Research Article

The possible protective effect of *Carthmustinctorius*(leaves) on anti-tuberculosis(Rifampin &Isoniazid)drugs –induced hepatotoxicity in rats.

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ABSTRACT

Anti-tuberculosis namely Rifampin (RIF) and Isoniazid (INH) yet represents a first-line drugs for the remedy and protection of tuberculosis disease, but various reactions frequently promote in patients administered this drug. However, Methanolic extract of leaves of *Carthmustinctorius* has hepatoprotective activity which was investigated against hepatotoxicity produced by administering a combination of two anti-tubercular drugs isoniazid and rifampicin.Thecurrent study was assessed the possible protective effect of *Carthmustinctorius* extract on anti-tuberculosis namely (INH&RIF)drugs.So,this study was aimed at examine the possibility protect effect and mechanism of *carthmustinctorius* extract against (INH&RIF)drugs-induced hepatotoxicity in rats.The present study esteem the in vivo role of methanolic extract of leaves of *Carthmustinctorius* in protecting the liver against injury via anti-tuberculosis namely (INH&RIF)drugs in rats and further explores the underlying mechanisms. We postulate that *Carthmustinctorius* extract might protect the liver injury from anti-tuberculosis (INH&RIF)drugs. Moreover, forty male albino rats were haphazerdly divided into 4 groups: Control group, INH + RIF treated group, (*Carthmustinctorius* extract 250g/kg/daily for 28 days+ combination of RIF+ INH) treated group, and last sole of treated *Carthmustinctorius* extract 250g/kg/daily for 28 days. The INH + RIF were orally administered at dose levels of 50mg/ kg–b.wt., daily for 4 weeks. As well as these tissue of liverinjuries were quickly separated and fixed in 10% formalin and exposed to histopathological studies. Statistical analysis was carried out using t-test and analysis of variance (ANOVA).Administration of Anti-tuberculosis drugs namely (RIF& INH) to rats induced marked hepatotoxicity as evidenced by significant rise in serum levels of ALT, AST, ALP and total bilirubin at ($p< 0.05$). The current study shows that the effectof methanolic extract of leaves of *Carthmustinctorius*on some blood serum criteria of liver functions of anti- tuberculosis drugs. The study showed that relative lung weight to animals body weight was significantly increased at ($P<0.05$) in (RIF& INH) treated animals in comparison to the other groups. The results of present study powerfully indicated that methanolic extract of leaves of *Carthmustinctorius* has hepatoprotective action against RIF&INH-induced hepatic injury in rats.

Key words;*Carthmustinctorius*, antituberculosis drugs, (RIF& INH), histopathological, body weight, biochemical parameters, hepatotoxicity.

INTRODUCTION

The liver is a prime organ for metabolism of strange substances and also functionally intervene between the site of resorption and the systemic circulation. Furthermore, it has major ability to detoxicate toxic substances and synthesize beneficial principles [(1)].These conditions submit the liver not only the most important organ for detoxification of foreign substances but also a great object of their toxicity. On other hand, Liver arrange many important metabolic functions, and any injury causes deformation of these metabolic functions[(2)]. Moreover, Hepatotoxic agents can interact with the basic cellular compositions and based on that induce roughly all types of liver lesions. Toxins and remedy are among the basic etiopathogeneticfactors of acute liver failure in Western countries [(3)]. Nevertheless, chemical toxins inclusive (acetaminophen, carbon tetrachloride, galactosamine and thioacetamide..etc.) are often applied as the model substances causing experimental hepatocyte damage in both in vivo and in vitro situations[(4,5, 6)]. Several reactions may result in the formation of intermediate metabolites that are away more toxic than the parent substrate and may result in liver injury. And when disturbance in normal functions of liver and kidney arise, metabolic disorders occur. that is to say, if cumulating of toxins in body fluids is quickly than their metabolizing capability by liver, hepatic damage and hepatic disease happen. In central control of metabolism of drugs and their toxic intermediates, P450 enzyme plays crucial role. For example, P450 enzyme activity increment several folds during metabolism of chemical agents like phenobarbital, phenytoin, carbamazepine, primidone, ethanol, glucocorticoids, rifampin, griseofulvin, quinine and omeprazole, while in contrast, P450 enzyme activity stops during metabolism of isoniazid,

