

# Anti-diabetic activity and hepatoprotective effect of *Chrozophora tinctoria* (L.) Rafin leaves aqueous extract in STZ induced diabetic rats model

Mohammed A. Auda<sup>1</sup> and Muna Hasson Saoudi\*<sup>2</sup>

<sup>1</sup>Chemistry Department, College of Science, Thi Qar University, Iraq

<sup>2</sup>Chemistry Department, College of Science, Al Muthanna University, Samawah, Iraq

## Abstract

The responses of streptozotocin (STZ)-induced diabetic rats for the aqueous *Chrozophora tinctoria* (L.) Rafin leaves extracts were studied in this new work. To achieve this aim, Physiological, biochemical and histological parameters of (STZ) diabetic rats are evaluated. Rats are distributed into five groups: Control, diabetic rats and Three diabetic groups received orally different doses of (50, 70, and 90 mg/kg body weight (BW)) from the target extract for aperiod of 35 days. When the therapy is ended, blood samples, liver tissues were taken and glucose, insulin, ALT, AST, ALP were determined as well as liver histology. In general, the results of this study show that the liver tissue damage serum ALP, AST ALT activities and blood glucose levels were remarkable normalized. Furthermore, it was observed that the BW of diabetic control group is decreased. However the BW is elevated slightly in the diabetic treated groups as well as serum insulin. In addition, the extract improves the liver function and reduces lesions associated with diabetic state in STZ induced rats. Moreover, the effect of oral administration of *Chrozophora tinctoria* (L.) Rafin at a dose of 90 mg / kg body weight was more efficacy than the 50 and 70 mg/kg body weight. The results indicate the extract exhibit protective effect on liver tissues, and prove its potentials as an antidiabetic and hepatoprotective agent.

**Keywords:** *Chrozophora tinctoria* (L.) Rafin; diabetes; streptozotocin; liver function

## INTRODUCTION

Diabetes mellitus is an endocrine disorder recognized as a syndrome resulting from a variable interaction of hereditary and environmental factors [1]. It is associated with insulin resistance (type 2, DM2) or with absolute or relative deficiency in the secretion of insulin (DM1) [2,3] as well as defecting in carbohydrate, protein and lipid metabolism [4]. The diabetes became a common disease in which more than 170 million people worldwide are affected [5]. In addition, It is a leading cause of morbidity and mortality due to diabetic complications such as heart disease, retinopathy, liver disease, peripheral neuropathy, nephropathy and stroke [4]. Hyperglycemia leads to these metabolic disorders and various complications [6]. Moreover, liver disease can cause death in persons with type 2 greater than of cardiovascular disease [7].

Many evidences indicate that the complications seen in diabetes as a result of free radicals production. However, the uncontrolled diabetes have species increased of auto oxidation of glycosylated proteins, damage membrane induction, cellular lipids proteins oxidation and activation of the sorbitol pathway [8]. Production of free radicals increases with Hyperglycemia leading to liver injuries related to carbohydrate metabolism disorder [9,10]. These injuries are represented by cellular necrosis due to increased oxidation and lipid accumulation in the hepatocytes [11]. Many complications are caused by defects in the body antioxidant defense systems [12], oxidative stress, DNA damage and cell death [13]. Natural antioxidants from plants repair these damages, and may be safe, an effective and economical alternative therapy for diabetes protection [14].

Medicinal plants have been largely used in treating various diseases as recommends by WHO [15]. *Chrozophora tinctoria* (L.) Rafin which grows in deep soils and sandy plains in Al-Salman District, a district of Al-Muthanna

governerate, Iraq, belongs to Euphobiaceae family. This plant was used for coloring Dutch cheese and certain liquors. Traditionally it is used for the treatment of warts [16]. It was also used as an emetic, cathartic, and for fever treatment [17]. Analysis of *Chrozophora tinctoria* (L.) Rafin showed that it contained flavonoids, alkaloids, diterpenoids, xanthenes, coumarins, chromones, diterpenoids, and phenylpropanoid glycosides [18, 19]. This report is the first on investigating the protective effect on the liver injury in STZ-induced diabetic rats by examining the protective effects of varying doses of aqueous *Chrozophora tinctoria* (L.) Rafin extract on liver enzymes and histopathological changes possibly occurring in diabetic experimental rats which may serve to fill the knowledge gap about the possible effective treatment of DM.

## MATERIALS AND METHODS

### Plant Collections and Identification

The *herbal plant* was collected during the period of May to September (2017) from the Al-Salman desert which is located in Al Muthanna governerate, Iraq 200 km (124 miles) south of Samawah city. The plant has been identified by botanical Dr. Taha Yaseen Mhoder Al-Edany (Plant Taxonomy and Ecology, College of Agriculture, University of Basrah, Iraq).

### Aqueous Extract

Fresh leaves of *Chrozophora tinctoria* (L.) Rafin were dried in the shade for two weeks and then 250 g collected separately. The leaves were crushed gently to powder. In 2.0 L of sterile distilled water the powder was suspended for 24 hours at 50 °C [20]. The resulting solution was filtered and stored for the rats pharmacological study [21].

### Animal Models

The male rats were purchased from Biotechnology Research Center of AL-Nahrain University, Iraq. The rats

have 90 days of age and  $220 \pm 10$  g of weight. The rats maintained in a 12 h for both light and dark cycles at  $27^{\circ}\text{C}$  during the study in the animal house. The roles of National Institutes of Health policy were followed for animal care during the experiment. Control and treated rats were received food and water ad libitum.

