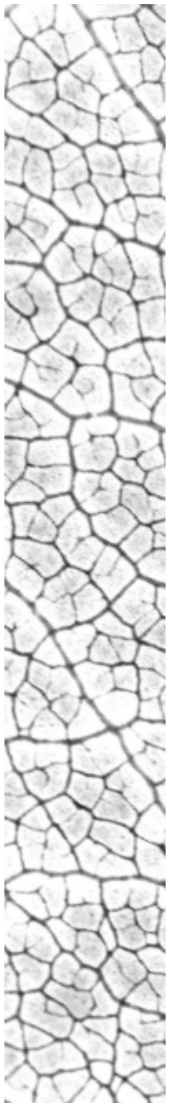

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# Histopathological and Biochemical study on the effect of flavonoids isolated from the plant *Curcuma longa* effective in liver enzymes (GOT, GPT) to female rats infected eggs diabetes induced in alloxan

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**Abstract**
The study was conducted in Faculty of Applied Science Department of Applied Chemistry / University of Samarra the study included to recognize the effects of flavonoids isolated from a Curcuma longa plant to minimize the damage caused by diabetes induced by alloxan in the histological structure of liver and the levels of the (GOT & GPT) in liver congeneric. The experimental divided to three group each group contains five rats. The one group is control and the second group is adiabatic animal by peritoneal injection Alloxan 50 mg/kg according to body weight it has not been treated, the final group was the diabetic animals and it has been processed with Alloxan 50 mg/kg and flavonoids compound 40 mg/kg and in the final of the experiment the animal killed for anatomy liver and cut 2gm of it to do histological sections from Liver for the study and measurement the concentration of enzymatic liver GOT & GPT.

**Keywords:** flavonoids, alloxan, liver, GOT & GPT

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## INTRODUCTION

The liver is an important organ (Li et al. 2015). Primary cells suffer lesions induced by oxidative stress in the liver are parenchymal (Sanchez-Valle et al. 2012). The liver works to regulate the level of glucose in the blood and the need for the body to store more animal starch with glycogen, which is converted into glucose when necessary (West 2002), and organic flavonoid compounds with a chemical structure consisting of in: Three hexagonal rings and three hydroxyl groups and two oxygen atoms, the compounds characterized the flavonoids with their inhibitory activity of certain enzymes and antioxidants (Dragan et al. 2007), and also function as a proof of the phenomenon of fat oxidation associated with diabetes and, consequently, necrosis and liver cell damage (Majumdar et al. 2008). Alloxan is a toxic glucose analog widely used to induce experimental diabetes in animals. It specifically annihilates the creator cells of insulin in the pancreas of the creatures and seems to establish a redox cycle with the disposition of superoxide radicals and undergo the decomposition of hydrogen peroxide with the development of hydroxyl radicals (Szkudelski 2001, Vanhorebeek et al. 2005, 2009). Plants have always been an exemplary source of medicines and mainly the currently available drugs have been obtained directly or indirectly from botanical products (Ojo et al. 2013). Curcuma longa is a perpetual rhizomatous herb that has a place with the family Zingiberaceae, a local of South Asia and commonly known as turmeric. In Malaysia, commonly known as Kunyit, the turmeric plant is a well-known element for preparing culinary dishes. In addition, it is used as a homegrown cure due to the common conviction that the plant has therapeutic properties. In the prescription of the society, the juice of rhizome of C. longa is used in the treatment of numerous ailments, for example, anthelmintics, asthma, gonorrhea and urine, and its essential oil is used in the treatment of carminatives, stomachs and tonics (Phansawan and Poungbangpho 2007). In traditional medicine, several plants and herbs have been used experimentally to treat liver disorders, including liver cirrhosis (Alshawsh et al. 2011). C. longa possesses antioxidant (Maizura et al. 2011), anti-tumor (Kunnumakkara et al. 2007), antimicrobial (Kim et al. 2005), anti-inflammatory (Kohli et al. 2005), wound

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healing (Panchatcharam et al. 2006), and gastroprotective activities (Miriyala et al. 2007). The past investigations have likewise demonstrated that the fluid concentrate of C. longa has hepatic defensive action against carbon tetrachloride lethality (Sengupta et al. 2011). Diabetes mellitus is a non-communicable, a disease which has been shown to improve with medicinal plants. It is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fats and protein metabolism resulting from defects in insulin secretion, insulin action or both (West 2002) Diabetes mellitus is a non-communicable disease, a disease that has been shown to improve with medicinal plants. It is a metabolic disorder characterized by chronic hyperglycemia with disorders of carbohydrates, fats and protein metabolism as a result of defects in insulin secretion, the action of insulin or both (West 2002). Genetic and ecological causes that cause abnormal levels of glucose (hyperglycemia) (Dragan et al. 2007). In this study, we evaluated the hepatic protective effect of the flavonoids of the rhizomes of C. longa against hepatic cirrhosis GOT and GPT in rats.