amiodarone, cimetidine, erythromycin, ketoconazole, metronidazole, sulfonamides and quinidine[7].Tuberculosis (TB) is one of the large communicable diseases, and nearly 2 million people die every year[8]. TB is ranked seventh among all illness[9].Nevertheless, drug-induced liver toxicity is a common cause of liver injury. It accounts for approximately one-half of the cases of acute liver failure and mimics all forms of acute and chronic liver disease [10]. An estimated 1000 drugs have been implicated in causing liver disease. Although drug-induced liver injury subsides after cessation of treatment with the drug, this represents an important diagnostic and therapeutic challenge for physicians [11]. However, Tuberculosis is an infectious, potentially fatal disease that is caused by bacteria and usually affects the lungs. It is generally difficult to catch. It is contracted by inhaling air that contains droplets contaminated with TB bacteria. Tuberculosis may be prevented by avoiding close contact with people who have active TB, and by staying healthy so the body can fight off infection. A vaccination called BCG helps prevent TB in many parts of the world, but it is not widely effective. Although uncomplicated TB can be defeated with antibiotics, it takes six to nine months of treatment with powerful drugs that often have unpleasant side effects[12].Several commonly used antituberculous drugs are potentially hepatotoxic and can cause severe, and even fatal, hepatitis. Apart from an evaluated hepatotoxicity of 1%–0.1% [13,14]. Where, toxicity was not predictable because, in the 1970s and 1980s, the rates of drug-induced liver dysfunction did not increment when pyrazinamide was added to medicinal regimens including isoniazid and rifampin [15].Moreover, three of the first-line anti-TB drugs i.e. isoniazid, rifampicin and pyrazinamide and all classes of antiretroviral drugs are also known to cause liver injury[16,17].However, many recent studies have suggested that the antituberculosis drugs(Rifampin, isonaized, Pyrazinamide) have adverse effects on the human body[18-21].Usually, Rifampicin has bactericidal activity versus M. tuberculosis by inhibiting bacterial DNA-dependent RNA polymerase (140). Isoniazid is a prod rug activated by bacterial catalase-peroxidase (KatG) and murder actively growing tubercle bacilli by inhibiting the biosyn-thesis of mycolic acids which are great components of cell wall of M. tuberculosis [22,23]. Although toxicity is low when administered alone, liner injury caused by rifampicin has been observed in patients with underlying liver diseases [24]. Pyrazinamide is found to induce a cytolytic hepatitis by direct toxicity, most notably after long periods of treatment. Some cases of fulminant liver failure has been reported mainly at relatively higher dosages, 40-50 mg/kg per day [24,25].However, Plants are beneficial source of a wide scope of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food supplements[26].Different medicinal plants extracts possessed hepatoprotective activity due to their contents of flavonoids, terpenoids, phenolic acids, stilbenes, alkaloids, antraquinones, curcuminoids, capsaicinoidsand chromenes[26].Because of involvement of oxidative stress in the mechanisms of hepatic injury, the antioxidant properties of medicinal plants were involved in the mechanism of their hepatoprotective activity. They inhibited hepatic oxidative stress by many mechanisms[27].MECT, in all doses caused significant decrease in AST, ALT, ALP, and total bilirubin levels and elevated the level of GSH[28].Hepatoprotective activity of methanolic extract of leaves of *Carthmustinctorius* (MECT) was investigated versus hepatotoxicity produced by administering a mixture of two anti-tubercular drugs isoniazid and rifampicin for 24 days by oral route in rats[28].Number of studies were cleared that the histopathologicalalterations weredetected that there were many hepatocytes with apparent adipose degeneration. Hepatic pathological damage in Safflower injection group was inconsiderable than that in chronic hypoxia and hypercapnia for four weeks group. Other workers show MECT, in all doses caused significant decrease in AST, ALT, ALP, and total bilirubin levels and elevated the level of GSH[29].Therefore, this work was designed to highlight impacts of hepatoprotective activity of *Carthamustinctorius*, methanolic leaf extract and using INH and RIF- -induced hepatic injury model in rats, and explores the plausible underlying antioxidant mechanism.

Material and methods

Experimental animals

Six wistar albino rats weighning (150-250g), they were get from the animal house/college of science/University of Thi-qar at april/2018, and were housed in well ventilated polproplene cages, under standard conditions (temperature 24±4C0, relative humidity 60- 70% and 12 hrs. dark light cycles). The animals stayed for one week in a climate controlled room before the beginning of the study. Allowed free diet ad libitum. and liberally supplied with water.

