Effect of Different Doses of Paracetamol on Liver Enzyme Activities in White Albino Male Mice

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ABSTRACT

A study investigated the effects of paracetamol on the level of liver enzyme activities that induced hepatotoxicity in white albino male mice. In this study forty mice where used, divided to four group, three of them where ingested orally different doses of paracetamol for three month and compared with control group, also GOT, GPT, ALP and GGT where measured.

Biochemical analysis showed highly significant difference in serum GOT, GPT, ALP and GGT in all experimental groups as compared to control group. In conclusion the paracetamol induced hepatotoxicities in mice in prolong period treatment.

Key words: paracetamol, Hepatotoxicity, GOT, GPT and GGT.

تأثير الجرع المختلفة للبراسيتامول على مستوى انزيمات الكبد في الفئران

الخلاصة

تهدف هذه الدراسة الى التحري عن تاثير البراسيتامول على الكبد من خلال قياس مستوى انزيمات الكبد وهي انتقال الامين لحامض الكلوتاميك و انتقال الامين لحامض البايروفيت و انزيم الفوسفاتيز القاعدي. شملت الدراسة على اربعة مجاميع من الفئران المختبرية واحتوت كل مجموعة على عشرة فئران. تم تجريع المجاميع الاولى والثانية والثالثة بجرع مختلفة من البراسيتامول (8,4,2 ملغم/مل/يوم) على التوالي و اعتبرت المجموعة الرابعة مجموعة سيطرة للمقارنة ولمدة ثلاثة اشهر. تم قياس مستوى مصل الانزيمات (GOT GPT, ALP,GGT) و لوحظ ارتفاع معنوي في مستوى الانزيمات مقارنة مع مجموعة السيطرة عند مستوى احتمال $p < 0.001$ وهذا يدل على التاثير السمي على خلايا الكبد عند استعمال الدواء لفترات طويلة.

INTRODUCTION

Paracetamol is a widely used as analgesic and antipyretic drug, It is commonly used for the relief of headaches and other minor aches, pains and is a major ingredient in numerous cold and flu remedies [1]. Prolonged daily use increases the risk of upper gastrointestinal complications such as stomach bleeding and may cause kidney or liver damage, Paracetamol is metabolized by the liver and is

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hepatotoxic, while chronic users of paracetamol may have a higher risk of developing blood cancer [2].

Liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infections and chronic alcoholism. If during all such exposures to the above mentioned challenges the natural protective mechanisms of the liver are overpowered, the result is hepatic injury [3].

Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like sGOT, sGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase, are elevated [3]. It is the key organ of metabolism and excretion has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents [4]. Hence, this organ is subjected to variety of diseases and disorders, several hundred plants have been examined for use in a wide variety of liver disorders, In overdose it produces a centrilobular hepatic necrosis. Toxicity is believed to occur by initial hepatic metabolism by cytochrome P450 enzymes to the highly reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI), after a therapeutic dose NAPQI is efficiently detoxified by GSH. When taken in overdose NAPQI leads to depletion of hepatic GSH levels and covalent binding to hepatic cellular proteins to form 3-(cystein-*S*-yl)-acetaminophen adducts [5]. Covalent binding has been shown to correlate with development of necrosis; Subsequent events important in the toxicity are poorly understood [6]. The incidence of Paracetamol poisoning and the severity of the outcomes vary extensively throughout the world which is mainly metabolized in the liver to excretable glucuronide and phosphate conjugates. However, once intracellular GSH reserves are depleted, multiple mechanisms ensure ultimate cell death [7].

Serum glutamic oxaloacetic transaminase (sGOT) is a pyridoxal phosphate dependent transaminase enzyme (EC 2.6.1.1), It is catalyzes the reversible transfer of a α-amino group between aspartate and glutamate and, as such, as an important enzyme in amino acid metabolism. GOT is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health [8].

Serum glutamic pyruvic transaminase (sGPT) (EC 2.6.1.2) an enzyme that is normally presents in liver and heart cells [9]. It is released into blood when the liver or heart is damaged. The blood sGPT levels are thus elevated with liver damage or with an insult to the heart. Some medications can also raise sGPT levels like diclofenic sodium, aspirin and paracetamol [9].