## MATERIALS AND METHODS

### Preparation of Flavonoid Extract

The turmeric plant purchased in local markets (city of Samarra), has been classified by the department of biology, college of education, University of Samarra. Then, the plant was cleaned well of impurities and ground to a fine powder using a blender. After that, the powder was extracted with ethanol (70%) using a Soxhlet apparatus. Then, the ethanol extracts were collected and evaporated by a rotary evaporator at room temperature. The gummy residue was dissolved using 50 ml of hot methanol (45-50 ° C) followed by vigorous mixing. This step resulted in the production of precipitated products of brown color. Then, the precipitated products were filtered and the solid material was collected. This product, called flavonoid compounds, was used for further fractionation and identification of flavonoids (Harborne 1973).

### Animal Models

#### *Animals and induction of diabetes*

All the experimental protocols will be carried out in accordance with the guidelines for the care and use of experimental animals. Healthy female albino rats were chosen. They lived in well-ventilated cages under normal environmental conditions (temperature and humidity). The animals were fed a commercial balanced diet and tap water. The animals were divided into three groups (five animals in each), all were standard food and water. Group I (control), Group II (DM induced by alloxan) The animals were injected with alloxan subcutaneously (50 mg / Kg), Group III (treatment group) The animals were injected with alloxan (50 mg / Kg) and Flavonoid extracts were administered daily (40 mg / Kg). Rats with moderate diabetes who have hyperglycemia (blood glucose level> 200 mg / dL) were used for the experiment. At the end of the experiment (10 days), the animals in the three groups fasted for 12 hours and collected blood samples.

### Enzymatic Assays: GOT, GPT

The laboratory animals liver (rat) was taken and an addition of solution normal saline and then mash using homogenizer (Chosh et al. 1983). The sample was separated by centrifugation device using the device type Abendorf tube 4000 r / min for 20 minutes the clear where taking and saved under a temperature of 4 °C until use.

#### *Measure the effectiveness of both the enzymes GOT and GPT in the liver*

The effectiveness of the enzymes have been identified by the way in which (Reitman and Franker 1957) use two test tubes for each sample, the first is a sample and the other is a blank, the first control sample and the second sample was prepared according to the method described by the company processed Randox, U.K was to know the effectiveness of the two enzymes by using a special table for each enzyme of these enzymes.

### Histopathology

The liver was harvested and fixed with 10% formalin and processed by a paraffin method, cut to a thickness of six microns using a rotary microtome and stained with hematoxylin and eosin (H & E) (Vacca 1985). The sections were examined by the histopathologist with the olumpis microscope (Japan). The photos were taken by a digital camera (sony-Japan 14 Megapixel). The results were evaluated using the ANOVA analysis of variance and the regression analysis using the SPSS programmer.

## DATA ANALYSIS

The values were calculated as mean ± S.E.M of at least three determinations. The statistical significance of the data was assessed by the unpaired t ‘test to determine the differences between the groups and the values of $p \leq 0.05$ were considered significant.

## RESULTS AND DISCUSSION

Alloxan is a toxic glucose analog widely used to induce experimental diabetes in animals. It specifically decreases the insulin-releasing cells in the pancreas in the creatures, and it seems that a redox cycle accumulates with the disposition of superoxide radicals and undergoes the decomposition of hydrogen peroxide with the development of hydroxyl radicals (Szkudelski 2001, Vanhorebeek et al. 2005, 2009). Enzymatic activities in tissues are generally used as a “marker” to determine the early toxic effects of foreign compounds

**Table 1.** The effect of flavonoids isolated from the plant turmeric on enzymatically GOT, GTP in serum liver homogenized female rats treated in Alloxan

| Parameter | GOT IU/ml Liver Homogenized | GPT IU/ml Liver Homogenized |
|---|---|---|
| Control | 24.925 ± 1.5275 A | 62.00 ± 5.00 A |
| Alloxan mg\kg50 | 16.9667± 1.5275 C | 14.00± 2.00 C |
| Alloxan + flavonoid extract mg/kg 20 | 23.125± 5.6862 B+C | 27.6667 5118 B |