#### Diabetic Model rats and treatment

The male rats intraperitoneally injected by a single dose of 55 mg/kg BW STZ (Sigma-Aldrich) that dissolved in saline solution [22]. The normal control group of rats received an equivalent amount of saline solution. After two days of injection and by (using the glucometer ACCU-Check, Roche Diagnostics Corporation, USA), the levels of fasting blood glucose were checked. The rats were considered diabetic with fasting blood glucose over 11mmol/L [23]. Extract *Chrozophora tinctoria* (L.) Rafin solution was administrated with different selected doses as daily drink. The doses were daily prepared in order to avoid oxidation and rancidity and unconsumed food over 24 h. Animals used as normal control received standard rat pellet with ad libitum, distilled water till the end of the experiment.

#### Animals Experimental Design

Twenty-five male rats were randomly divided into five groups and placed in cages according to the groups, containing 5 rats per group. Group I, considered as the normal control, given standard food and water for a period of 35 days; Group II, served as the diabetic control, given Streptozotocin 55mg/kg b.w. as a single dose; Group III, received Streptozotocin (55mg/kg b.w.) and given leaves aqueous extract of *Chrozophora tinctoria* (L.) Rafin 50mg/kg b.w. administrated orally every day for a period of 35days; Group IV, received Streptozotocin (55mg/kg b.w.) and given leaves aqueous extract of *Chrozophora tinctoria* (L.) Rafin 70mg/kg b.w. administrated orally every day for a period of 35 days. Group V, received Streptozotocin (55mg/kg b.w.) and given leaves aqueous extract of *Chrozophora tinctoria* (L.) Rafin 90mg/kg b.w. administrated orally every day for a period of 35 followed by receiving standard food and water for diabetic control and the treated diabetic rats. After 35days, the rats were fasted for 12-hours and sacrificed by inhalation mild diethyl ether. Blood samples were obtained by means of heart puncture, plasma was separated and stored for glucose, insulin and liver enzymes determination, liver tissues were collected for histological study.

#### Acute Toxicity Study

Fasted adult rats were allocated in five groups of eight animals per group. Aqueous *Chrozophora tinctoria* (L.) Rafin extract was administrated to experimental rats. The groups treated orally dosed with *Chrozophora tinctoria* (L.) Rafin (50, 100, 200, 400, 500, 700, 900, 1000 mg/kg) of the extract respectively. The selected doses never showed physical signs of toxicity, or body weight changes up to 14 days. No death in animals was observed up to 950 mg/kg of aqueous *Chrozophora tinctoria* (L.) Rafin extract. So, 50, 70 and 90 mg/kg doses of body weight were selected as effective doses for medication experiments [24].

#### Estimation of glucose, insulin, ATP, AST and ALP

The blood glucose was estimated using a glucose enzymatic-colorimetric test kit (Glucose-TR. SPAIN).

Insulin levels were determined by using (ELISA) kit (Accu Bind-Elisa -microwells-USA) according to the instructions. Determination of selected Liver enzymes, Alanine aminotransferase ALT, Aspartate transferase AST, and Alkaline phosphatase ALP tests were estimated using BIO RAD ( LiquiCHEK<sub>TM</sub>)kit -USA.

#### Tissue Collection and Histopathology

At the end of the experiment, the tissue sections were excised from rats livers according to [25,26]. The livers were fixed in 10% buffered formalin solution. After that, the tissue samples were embedded in paraffin block wax for histological study. Sections of 4-6  $\mu\text{m}$  thickness were taken using rotary microtome (Lecia), stained with hematoxylin and eosin (H&E) [27]. The stained slides were then examined with APCAM-5 USB 2 digital cameras attached to a computer monitor.

#### Statistical Analysis

The data were presented using one way ANOVA and Newman-Keuls Multiple Comparison Test, N=5, NS (NON SIGNIFICANT) \*P<0.05, \*\*P<0.01, and \*\*\*P<0.001.

## RESULTS

#### Body Weight and Blood Glucose Levels

As shown in Figure 1(a) the administration of *Chrozophora tinctoria* (L.) Rafin aqueous extract cause slightly changes on body weight in diabetic control and all the treated groups. A significant loss of body weight in the diabetic control group rats was observed when compared to control healthy group. In addition, there was a significant gain of body weight in the treated groups. The maximum gain was at dose of 90 mg/kg. Figure 1 (b) showed oral administration of *Chrozophora tinctoria* (L.) Rafin extract (50, 70 and 90mg/ kg BW) to STZ diabetic rats during 35 days. It was observed that the serum glucose level was significantly reduced. Fasting glucose of STZ induced diabetic rats were significantly increased in comparison to healthy rats. The maximum effect of the plant extract for lowering glucose levels was at dose of 90 mg/kg (group VI) as compared to non-treated group.