Experiment materials

liver functions biochemical parameters were evaluated use criteria kits. Rifampicin(RIF) and isoniazid(INH) were purchased from sigma chemicals and all other chemicals used for this study were of analytical grade. The plant extract was bring from the local market of Basra city/Iraq.Identified by the plant "herbium college of science " Basra University.

Experimental design

Atotal of (40) rats were divided randomly into four groups of ten each. Group A (negative control) Normal control (0.5ml/kg distilled water, p. o), group B (positive control) INH&RIF drugs treated, group C (INH&RIF drugs + plant extract mg/kg p.o), group D (plant extract (mg/kg). and is as under:

The following are the totals of the animals on which the experiment was carried out:

Group A – negative control (0.5 ml/kg d.w. daily for 28 days, p. o)

Group B - Toxicant (positive control) (INH&RIF 50mg/kg.daily for 28 days, P.O.)[30].

Group C –(plant extract 250 mg/kg p. o[31] + INH&RIF drugs50mg/kg. daily for 28 days,P.O)

Group D - Representing animals orally administered plant extract alone at dose level of 250 mg kg–1 b.wt., daily for 4 week.[(28)].

Twenty-four hours after the last doses of the treatments animals, the rats were anesthetized lightly by chloroform inhalation, then killed by neck dislocation, and blood samples were drawn by heart Puncture of each sacrificed animal. The samples were collected in plastic test tubes and admitted to stand for 2 h to ensure complete clotting. Thenblood samples were centrifuged at 3000 rpm for 20 min, where sera was separated and kept in vials to be used for the biochemical study.However, the study was divided into; Calculation of relative weight of liver / Body weight., biochemical and histopathological study.

Relative Organ Weight

Livers were dried by filter paper and weighted to determine their relative weight (gm⁄100gm of animal body weight). Relative Organ Weight On last day of experiment of the treating period, all the animals were bullet of mercy by dislocation under chloroform anesthesia. livers, lungs were carefully dissected out and weighed in grams (absolute organ weight). The relative organ weight of each animal was then calculated as in under[32].

$$\text{RelativeOrgan Weight} = \frac{\text{Absolute organ weight(g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

Table 1: Shows Body weight and relative liver weights of control and the rats treated with INH+RIF, carthmustinctorous extract 250mg/kg and combination (mean±SD).

No. groups	Body weight starting(%)	Relative Liver weight/100 gbody weight
Group A	147.01 ± 19.11	3.63 ± 0.03
Group B	153. 06 ± 2.10*	4.19 ± 0.26a
Group C	150.20 ± 3. 33*	3.46± 0.19b
Group D	149.0.9 ± 4.01*	3.87 ± 0.29^{c}

Data were calculated as relative weight of liver to 100 g animal body weight

Data are presented as mean (SD) of 10 animals/ group.

*No significantly different among group.

$^{a}P < 0.05$ INH + RIF *vs control group ;* $^{b}P < 0.05$ combination vs control group and induction (group 3); $^{c}P< 0.05$ plant extract (group 4) vs Group(1,2)

Table 2:- The effect of oral administration of turcarthmustinctorousextract 250mg/kgonserum liver enzymes (ALT, AST, ALP) and total bilirubin in rifampin (IRF) and isonaized (INH)- induced hepatotoxicity in rats. Value was expressed as mean ± SD(n=10).

Parameters Groups	S. ALT IU/L	S. AST IU/L	S. ALP IU/L	Total bilirubin mg/dl
Group A	60.8±4.01^{a}	88.09± 5.14^{a}	287.01±12.18^{a}	3.01 ± 0.01^{a}
Group B	102.5± 3.22^{b}	116.04±10.15^{b}	367.29±33.04^{b}	5.09±0.38^{b}
Group C	83.4±4.08^{c}	104.02±7.01^{c}	326.22±9.23^{c}	3.43±0.02^{c}
Group D	64.6± 2.14^{NS}	92± 2.78^{NS}	291.31±7.04^{NS}	3.77± 0.09^{NS}

-All values expression by mean± SD, (n=10 in each group).

-Data are statistically significant at P<0.05. NS: not significant.

-Group 2, 3 and 4 were compared with group 1 (normal control) and group 3was compared with group 2 : different letters means significant, NS: not significant.