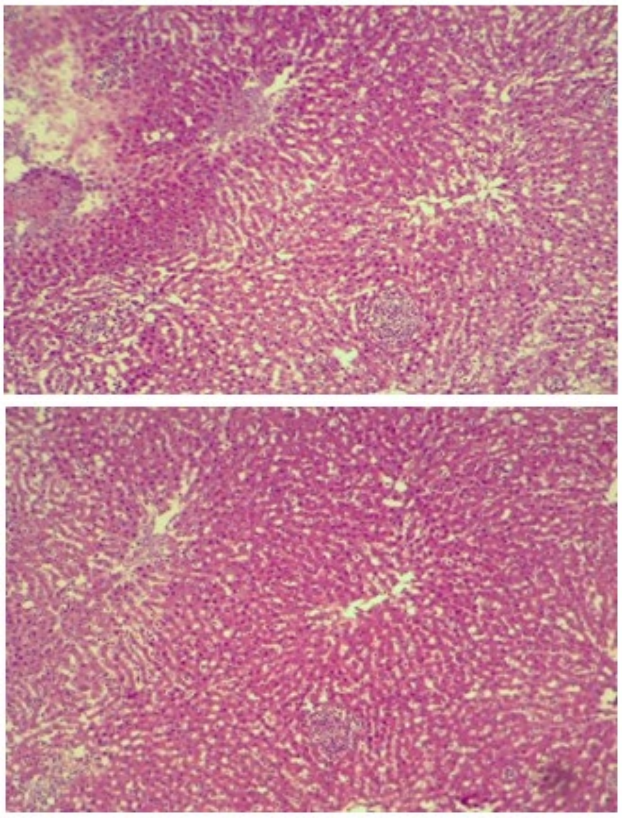
Like similar letters in vertical form indicate the lack of a significant difference in the level of significance P<0.05
Like similar letters in vertical form indicate a significant difference in the level of significance P<0.05

administered to experimental animals (Adesokan and Akanji 2004, Adesokan et al. 2009, Akanji and Ngaha 1989, Ojo et al. 2013, 2014, Sokeng et al. 2005, Weaver et al. 1978). Glucose is the key physiological regulator of insulin secretion; indeed, short-term exposure of, B-cells to in-creasing glucose concentrations induces proliferation in a concentration-dependent manner. In addition to its effect on,-cell turnover, hyperglycemia also impairs, B-cell secretary function. This glucotoxic effect is evident before apoptosis leads to a significant decrease in, B-cell mass (Donath and Halban 2004). Results of statistical analysis showed a decrease significantly (P<0.05) in the effectiveness of the enzyme GOT in liver homogenized Group (2) diabetic Decreasing (16.9667± 1.5275) The unit / liter , Units / liter compared to its effectiveness in the control group liver homogenized and adult (24.925 ± 1.5275) , while noted for high moral (P<0.05) in the group of infected rats and treatment compound flavones Decreasing (23.125± 5.6862) The unit / liter when compared to the group liver homogenized Diabetic Rats ,The results of the effectiveness of the enzyme GPT get the results show significantly decreased (P<0.05) in the liver homogenized Diabetic Rats Decreasing (14.00± 2.00) The unit / liter when compared to the rate of effectiveness in the control group liver homogenized and adult (62.00 ± 5.00) The unit / l , has been observed for high moral (P<0.05) in the effectiveness of this enzyme in rats with treatment group and compound flavones Decreasing (27.6667 ± 3.5118) Compared to a rate of effectiveness in liver homogenized diabetic rats group as in **Table 1**.

The significant decrease (P<0.05) in the effectiveness of the GOT rate, the GPT in the congeneric liver of the group infected with diabetes, may be largely due to a greater leakage of enzymes from the liver cells into the bloodstream, where there is GOT , GTP in the cytoplasm and organelles of liver cells and in large quantities and in case of hepatocyte lesions or enzymes released by death in the bloodstream, so the decrease in their focus in the liver homogenized reflects the severity of the damage in the liver (Prakasam et al. 2004). The results of the statistical analysis for a significant rise in GOT rate , GPT in congeneric liver in flavonoid treated group and this could be due to the role of a compound flavonol antioxidants where he works to minimize the damage caused by the intensity of Oxidative by raising the level of GSH and reduce GOT, GTP in serum (Jagadeesan and Kavitha 2006), the high rate of GOT and the GPT in the liver homogenized guide the return to normal liver cells by treatment in flavonoids in reducing the damage caused by the treatment in alloxan.