#### Lethal Dose (LD<sub>50</sub>)

Normal rats were treated with seven doses selected (50, 100, 200, 400, 500, 800, 1000 and 1600 mg/kg. B.W) for the toxicity study (Figure 3). After 14 days, the morphological changes were observed and the number of dead animals was counted. The acute oral LD<sub>50</sub> value of *Chrozophora tinctoria* (L.) Rafin was calculated as 995mg/kg body weight. The functional biomarkers were analyzed for the determination of toxic changes during the extract administration. There were no toxic changes observed at a concentration of 100, 200, and 800 mg/kg of body weight for 30 days. Thus, it could be concluded that the *Chrozophora tinctoria* (L.) Rafin was nontoxic till the dose range of 995 mg/kg B.W. as shown in Figure 5. The doses of *Chrozophora tinctoria* (L.) Rafin extract used in experiment were 50, 70, and 90 mg/kg body weight because the doses were considered safe during the toxicity treatment, using the aqueous *Chrozophora tinctoria* (L.) Rafin extract.

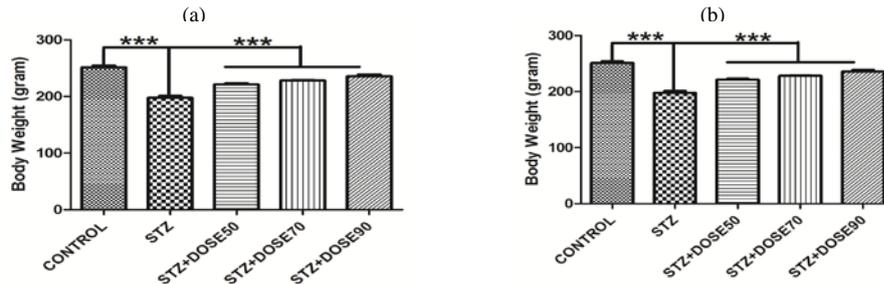


Figure 1: Effect of *Chrozophora tinctoria* (L.) Rafin extract at (a) different concentrations on body weight in normal and experimental rats. (b) different concentrations on blood glucose in normal and experimental rats.

**Insulin Levels and Liver Enzymes**

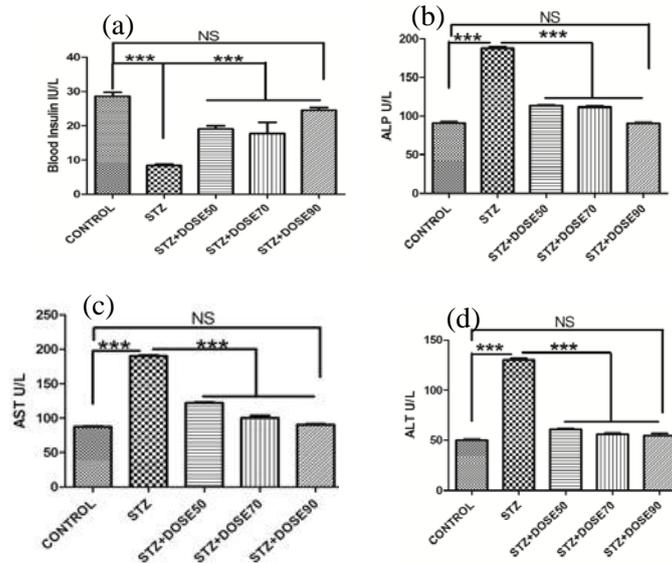


Figure 2(a) showed fasting insulin levels of control group and (STZ)-induced diabetic rats. The insulin levels of non-treated group were significantly decreased when compared to the healthy rats (group I) while the three treated groups showed promotes of insulin levels. The maximum rise of insulin levels near normal value was at a dose of 90 mg/kg B.W. (for a period time 35 days) in comparison to the other groups treated with 50, 70 mg/kg. The administration of *Chrozophora tinctoria* (L.) Rafin in STZ treated groups shows similar decrease in the AST, ALP and AST level, while level of serum AST ALP and AST in STZ-induced diabetic rats (groupII) observed significantly increased as compared to those in healthy group. Through administration of different concentrations of *Chrozophora tinctoria* (L.) Rafin (50 mg/kg and 90 mg/kg) the level of serum liver enzymes lowered to a good value. The maximum enhancement of serum liver enzymes was observed in group given *Chrozophora tinctoria* (L.) Rafin extract of 90 mg/kg as shown in Figure 2(b, c, and d).

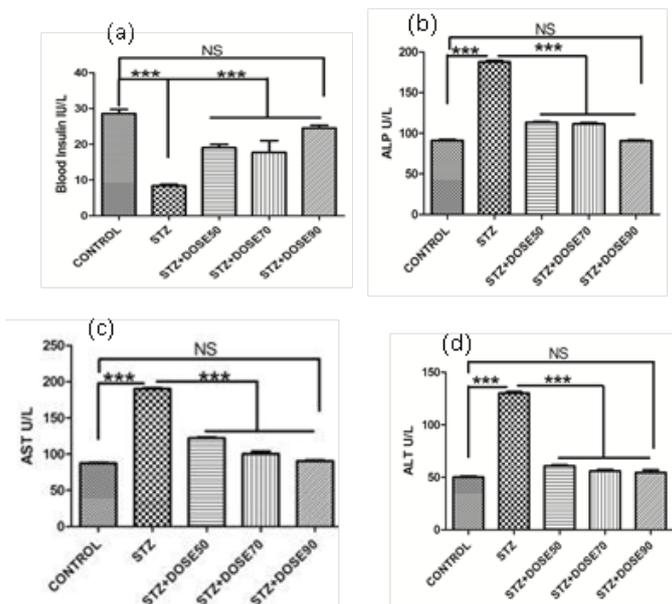


Figure 2: Effect of *Chrozophora tinctoria* (L.) Rafin extract at: (a) different concentrations on blood insulin in normal and experimental rats.(b, c, and d) different concentrations on the liver enzymes ALP, AST, and ALT respectively.

