysedstatistically using SPSS . P value of < 0.05 was considered as statistically significant.

Results

Treatment of male rats with paracetamol led to significant increase the activities of serum enzymes level GPT,GOT, ALP levels compared with the normal rats, In contrast allopurinol treated rat prevented the liver damage as judged by the decreased enzyme levels as compared to paracetamol-induced liver damage. (table1)

Histopathological studies of liver tissues of the normal animals showed normal hepatocytes with central vein, cytoplasm, and nucleus (figure 1). Damage of parenchymal cells, hemorrhagic necrosis of hepatocytes, and necrosis seen around central vein were observed in paracetamol treated rats (figure2). The liver sections of the rats treated with allopurinol followed by Paracetamol intoxication showed a sign of protection (figure3).

Table 1. effects of oral allopurinol on liver function test of male rats with paracetamol induced hepatotoxcity

Group	AST u/l	ALT u/l	AlPu/l	Protein gm/dl	Bilirubinmg/dl
Control	0.73±0.32	8.20±2.12	60.56±3.17	31.50±2.96	37.59±6.42
Paracetol(300mg\kg)	1.66±0.59*#	7.34±0.59*#	173.77±5.29*#	190.69±8.39*#	132.96±5.27*#
Allopurinol(100mg\kg)	1.18±0.21*	5.33±1.15*	96.77±6.25*	102.47±6.99*	73.40±3.24*

Values are expressed as mean ± SEM. for six rats in each group

Single dose of100mg/kg

* P< 0.05 represented the significant differences between treated groups and control group

# P<0.01 represented the significant between treated groups only.

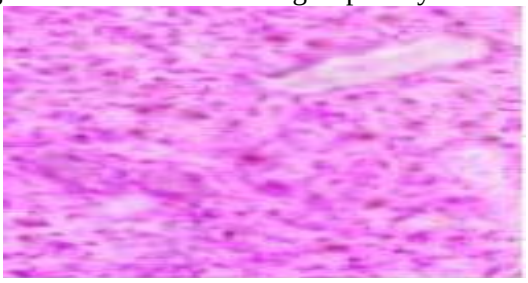

Figure1. Section of liver tissue of control rat showing normal histology stained with H&E(100X)

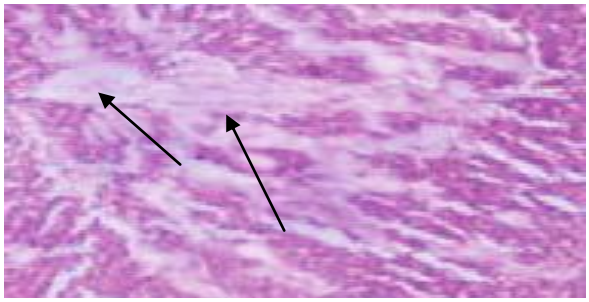

Figure 2. Section of liver tissue of paracetol treated group without allopurinol rat showing loss of normal liver histology (enlargement of many hepatocyte (2+) with inflammatory foci (3+)) section stained with H&E (100X)

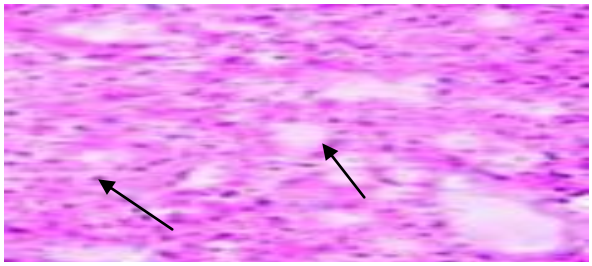

Figure3. Section of liver tissue of allopurinol treated rat showing normal hepatocyte with regenerating hepatocyte (aminimal hepatocyte enlargement (1+) with minimal inflammation (1+)) section stained with H&E(100X)

Discussion

The present study was conducted on adult male albino rats. Males have been chosen in this study to avoid the hormonal changes, which may be faced in females and may affect the results .Liver have been chosen in this study because it is target organs for drug toxicity .The liver is a specialized organ in terms of its metabolic, synthetic and detoxifying function .Liver and kidney are the primary target for a variety of noxious agents inducing inflammation, necrosis and fibrosis[25].

The present study investigated the hepatoprotective effect of allopurinol on experimental liver injury using PCM induced hepatotoxicity models in rat.PCM resultsin hepatotoxicity in men as well as in experimental animals. So the PCM - induced hepatotoxicity was selected as experimental models of liver injury in present study. PCM is metabolized to a toxic reactive metabolite N-acetyl-p-bezoquinone imine (NAPQI) by cytochrome P-450 which is further reported to cause massive oxidative stress and finally liver cell death.[26-28] The elevated levels of serum enzymes are indication of cellular leakages and loss of functional capacity of cell membrane in liverIt has been established that serum biochemical parameters such as AST, ALT, ALP etc levels were elevated in paracetamol-induced hepatotoxicity[29]During the assessment of liver damage by paracetamol the determination of enzyme levels such as AST, ALT is widely used. AST found in mitochondria of hepatocytes. Necrosis of liver cells release the enzyme into circulation and it can be measured in the serum. High concentrations of AST showed liver damage. ALT is more specific to the liver, and it wasa better parameter for hepatic injury. High levels of AST indicate the cellular leakage as well as loss of functional ability of cell membrane in liver[30]. Serum ALP and bilirubin is also related with liver cell damage. High concentrations of ALP and TB were shown serious hepatic damage in

Paracetamol treated rats. The decrease in the levels of total protein (TP) observed in the Paracetamol treated rats suggested that the decrease in the number of hepatocytes which may result in decrease in hepatic capacity to synthesize protein. In the present study, pretreatment with allopurinol (100 mg/kg, p.o,) there was significant ($p<0.05$) reduction in the elevated liver enzymes as compaired with paracetamol treated group reduced the elevated serum levels of AST, ALT, ALP, TB, and significantly ($p<0.05$) elevate the level of TP, indicates that it offered protection by preserving the structural integrity of the hepatocellular membrane against paracetamol induce hepatotoxicity This experimental results indicate that allopurinol have stabilizes the plasma membrane as well as helped in healing of the hepatic tissue damage.

ROS play a major role in production of microvascular and parenchymalcell damage associated with paracetamol induced hepatotoxicity. One cellular defense mechanism for coping with oxidativestress is enhancement of expression of a selective set of genes that encode antioxidant enzymes via activation of several cytoplasmicredox-sensitive transcription factors. This leads to enhanced productionof the GSH needed for rapid scavenging of ROS allopurinolpre. treatment showed a significant reduction in oxidative damage through increase of the content of GSH[31] and allopurinol had a protective role against I/R induced oxidative stress through increase of GSH [32,38]. In the present study allopurinol significant lyattenuate a decrease in hepatic GSH contents, which was abolished by paracetamol toxicity this finding is in agreement with Lee et.al[33]

Conclusion and recommendation

Our results demonstrate that:

1. paracetamol is capableof inducing marked alterations in biochemical parameters (liver enzymes)
2. allopurinoll administeredbeforeparacetamol exposure, minimizedparacetamol-associated hazards as shown by histopathological finding Therefore, allopurinol could be beneficial foralleviating paracetamol toxicity.
3. Further studies and other parameter to measure oxidative strees arerequired, to confirmthese protective effects

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