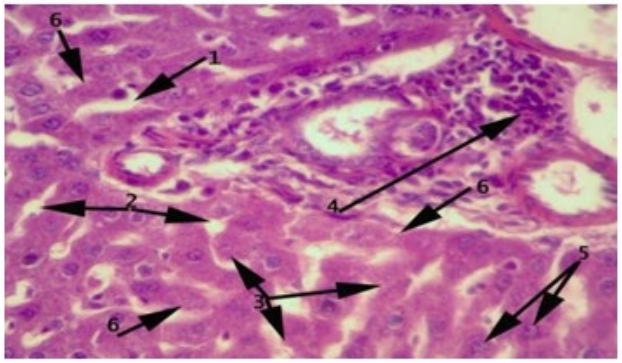
The sections were processed in a series of alcohol-xylene and stained with hematoxylin and eosin. The slides were studied under a light microscope to detect any damage / histological protection.

**Group I**

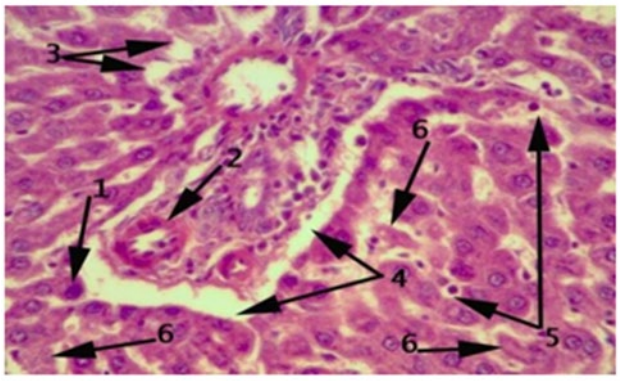


Rat liver control group, Stain H&E (100X). Images show the rat liver normally a component of the central vein is surrounded by liver cells, which are arranged in plates, as well as the presence of Sinusoids that separates the platelets

**Group II**



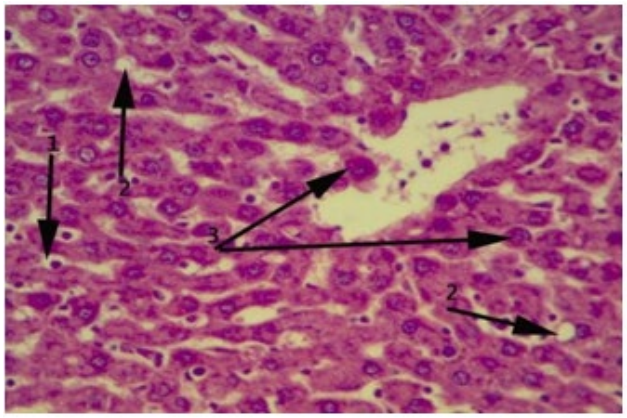

Rat liver treated with alloxan showed: (1) Necrosis cytoplasim, (2) vaculative, (3) Apoptosis, (4) Infiltration Lymphocyte, (5) karyolysis, (6) fibrosis, Stain H&E



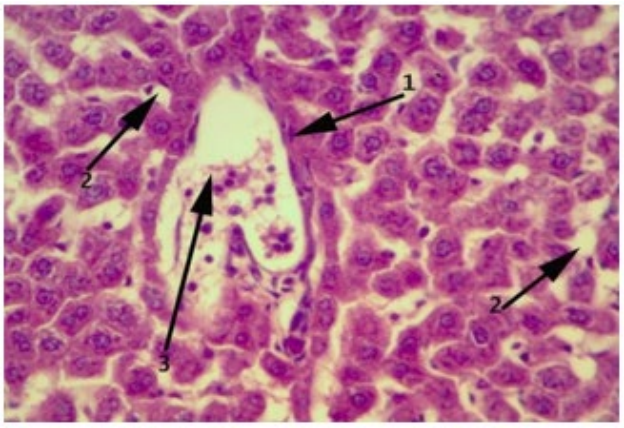

Rat liver treated with alloxan showed: (1) Auxes nucleus (2) Hemorrage, (3) Vaculation, (4) Discoid Necrosis, (5) Necrosis cytoblasim, (6) fibrosis, Stain H&E (400X)