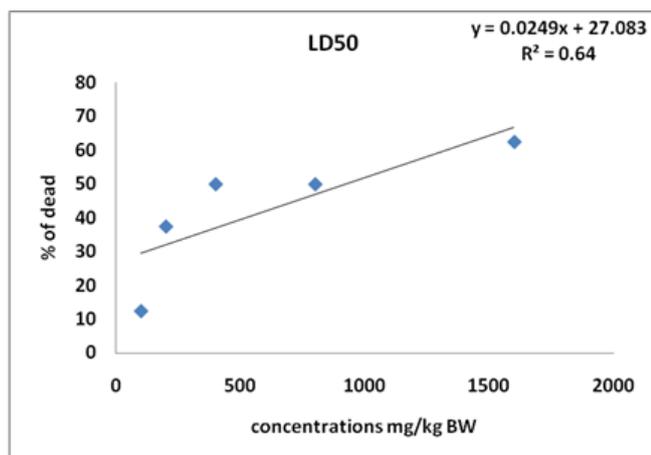


Figure 3: LD<sub>50</sub> dose response curve of *Chrozophora tinctoria (L.) Rafin*.

### Histopathological Findings

Liver sections of treated diabetic rats reveals noticeable degree of hepatoprotection against the hepatic changes. The capability of herbal plant *Chrozophora tinctoria (L.) Rafin* to protect liver seems to be in proportion to increasing dose of the aqueous extract as compared to the STZ non-treated group. The section of rat liver of normal control group showed normal hepatocytes which arranged as hepatic cords with prominent nuclei, normal portal area, normal hepatic artery and portal vein. The liver parenchyma was normal structure, no inflammatory cells aggregations, no fatty degenerations, no blood conjuration with normal portal area (as shown in Figure 4). While (Figure 5) showed the tissue section of liver after treated with (STZ). The histological results of liver after injection with STZ noted abnormal hepatic cords with wide cystic dilation and aggregation of inflammatory cells beside the cystic dilation. The tissue section showed abnormal portal area with prominent spaces between abnormal hepatocytes. The liver sections obtained from STZ-diabetic rats treated with

different doses of *Chrozophora tinctoria (L.) Rafin* (50 mg/kg, 70 mg/kg and 90 mg/kg) showed less pathological changes and improved liver hepatocytes. In (Figure 6) the tissue section of liver after treated with extract of 50 mg/kg showed the hepatocytes have prominent nuclei, reduced fatty degenerations in some locations of liver parenchyma. The portal area was irregular in shape with some cluster of inflammatory cells near portal area and the hepatocytes arrangement as short hepatic cords, surrounded by some of kupffer cells. The tissue section of liver after treated with extract 70 mg/kg showed in (Figure 7). Normal liver parenchyma without fatty degeneration was observed, the portal area was normal structure with prominent bile duct. Histological results noted disappeared of the clusters of inflammatory cells, the most hepatocytes arrangement as long hepatic cords. The central vein was normal in shape and lumen but reduced in blood flow so, the hepatic artery have prominent lumen without blood. Current results showed prominent bile duct through the liver parenchyma. Tissue section of liver after treated with extract 90mg/kg is given in (Figure 8). The liver showed normal arrangement of hepatic cords, which consist of normal hepatocytes. The hepatocytes have clear nuclei with normal cytoplasm. The tissue section of liver showed completely the absence of inflammatory cells, no fatty degeneration, generally the liver parenchyma have very long hepatic cords with normal distribution of kupffer cells between hepatic cords. The portal area was normal in shape. The bile duct appeared filled with secretion. The central veins have blood cells compared with previous groups. Current result of liver showed normal spaces between the hepatic cords. The tissue results of liver after treated with 90mg/kg were similar to tissue structure of control group generally. Highest improvement as well as hepatoprotective effect exhibited in the dose of 90 mg/kg as mention in (Figure 7), as compared to the control group in (Figure 4).