Alkalin phosphatase (ALP) (EC 3.1.3.1) are a group of relatively non specific enzyme ,it is found in all tissue of the body, serum, cell membrane, liver, bile duct, palcenta, chromosomes and intestinal epithelium. Moderate elevation of ALP may be attributed to Hodgin disease, congestive heart failure and abdominal bacterial infection, while high level of ALP occur in case of hepatitis, obstructive liver disease and DM [10].

Serum Gamma GlutamylTransferase (GGT) (EC 2.3.2.2) is the enzyme which responsible for the extra cellular catabolism of (GSH-Gamma Glutamyl-Cysteinyl-Glycine), the main thiol intracellular antioxidant agent (11) and the larger function of enzyme is located in the cell membrane and may act to transport amino acid and peptide into the cell across the cell membrane in the form of gamma glutamyl peptidase. In one study only 32.4% of the patient with high GGT level had

hepatobiliary pathology, In addition to pancreatic event, myocardial infraction, diabetes mellitus, chronic obstructive lung disease which can increase GGT level [8].The aim of the present study was to observe the side effects of the drug (paracetamol) at doses (2, 4 and 8 mg/ml/day) on liver enzymes activities in white albino male mice by observing GOT, GPT, ALP and GGT after three month of the treatment.

MATERIAL AND METHODS

PREPARATION OF THE DRUG

Two hundreds mg paracetamol tablets has been dissolved in 20 ml of distilled water forming 10 mg/ml, then 2, 4 and 8 ml of this stock solution were taken and added to 8, 6 and 2 ml distilled water respectively to prepare the concentration of 2, 4 and 8 mg/ml that used to dosed T1, T2, T3 animals groups at regiment 0.1 ml/10g B.W.

Laboratory animals and sample collection

A total of 40 white albino male mice weighting 30-35 gm aged of 3-4 months were used in this study. All mice were kept under constant environmental conditions (24 to 26°C and 55 to 60% humidity) with a 12-hour light/dark cycle. They were housed in polypropylene cages with wood dust and given free access to food and tap water *ad labium*.. Experimental groups were classified into four groups; each group comprised of ten animals. The first group (C) was the control group which has not received the drug; the second group (T1), third group (T2) and fourth group (T3) have had received orally 0.1 ml/10g B.W. of 2,4 and 8 mg/ml/day of paracetamol respectively. All group dosed paracetamol for three month. Then the mice were killed by decapitation after three months of receiving the drug. The samples of collected whole blood were left for 15 minutes at room temperature for clotting, then centrifugation at 10 rpm at 10 min and separation of the serum. The serum measurements at the same day of collection of serum GOT, GPT, ALP and GGT were done for the control and treated animal groups.

Assay Methods

GOT and GPT were measured by kit method (Reitman-France colorimetric method, linear chemical, S.L, Spain), the assay method is aspartate aminotransferase GOT and alanin aminotransferase catalyzes the transfer of the amino group from aspartate or alanine to oxoglutarate with formation of glutamate oxaloacetate for GOT and glutamate pyruvate for GPT [12].

Assay of ALP activity is estimated by kit (BIOMERIEUX, 61511, France), phenol is released by enzymatic hydrolysis from phenylphosphal. The librated phenol is measured by spectrophotometer in the presence of 4-aminoantipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction [13].

The activity of GGT was determined by kinetic methods using a special kit (BIOLABO SA Reagents, 021602, maziy France). The assay method is based on transport of gamma glutamyl group from gamma glutamyl P-nitroanilide to glycylglycine by GGT enzyme leaving the yellow product of p-nitroaniline, the analytic method was performed at 30 C by measuring the absorbance change with spectrophotometer [14]. The rate of formation of p-nitroaniline is directly proportional to GGT activity in the specimens is measured at 405 nm [14].

Statistical Data Analysis

Data were statistically analyzed using SPSS statistical software (version 11.5) by one way ANOVA test. The values are giving as mean ± SD.

RESULTS

In this study 40 white albino male mice were used to study the toxic effect on liver enzyme activities via measurement of serum glutamic oxaloacetic transaminase (sGOT), serum glutamic pyruvic transaminase (sGPT), Alkalin phosphatase (ALP) and serum Gamma GlutamylTransferase (sGGT) which demonstrated in table1 and in Figures (1 to 4).