In the second group note of the images occurrence of histological lesions represented atrophy liver cells and infiltration of lymphocytes into the liver tissue. In the second note of the images occurrence of histological lesions represented atrophy liver cells and infiltration of lymphocytes into the liver tissue. Tissue sections show a nuclear karyolysis in some cells of the liver and the other suffered from the Apoptosis of the nuclei and also section clearly shows thickening nuclei and picnotic nucleus and dilated Sinusoids. Cirrhosis of the liver caused by the presence of scar tissue replaces healthy tissue, causing obstruction of blood flow through the portal vein and affects the normal functioning of the liver. Damage to liver tissue impedes blood flow at its natural vessels. In addition, these cell secretes a substance known as transformational growth factor beta 1 TGF-β1 a substance that leads to Cirrhosis response and proliferation of connective tissue. The presence of infiltration of inflammatory cells of the lymph around some blood vessels. This shows for inflammation, as happened necrosis focal and discoid in the liver cells and found some of them degenerate Vacuolated. Some showed nuclei increase in size Auxes of Nucleus and a number of Red blood cells prevalent in the tissue a sign of bleeding Haemorrhage. The present investigation indicated that a single dose of alloxan (150mg / kg) intraperitoneal for adult male albino rats (210-220g) was adequate to induce histological changes in the liver of diabetic rats induced by alloxan with characterized appearance, enlarged and swollen hepatocytes (Sugimoto et al. 2005).

**Group III**




Rat liver treated with alloxan showed: (1) Cytoblasim necrosis, (2) vaculation, (3) Auxes nucleus, Stain H&E (400X)




Rat liver treated with alloxan showed: (1) Infiltration lymphocytes, (2) vaculation, (3) Fibrin Deposition, Stain H&E (400X)

Notes in this group improved in a number of nuclei and the beginning of the reform of the central vein wall damage due to this extract therapeutic role Because liver. The liver assumes an imperative role in the upkeep of blood glucose levels by directing its digestion. Alloxan causes a significant increase in the activity of SAST and SALT, according to which it is directly related to the changes in the metabolism in which the enzymes are involved. Expanded transaminase exercises, which are active without insulin due to the accessibility of amino acids in the blood of DM and are also in charge of expanded gluconeogenesis and keto-ketogenesis (Batran et al. 2006).

Cirrhosis occurs as a result damage of actrocytes, These cell secretes a substance known as transformational growth factor beta 1 TGF-β1 a

substance that leads to response proliferation of connective tissue, The fibrous tissue packs (hurdles) separating the nodules of liver cells, which resolved eventually replace full histological structure of the liver; leading to reduced blood flow in all organs of the body rate. It is noteworthy that high blood pressure in the portal vein is the reason behind more complications unit to liver cirrhosis. The high blood glucose level of rats treated with alloxan resulted in necrosis of the liver cells may be due to a lack of blood supply to the liver resulting from the get clogged in the hepatic artery , which leads to a lack of oxygen hypoxia and this shortage causes the liberation of enzymes lysosomes and secretory materials Others this explains some of the necrosis of liver cells (Macseen and Whaley 1992), or that the damage may be the result of an act of free radicals, where many of the studies suggest that diabetes is a disease of spaces that result in the generation of large numbers of free radicals (Kecui and Keyixu 2004) and that interact with the most important cell components such as DNA or DNA undiminished oxygen with the cell membrane, resulting in percentage and thus its inability to carry out their functions (Valko et al. 2006). And the appearance of the output inflammatory areas on the occurrence of necrosis of the cells of the liver, which in turn leads to obtain inflammatory response, phosphorylation resulting from free radicals gathered cause crashes liver cells or it may be a result of the impact of toxic to alloxan, and also that the oxidation of lipids of the cell membrane leads to the occurrence of inflammatory response, the expansion of sinusoid may come back to get weak in the venous flow in the hepatic vein or may be a high pressure pyloric vein to the liver is another reason for the occurrence Expansion of sinusoid. While histological study of Beit liver rats with diabetes and treatment in al flavone, to a slight expansion in the sinusoid and remnants of necrosis, suggesting that the flavonoids an important role, where he works as a proof of the phenomenon of fat oxidation associated with diabetes and consequential them from necrosis and damage to the cells of the liver and thus regulate tissue liver (Sanchez-Valle 2012), and is a flavonoid antioxidants as it works to protect cells from the damage of vandalism resulting from the reaction of free radicals Free radical (Arikan et al. 2009), as flavone ability to inhibit its effectiveness and is characterized by its composition process and thus reduces or prevents damage to biomolecules (Giovannucci et al. 2006).

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