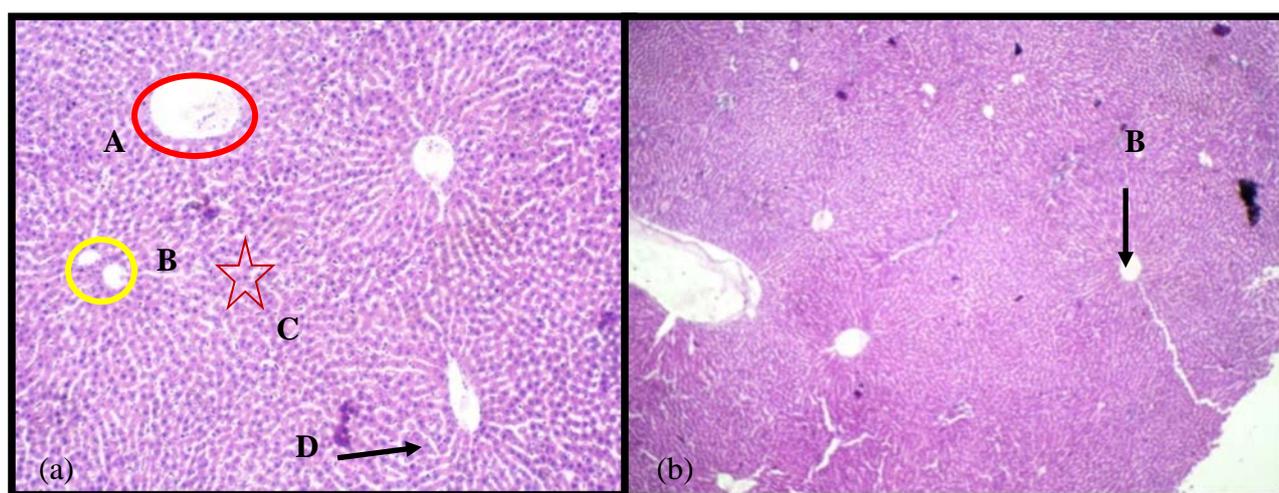


Figure 4: Photograph of liver in control group showed: A-Central vein, Portal triad, B- Hepatic cord, C-prominent bile duct. D- liver parenchyma. H & E (a-200X), (b-100X).

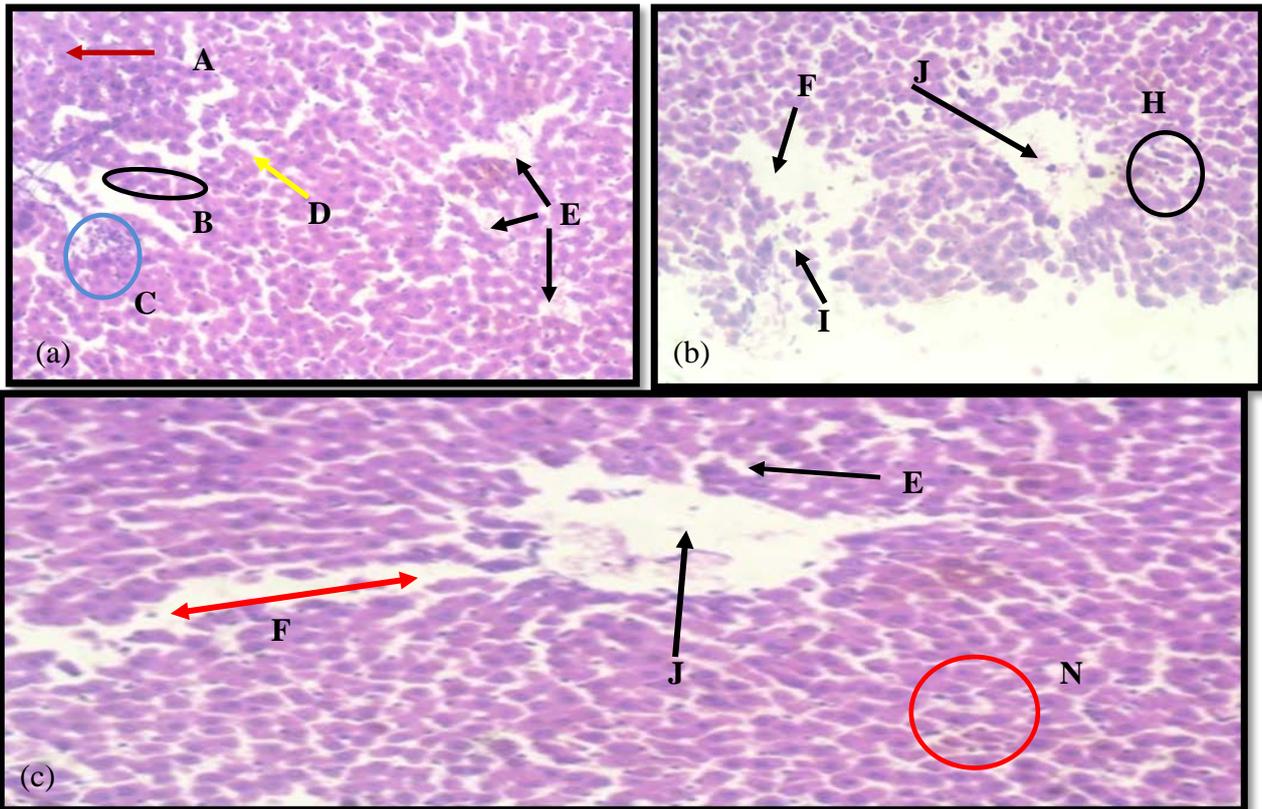


Figure 5: Photograph of liver in STZ Diabetic Rat liver showed : Inflammatory cells (A), destruction hepatic cord (B) necrosis (C), abnormal hepatic cord (D), fatty degeneration (E), wide cystic dilation (F), abnormal portal area (J), aggregation of inflammatory cells (H), acute hepatic degeneration (I). H & E 200X (a), (b) and (c).