Biochemical assays

The following parameters were evaluated colorimetrically, Serum pathophysiological enzymes including; (alkaline phosphatase (ALK), Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALK)) and total bilirubin.

Histopathological examination

Taking portions from the livers then fixed with neutral buffered formalin 10%for next histological study. They were embedded in paraffin wax and then sectioned. (4-5 µm thick) were taken from each tissue. Liver sections were stained with hematoxylin and eosin.[33].

Statistical analysis

Statistical analysis of got data was carried out through student's-t test which was used to determine the significance among the treated group in comparison with control and induction group and between each other. The results in all above were accepted as statistical significant when the P value less than ($p< 0.05$).Data were expressed as mean ± SD.Statistical analyses were performed using SPSS (Version 15) [34].

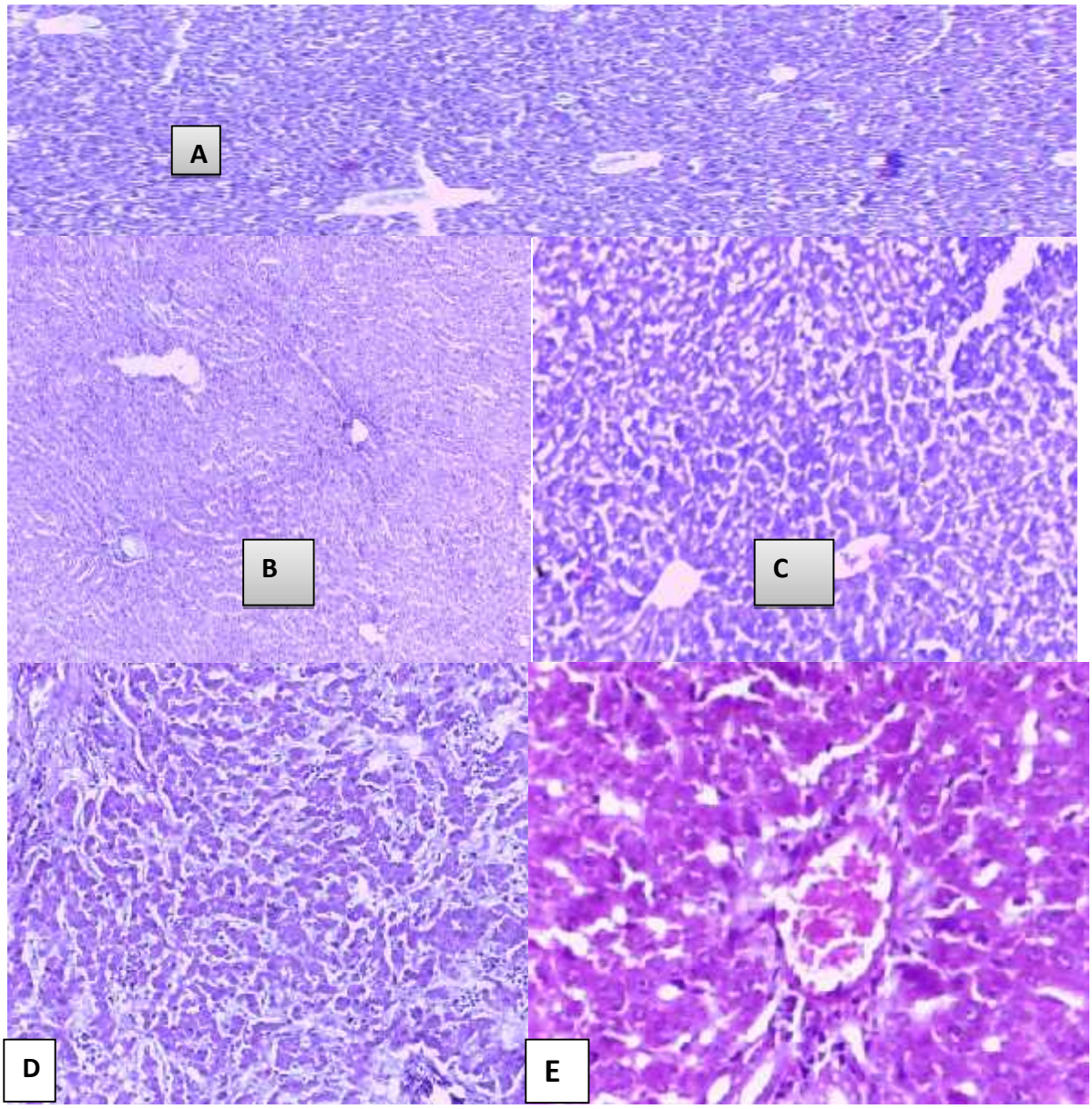

Figure: (A,B,C,D,E): impact extract of carthmustinctorous at 250mg/kg against anti - tuberculosis drugs(INH&RIF) - induced hepatotoxicity in normal rat liver.(A) Photomicrograph of control rat liver section exhibit normal hepatic architecture, sinusoidal spaces, central vein and surroundings hepatocytes, few of those cells bi-nucleated, hepatic cord cells arrange in strands, normal lobular structure with hepatocytes arranged in hepatic cords radiating around the central vein, separated by obvious blood sinusoids which is formed of endothelial lining cells and contains phagocytic Kupffercells (H&E, 200X). (B-D)liver sections of anti - tuberculosis drugs(INH&RIF) showing sever damage of liver architecture,disorganized hepatocytes cord, multiple areas of necrosis, marked infiltration of inflammatory cells mostly (PMNs), hepatocytes degeneration and cytoplasmic vaculation, edema, few bundles of fibrous tissue. disorganized hepatocytes cord, some hepatocytes were pyknotic, congested

central vein few bundles of fibrous tissue, dilatation in hepatic sinusoids with indistinct cell boundaries(H&E, 400X).(E) sections in rat treated INH+RIF drugs with *Carthmustinctorius* extract (group 3)revealed markeda meliorate in hepatic cellsaround the central vein and prominent progression around the portal area exhibit marked ameliorate of hepatic tissue with normal hepatocytessradiating around central vein, few number of inflammatory cells, Total disappearance of fibrous tissue (H&E, 400X).

Results

As shown in table 1;By contrast to the control group, liver/body weight index significantly increased after INH+RIF drugs sole treated(4.19 ± 0.26, $p < 0.05$)compared to normal control(3.63 ± 0.03, $p < 0.05$). Conversely, daily treatment both of *Carthmustinctorius* extract and INH+RIF drugs displayed significantly decreased(3.46 ± 0.19, $p < 0.05$) liver/body weight index compared to induction group(4.19 ± 0.26, $p < 0.05$) which is approaching to normal value. On the other hand, the results was appeared that body weights of control and the rats treated with INH+RIF, and combination no significantly differences among group (table 1).In the present study, the results as shown in