Results are illustrated in table 1 represented data of the serum enzymes activity of GOT, GPT, ALP and GGT which show increases in their activities in comparison with normal population groups which administered the drug for three months. Figures 1, 2, 3, and 4 summarizes the measurement of different biochemical parameters (GOT, GPT, ALP and GGT) activities in treated groups which dosed paracetamol in three different doses for three months ($p<0.001$).

DISCUSSION

Paracetamol, a widely used antipyretic and analgesic drug, produces acute hepatic damage on accidental over dosage. It is established that, a fraction of paracetamol is converted via the cytochrome P450 pathway to a highly toxic metabolite, N-acetyl-P-benzoquinamine (NAPQI) [15], which is normally conjugated with glutathione and excreted in urine. Over dose of paracetamol depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction [16] and development of acute hepatic necrosis. Several P450 enzymes play an important role in N-acetyl-P-aminophenol (APAP) bioactivation to NAPQI. P450 have been suggested to by primary enzymes for paracetamol bioactivation in liver microsomes. Studies demonstrated that paracetamol induced hepatotoxicity can be modulated by substances that influence P450 activity [17].

In the present study, administration of hepatotoxic doses of APAP to mice resulted in difference in serum enzyme levels when compared with control group. Results of previous studies indicated that high doses of APAP significantly raised the serum levels of GOT, GPT, ALP and GGT which was indicative of loss of functional integrity of cell membrane (18). However, ALT is considered more specific and sensitive indicator of liver injury [19].

Table (1) shows highly significant difference in serum GOT, GPT, ALP and GGT activity in treated group in comparison with normal population, this rises in enzymes is attributed to the hepatic damage caused by paracetamol administration was observed by recording enzymes levels in experimental groups due to hepatic injury, causing the leakage of enzymes by altered membrane permeability (20,21).

Figure (1) shows a significant difference in serum GOT activity in treated groups in comparison with normal population. Figure (2) demonstrates the effect of paracetamol on GPT enzyme activities in T1, T2 and T3 groups and shown highly significant difference in comparison with normal group, this agreement with ref (22).

Figure (3 and 4) illustrated toxic effect of paracetamol via measurement of ALP and GGT enzyme activities, the level of these enzymes was increase with increasing dose resulting in high level of ALP and GGT enzyme activities, this result agreement with ref [23] which refer to the paracetamol induced plasma membrane stabilization and hepatictissuedamagerepair.

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Table (1) Effect of different doses of orally administer paracetamol on serum liver enzyme activities in different experimental Groups in comparison with control group.

Groups	(Mean ± SD)			
	GOT activities (I.U/ml)	GPT activities (I.U/ml)	ALP activities (I.U/ml)	GGT activities (I.U/ml)
C	17.04±1.14 CV%=6.69	9.12±0.12 CV%=1.31	33.78±1.99 CV%=5.89	35.16±2.25 CV%=6.39
T1	24.99±2.16 CV%=8.64	15.63±0.19 CV%=1.21	46.03±2.29 CV%=4.97	48.88±3.19 CV%=6.52
T2	32.06±3.01 CV%=9.38	27.60±1.40 CV%=5.07	60.32±3.13 CV%=6.22	57.62±4.55 CV%=7.89
T3	47.82±3.99 CV%=8.34	39.00±2.22 CV%=5.69	73.61±3.99 CV%=5.42	69.16±5.00 CV%=7.22