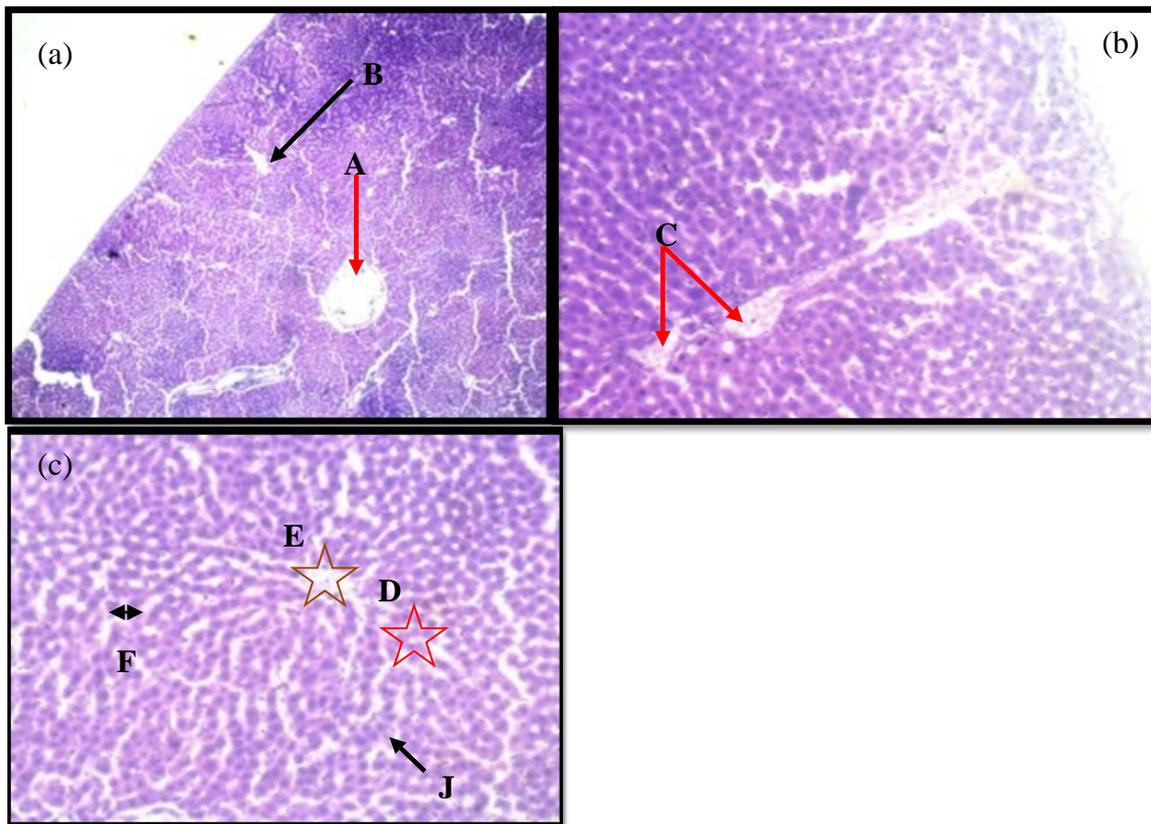


Figure 6: Photograph of liver in STZ Diabetic Rat liver treated with 50 mg /kg showed: central vein (A), abnormal portal area (B) fatty degeneration (C), cluster inflammatory cells (D), irregular central vein (E), short hepatic cord (F), kupffer cells (J). H & E 200X (a), (b) and (c).

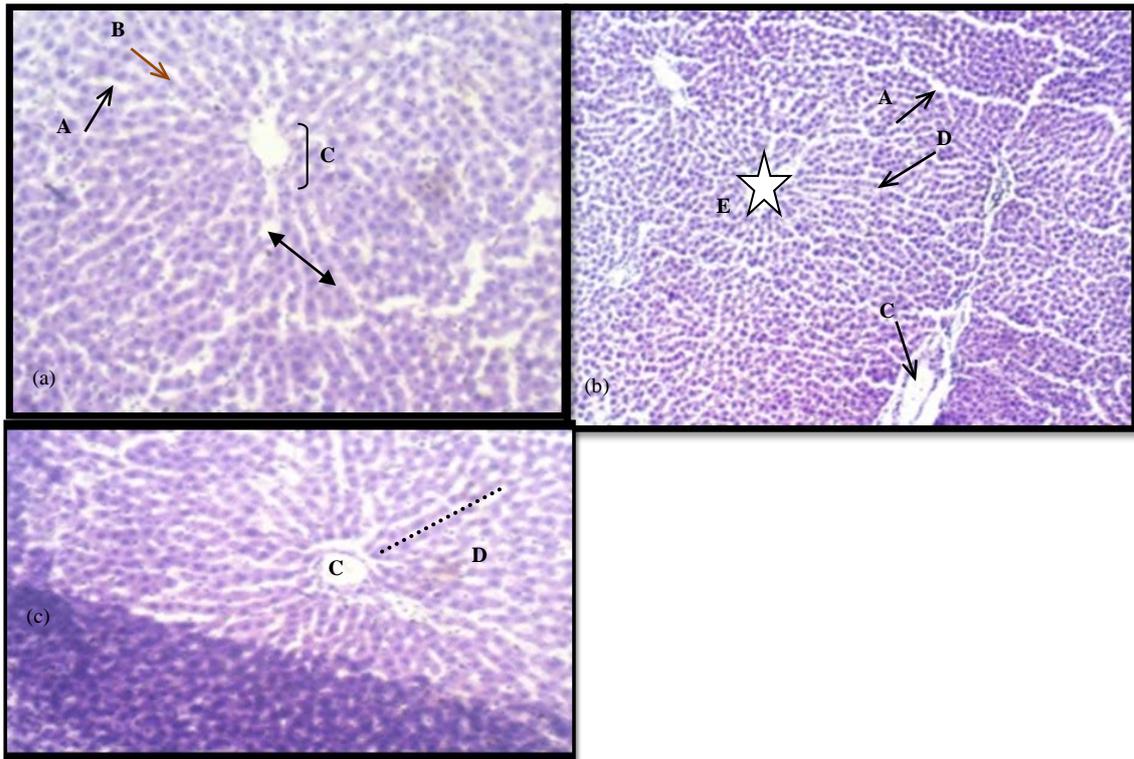


Figure 7: Photograph of liver in STZ Diabetic Rat liver treated with 70 mg/kg showed: prominent bile duct (A), kupffer cells (B), central vein (C) hepatic cord (D) portal area (E). H & E 200X(a), (b) and (c).