table(2) was appeared that in toxic group, the liver functional enzymes levelAlt,AST and ALPsignificant increase in the INH+RIF treated rats group(102.5± 3.22; 116.04±10.15;367.29±33.04) compared to the control (60.8±4.01; 88.09± 5.14; 287.01±12.18).However, the results in (Table 2) revealed that Administration of *Carthmustinctorius* extract 250mg/kg in combination with INH+RIF drugs were revealed a significant lower in these enzymes(83.4±4.08; 104.02±7.01; 326.22±9.23)in compare to sole INH+RIF treated rats (102.5±3.22; 116.04±10.15;367.29±33.04).Regarding other indicate for liver function is total bilirubin level, the study exhibited the same manner as results of hepatic enzymes levels aforementioned of all study groups. Furthermore, this study was appeared that the animals of group 4 which treated by *Carthmustinctorous* extract 250mg/kg for 28 days showed no significant changes compared with group 1 (control group) in the serum levels of ALT, AST, ALP and total bilirubin (table 2).**Histopathological changes**: Microscopic examination of hepatic parenchyma displayed that the Histological changes of the liver sections in rats under this study exhibited that the control group, no histopathological alterations. On the other hand, normal classical hepatic cellular architecture of sinusoidal spaces, central vein and surroundings hepatocytes, as well as showing polyhedral hepatocytes with eosinophilic cytoplasm arranged in strandsaround the central vein, few of those cells binucleated, clear-cut hepatic constitutional architecture arrange in strands,normal lobular structure with hepatocytes arranged in hepatic cords radiating surround the central vein and separated by obvious blood sinusoids which is formed of endothelial liningcells and contains phagocytic Kupffercellsn(figure 1).However, rats treated with INH+RIF drugs group, showed numerous histopathological changes when compared with the controlgroup, include severe damage in thehepatic architecture, prominent polymer phonuclear inflammatory infiltration cells almost every tissue section of the liver is covered, hepatocytes cytoplasmic vaculation, interlobularhemorrhage, dilatation in hepatic sinusoids with degeneration in the hepatocytes and moderately dilatation and congestion in central vein, disorganizedhepatocytes cord. In addition Liver section of rat treated with INH+RIFgroup, showed focal necrosis in the hepatic parenchyma, with odema, some hepatocytes were pyknotic, and fewbundles of fibrous tissue. Mostly hepatocytes merge with each other forming eosinophilic syncytial masses. ballooning degeneration of hepatocytes (BD) is seenwith vacuolated cytoplasm and indistinct cell boundaries. Moreover, another sections in rat treated INH+RIF drugs with *Carthmustinctorius* extract (group 3) showing marked ameliorate of hepatic tissue with normal hepatocytessradiating around central vein. and mild degeneration in the hepatocytes and slightly congestion in portal vein with partially inflammatory cells infiltration in the hepatic parenchyma, when compared to induction group(3 group).livers of rats group received *Carthmustinctorius* extract solely (group 4)showed no significant histopathological alterations.