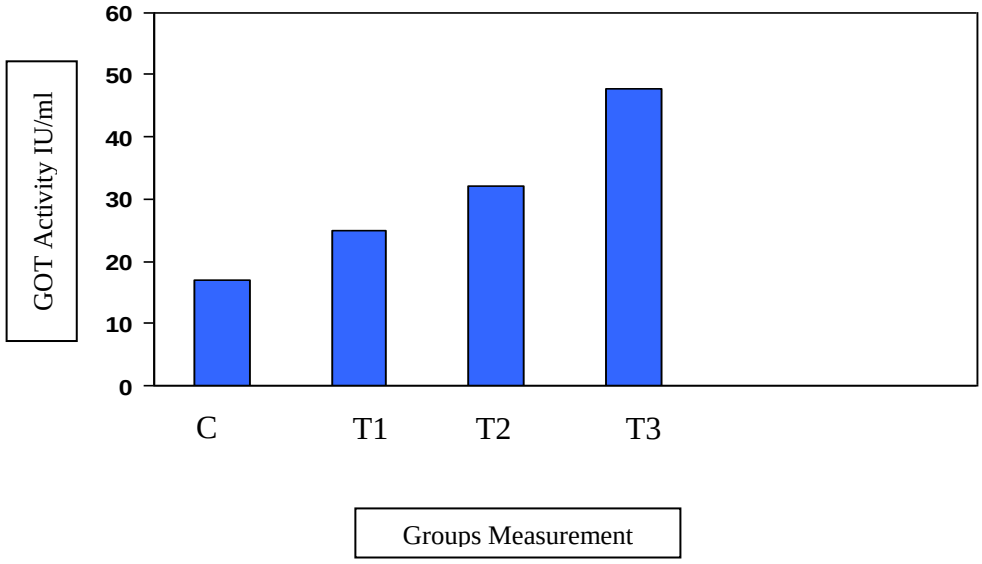

Figure (1) Effect of different doses of orally administers paracetamol on serum GOT in different experimental groups in comparison with control group.

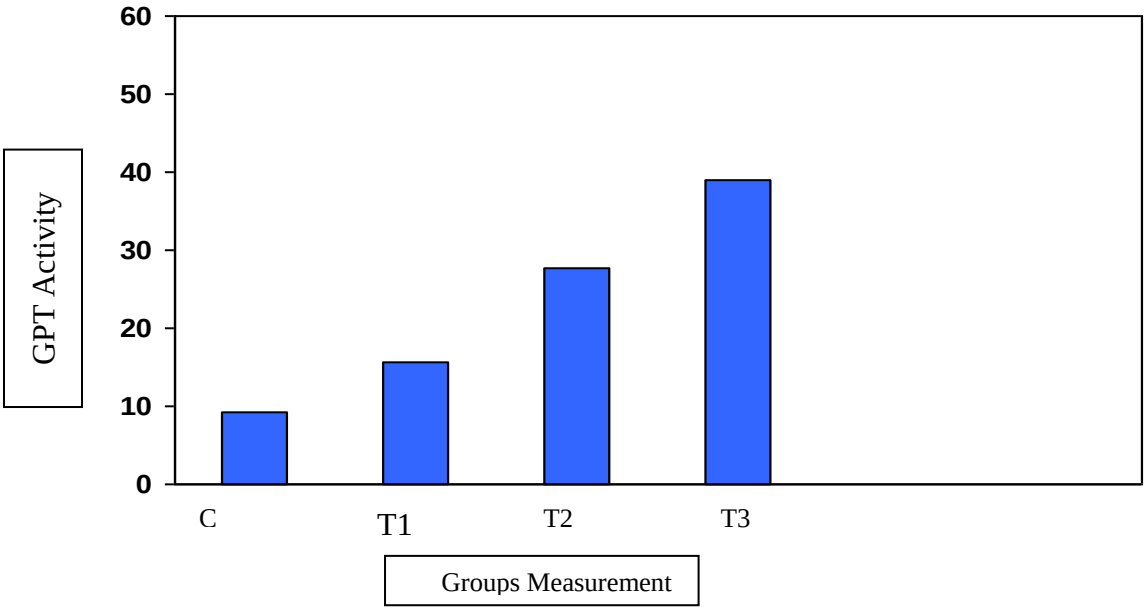

Figure (2) Effect of Different Doses of Orally Administer Paracetamol on Serum GPT in Different Experimental Groups in Comparison with Control Group.