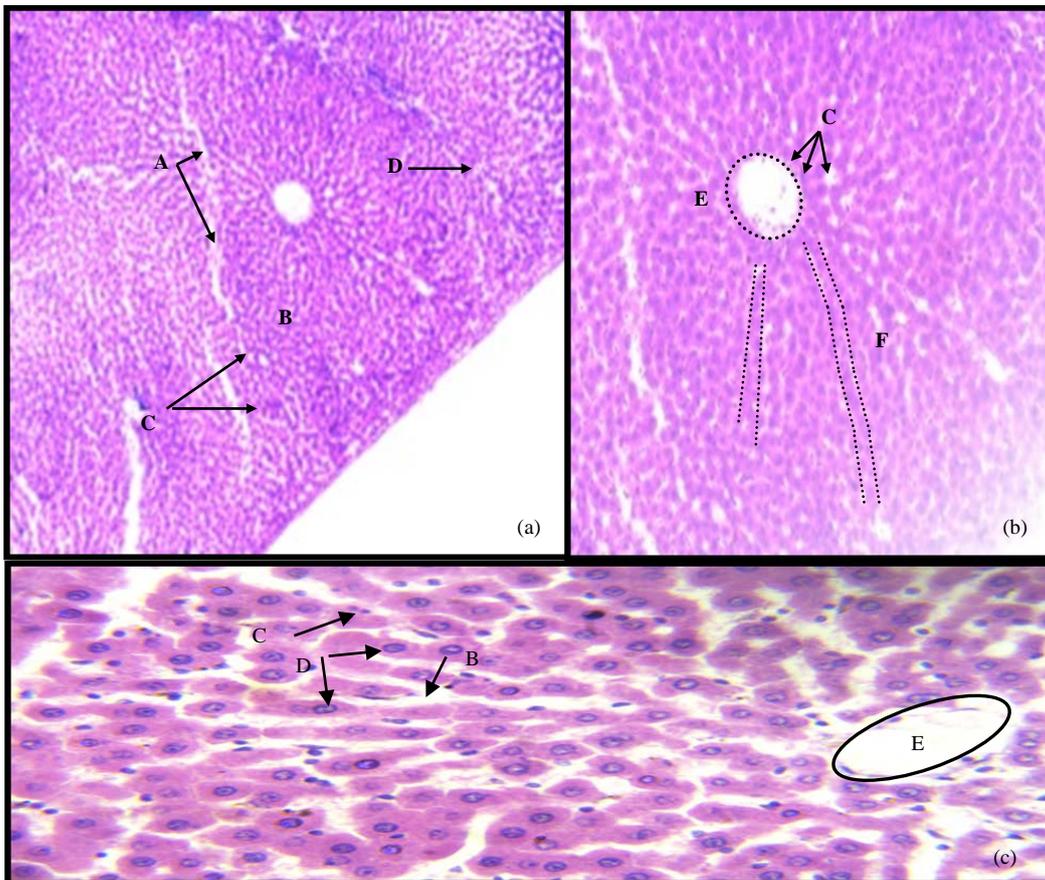


Figure 8: Photograph of liver in STZ diabetic Rat liver treated with 90 mg/kg showed prominent bile duct (A), liver parenchyma (B), hepatocytes (C), kupffer cells (D), central vein (E), hepatic cord (F), H & E 200X (a), (b) and (c).