Discussion

The major purpose of this study was to determine whether or not RIF&INH-induced hepatotoxicity in the rats model. However, The liver is the leading organ for maintaining the internal environment of the body. There is currently no other way to compensate of liver functions. The significant impact on the flow of nutrients to other body organs, in addition to controlling the metabolism of carbohydrates, protein and fat, Drugs were considered to be a major cause of liver damage. There are more than 900 kinds of medicines, toxin and herbs that have been reported to cause liver injury[35,36,37].On the other hand, The first line anti-tubercular drugs namely, Rifampicin, Isoniazid and Pyrazinamide are actually hepatotoxic drugs. These drugs are metabolized by the liver. Thus, although both INH, Rifampicin and Pyrazinamide itself when actually giving them together lead to liver damage and enhance the toxicity, which lead to increased incidence of hepatitis[37, 38].However, the results was appeared that body weights of control and the rats treated with INH+RIF, and combination no significantly

differences among groups but still lower than normal value of the control. but they did not normalize the level of body weights.These results were justified that may be that indicate to gastrointestinal toxicity of these animals and with reason decreasing ingestion of food by the animals [40]. Conversely, the rat group treated with INH&RIF drugs(Toxic group) showed a significant increase in the relative weights of liver. The administration of *Carthmustinctorius* extract (standard group) along with RIF&INH treated rats at the same period (28 days) led to significant decrease of the liver relative weightcompared with the toxic group. These results appeared that the plant extract has the ability in preventive of the rats against INH&RIF drugs –induced hepatotoxicity and improvement of the relative liver of the experimental rats. These results agree with previous studies on other plant extracts.[41,42].However, the results of this study showed that the animals which received dose of anti-tuberculosis disease which include (INH&RIF) drugs, caused severe liver tissue damage, which was revealed because of the increase in serum levels of ALT, AST and ALP enzymes. Since injury to liver cells changes their functional transition, causes membrane permeability, and leads to the leakage of enzymes into extracellular space [43,44]. Interestingly that the levels of these enzymes have returned to near -normal levels in the treated rats with *Carthmoustinctorius* extract It can stabilize the membrane of liver cells and thus prevent the leakage of these enzymes. However, preventing or blocking the production of free radicals and neutralization[45]. Thus, the possibility of protection of this plant against hepatic toxins can be other possible causes of the impact on healing. The liver, which is the main site of the protein synthesis and especially the albumin, in this study. It is always used to evaluate the efficiency of the liver for the synthesis of these proteins[8]. The increase observed in the level of albumin after the dosage of the animals in this plant extract suggests that the plant extract can prevent the reduction of potential albumins by stabilizing The endoplasmic reticulum and the re-synthesis of the protein or through the neutralization or equation of free radicals by the scavenger compounds. With regard to histopathological findings in this study that associated with INH&RIF drugs were appeared also agrees to most previous studies documented by many researchers which ameliorate included the same study despite the differences of plant extracts used to reduce the side effects of those drug aforementioned we can concluded that the protective effects methanol *Carthamustinctorius* extract(at dose 250mg/kg for successive 28 days) in combination with RIF&INH drugs showed evidently normal organ with slight necrotized hepatocytes and could be attributed to its antioxidant and anti-inflammatory effects.

Declarations

Ethics acceptance and approval to participate

This work was performed by the author empirically

Consent for publication

The manuscript didn't include any individual persons data.

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