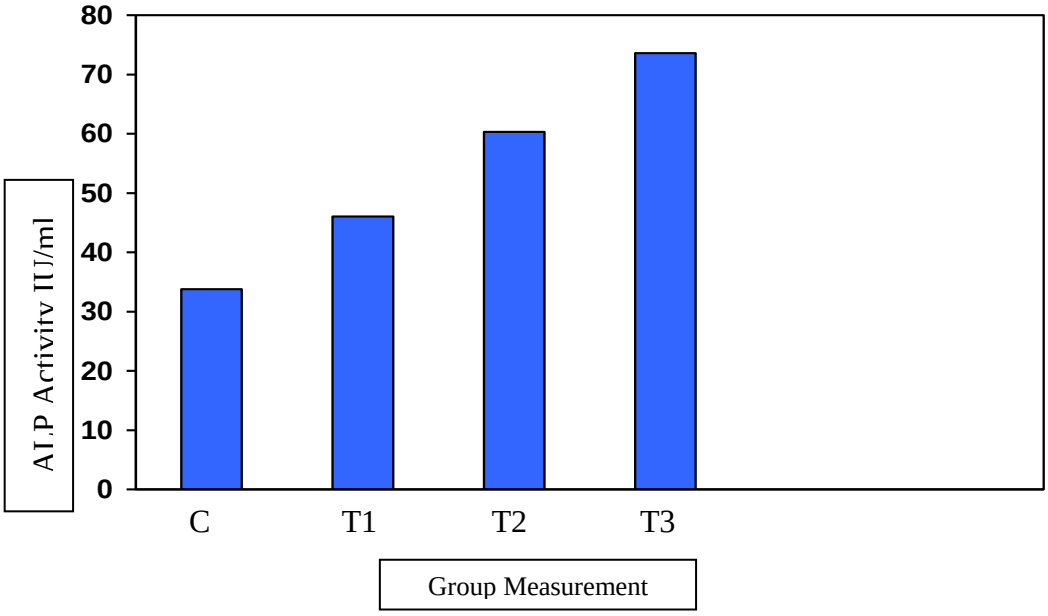

Figure (3) Effect of different doses of orally administers paracetamol on serum ALP in different experimental groups in comparison with control group.

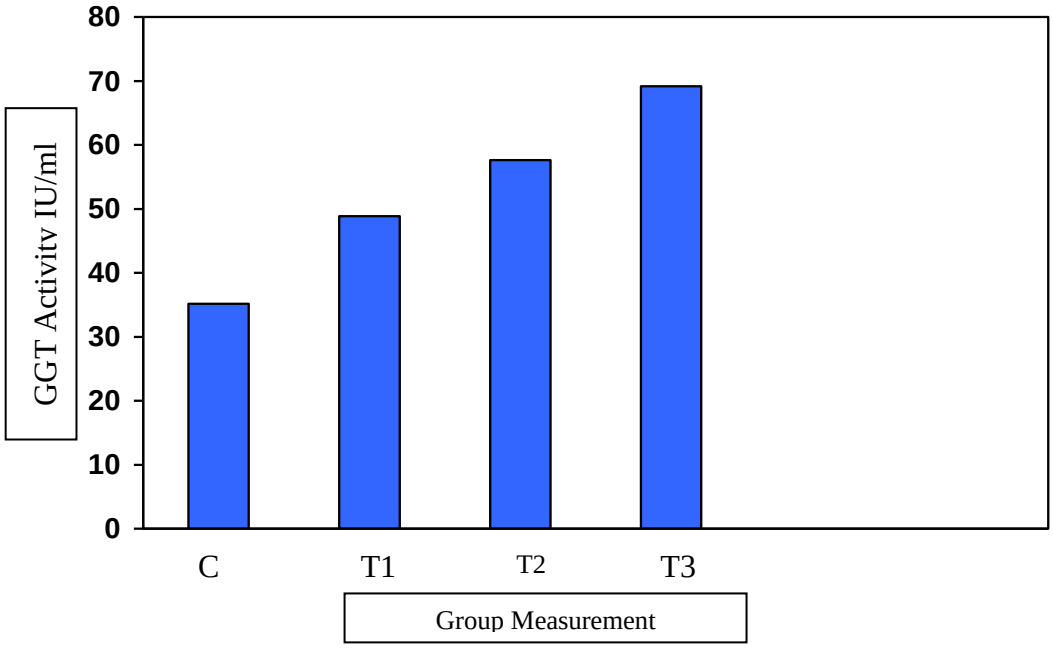

Figure (4) Effect of Different Doses of Orally Administer Paracetamol on Serum Ggt in Different Experimental Groups in Comparison with Control Group.