## DISCUSSION

In order to investigate the toxicological effect of the *Chrozophora tinctoria* (L.) Rafin extract prior to their oral administration in adult rats, different concentrations of the aqueous extract were tested. The aqueous extract had no any toxic effect in the general behavior, no death was recorded when given at the dose (50 to 900mg/kg). However, there was a dose-involved presents signs of toxicity and mortality at the higher concentrations. These results could be explained safety nature of the plant extract on rats as mention in (Figure 3). Body weight is one of the factors related to metabolic regulation for diabetes. STZ diabetic rats showed body weight loss might occur due to increasing in muscle wasting [28] or might be due to loss of tissue proteins [29]. In this direction, the reduced level of glucose is compensated by stimulating gluconeogenesis in cells, which causes a decrease in the body weight, as well as insulin deficiency inhibits all anabolic processes and promotes catabolic processes, generally leading further to body weight loss, through increase of glycosuria and polyuria [30]. However, body weight decreased of STZ diabetic rats might be due to a toxic effect of STZ. Treatment with *Chrozophora tinctoria* (L.) Rafin extract improved the reduction in body weight in diabetic rats, and maintained food and water intake. In (Figure 1 (a)) the administration of aqueous extract *Chrozophora tinctoria* (L.) Rafin to experimental diabetic rats showed improvement in body weight when compared to the non-treated group, which may be due to the protection effect of the extract or due to the bioactive constituents of *Chrozophora tinctoria* (L.) Rafin to maintain hyperglycemia. Administration of *Chrozophora tinctoria* (L.) Rafin to STZ-induced diabetic rats after 35 days showed a decrease of the plasma glucose level (see Figure 1 (b)), perhaps by the augmenting quantity of insulin in all treated diabetic rats. Additionally, the mechanism of action of aqueous *Chrozophora tinctoria* (L.) Rafin extract is unknown, involved in utilization of glucose and regulate insulin secretion. Plasma insulin levels in STZ diabetic rats were observed decreased significantly. Whereas treated of diabetic rats with *Chrozophora tinctoria* (L.) Rafin extract shows increases in plasma insulin level (Figure 2 (a)). Common biochemical markers to determine changes in liver function namely ALT, AST and ALP were also quantified. All these enzymes levels increase in activity during diabetes which indicates liver dysfunction [31]. The groups that administered (50, 70 and 90 mg/kg B.W.) of *Chrozophora tinctoria* (L.) Rafin showed a significant decrease in AST levels as compared to the non-treated group and this may lead to the possible hepato protective effects of oral administration of the plant extracts in diabetes rats (Figure 2c). The administration of plant extract effectively lowered the elevated ALT in all induced rats. In the diabetic groups given *Chrozophora tinctoria* (L.) Rafin extract a reduction in serum ALT was observed, in contrast to a significant elevation in the diabetic non treated group (Figure 2d). Similar effect was recorded with ALP. *Chrozophora tinctoria* (L.) Rafin extract could reduce ALT level, as well as reduced ALP level in all treated groups (Figure 2b). It is concluded that, *Chrozophora*

*tinctoria* (L.) Rafin extract showed improvement in liver function when used as antidiabetic agent in the treatment of diabetic rats. The liver has important role in the excretion and removal of toxic substances from the body cells. DM causes changes in this organ tissue, representative photo histological view from the HE-stained of liver tissue sections are observed in Figures (4, 5, 6, 7), liver section of STZ induced diabetic rats showed several liver alterations due to the lack of insulin. The major alteration including fatty degenerations in different locations of liver parenchyma, abnormal hepatic cords with wide cystic dilation and aggregation of inflammatory cells. The livers of the control untreated diabetic rats showed some of hepatocytes lost their nuclei, and others have clear cytoplasmic vacuoles. The tissue section showed abnormal portal area with prominent spaces between abnormal hepatocytes, similar to other experimentally induced diabetic animal models [31,32]. This damage is partially reversed by the *Chrozophora tinctoria* (L.) Rafin extract treatment. The liver tissue of the control group (Figure 4) did not show any histological alteration the section presented typical histological organization, normal portal areas in agreement with the description of [33]. In diabetic rats treated with *Chrozophora tinctoria* (L.) Rafin extract. The major changes detected in diabetic livers were hydropic swelling, disarrangement in hepatocytes, microvesicular vacuolization, granular degeneration, and necrotic cells [34]. Most of the plant extract significantly decreased the hepatic damages. The noted hepatocytes fatty degeneration might occur due to insulin deficiency and the mitochondrial abnormalities. Major metabolic diseases such as DM and atherosclerosis are inflammatory states, and the responses to these conditions are mediated by macrophages like Kupffer cells [35] which noted clearly in diabetic groups treated with aqueous *Chrozophora tinctoria* (L.) Rafin extract. Kupffer cells are mobile macrophages, adhering to the endothelial lining and located at periportal sinusoid. Kupffer cells are activated in response to over nutrition, whether a high-fat diet or a high-sucrose diet, which resulted in the fast development of hepatic insulin insensitivity leading to disorders in lipid metabolism. Kupffer cells execute two roles: either as a mediator of damage or as a protector during the regeneration and repair processes [36]. The hepatic histological observations here showed that the severities of injuries in the treated rats when compared to DC rats were maintained probably due to the presence of kupffer cells. There was no difference between liver section of treated group(90mg/kg)and the healthy group. Further histopathological and toxicological studies are necessary in order to indicate the ability of *Chrozophora tinctoria* (L.) Rafin as hepatoprotactant. In the present study, histopathological findings also aids the protective potential of *Chrozophora tinctoria* (L.) Rafin aqueous extract to stimulate the activity of insulin secretion during treated diabetes.

## CONCLUSIONS

The aim of current study was to evaluate the antidiabetic potential and hepatoprotective effect of the oral administration *Chrozophora tinctoria* (L.) Rafin aqueous

extracts enhancement of hepatic injuries in (STZ) induced diabetic rats. Our results show that the *Chrozophora tinctoria* (L.) Rafin extract effectively improve the damaged hepatocytes, resulting in the observed reduction of the lesions associated with diabetic state in (STZ) – diabetic rats and serves as protective effect against hepatotoxicity produced by diabetes, which may be attributed to the synergistic action of various active compounds present in this plant extract. *Chrozophora tinctoria* (L.) Rafin aqueous extract leaves possesses antidiabetic activity. Increased insulin secretion after treatment with *Chrozophora tinctoria* (L.) Rafin aqueous extract positively decreasing hyperglycemia. Furthermore, the effect of oral *Chrozophora tinctoria* (L.) Rafin extract at the dose 90mg/kg body weight was more efficacy than 50, 70 mg/kg body weight. However, further studies on main components

are necessary to find out the responsible mechanism of action of this plant extract in ameliorating diabetic hepatopathy.

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