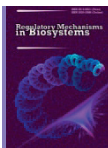

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Effect of safflower (*Carthamus tinctorius*) on the biochemical profile of blood serum and insulin levels in alloxan-induced diabetic rats

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Safflower or *Carthamus tinctorius* L. has found clinical applications in treatment of certain diseases which involve inflammation and oxidative stress. The aim of the present study was to detect the ameliorative effects of safflower on alloxan -induced tissue injury. To do so, serum samples were collected from rats exposed to alloxan (120 mg/kg/day) and a combination of alloxan and safflower extract (100 and 200 mg/kg/day). All three experimental groups were compared to the control group. The collected serum was analysed for measurement of fasting serum glucose (FSG), serum insulin level, total cholesterol (TC), triglycerides (TG), aspartate transferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP). Safflower significantly reduced the alloxan induced increase in FSG, TG, TC, AST, ALT, and ALP. Moreover, safflower significantly reversed the alloxan induced reduction in serum insulin. Interestingly, the greater the increase in safflower dose (100 versus 200 mg/kg/day), the better the outcomes of the measured parameters. Alloxan significantly induced pancreatic and liver injury, indicated by elevated glycemic and lipid profile alongside elevated liver enzymes (AST, ALT, and ALP). These defects were significantly ameliorated by safflower extract in dose dependent manner.

Keywords: safflower; *Carthamus tinctorius*; lipid profile; liver function test; glycemic parameters.

Introduction

The plant *Carthamus tinctorius* L. (also known as safflower or false saffron; belonging to the Compositae or Asteraceae family; native to Southern Asia, China, India, Iran, and Egypt) has a thistle-like appearance (Shirwaikar et al., 2010). The flowers of the plant are the active part and are collected during summer to be used for therapy of diseases (Zhou et al., 2014). Extract of the plant has been used due to its content of 200 compounds as active constituents including flavonoids, phenylethanoid glycosides, coumarins, fatty acids, steroids, and safflower polysaccharides (Asgarpanah & Kazemivash, 2013).

Safflower extract could be used for treatment of various diseases, including ischemic diseases due to its capacity to suppress oxidative and inflammatory reaction via the active components it contains (Wang et al., 2020). The mechanism is obscure, however, it has been explained in the context that safflower blocks inflammation, oxidative stress, and apoptosis (Qi et al., 2014; Deng et al., 2018). Moreover, safflower components also inhibit platelet activity and reduce blood viscosity and thereby thrombosis, alongside increased cardiac and cerebral vasodilator improving hemodynamic parameters (Fan et al., 2014; Sun et al., 2018).

The flavonoid content in safflower make it suitable to be used for neurological diseases via their anti-inflammatory activity and via reducing inflammation markers or pathways (Liu et al., 2013; Lv et al., 2016; Wang et al., 2018), providing neuroprotection (Wang et al., 2016). One relevant example of research conducted in this area demonstrated that kaempferol extracted from safflower provided protection against neuronal excitation induced by $CdCl_2$ via suppression of oxidative stress, inflammation, and apoptosis (Han et al., 2019; Yan et al., 2019; El-Kott et al., 2020). Hyperoside extracted from safflower provided neuroprotection in epilepsy via its antioxidant effects (Huo et al., 2014; Kwon et al., 2019; Cao et al., 2020). Moreover, flavonoids extracted from safflower provided improvement of Alzheimer's disease (AD) and Parkinson's disease via modulating oxidant/antioxidant status, reducing inflammatory markers, and neurotransmitters involved in the pathogenesis of these diseases (Ma et al., 2015; Ruan et al., 2016; Shi et al., 2018; Zhang et al., 2019; Hou et al., 2020).

Additionally, the flavonoids extracted from safflower have potentially provided anti-inflammatory activity against lung diseases (Song et al., 2013; Kim et al., 2014). Moreover, safflower extract has mitigated TGF-β1 expression, providing reduction in airway remodeling (Wang et al., 2014), exhibiting attenuated morphological lung changes in patients with lung fibrosis with different mechanisms including modulated α-smooth muscle actin (α-SMA) expression, Smad 3 phosphorylation and TGF-β1 type II receptor (Jin et al., 2016; Pan et al., 2017). This could potentially provide application in treatment of inflammatory diseases including COVID-19 due to safflower's content of antioxidant constituents and anti-inflammatory compounds (Huang et al., 2020; Hamidi et al., 2021). Similarly, the anti-inflammatory extract of safflower has provided protection against liver injury (Li et al., 2012; Dong et al., 2014), via liver protection and increased liver blood supply (Wang et al., 2021).

Materials and methods

A total of 28 male white albino rats (age 2–3 months; weight 170–200 g) were subdivided into 4 groups (7 each). The rats were kept in plastic cages in standard conditions (temperature 20–25 °C; 12 hr-light/dark cycle, free access to food/water).

To prepare the drugs before experimentation, alloxan powder (India MART, India) was reconstituted using distilled water at concentration 100 mg/mL and aliquoted and stored at –20 ºC to be ready for use. Safflower was purchased from local markets in dry form, the plant was ground and dissolved in distilled water at concentration of 250 mg/mL. Glucose powder (Thermo Fisher, UK) was dissolved at 5% concentration and used in the water bottles for the rats to avoid hypoglycemia due to diabetic induction.

The rats were subdivided into 4 groups (7 rats each); these groups were: the control group was given distilled water on a daily basis; the DM group was given alloxan 120 mg/kg/day; the DM+100S group was given alloxan 120 mg/kg/day and safflower extract 100 mg/kg/day; the DM+200S group was given alloxan 120 mg/kg/day and safflower extract 200 mg/kg/day.

Blood samples were collected from all rats from the tail using small needles. The collected blood was transferred into Eppendorf tu-

bes and collection was conducted at day 10, day 20, and day 30 following initiation of intervention. The serum was separated and serum tubes frozen at –20 ºC for future analysis. The serum was analysed based on the manufacturer's instruction supplied in the kit of Mindray (China) using Mindray BS-200 analysis machine.

Measurement of glucose. The principle of assay in case of glucose was based on oxidation of glucose molecule by glucose oxidase, generating hydrogen peroxide and gluconic acid. The peroxidase supplied in the kit catalyses the interaction of chromogen with generated hydrogen peroxide producing a colored compound to be quantified at OD 505 nm using the plate reader (Mindray, China), which is proportionate with the original glucose concentration.

Measurement of insulin. The principle of chemiluminescent series insulin assay is based on competitive binding immunoenzymatic assay to determine the level of insulin. The concentration can be extrapolated to the calibration curve of the standard and this is automatically calculated by the analyzer machine (Mindray, China) from the standard provided in the kit of Insulin (Insulin CLIA 111, China).

Calculation of HOMA-IR. HOMA-IR = glucose (mg/dL) × insulin (mU/L)/405.

Measurement of cholesterol. The principle of assay is based on the enzymatic colorimetric, in which cholesterol converted via cholesterol oxidase supplied in kit components to cholest-4-en-3-one and hydrogen peroxide. The generated hydrogen peroxide interacts with chromogen in the presence of peroxidase supplied in kit conponents, leading to production of colored compounds to be quantified at OD 600 nm using the plate reader (Mindray, China), which is proportionate with the original cholesterol concentration.

Measurement of triglycerides. The principle of assay based on the enzymatic colorimetric, in which lipase enzyme (supplied in the kit components) digests triglycerides in the serum sample to glycerol and fatty acids. The glycerol kinase enzyme (supplied in the kit components) digests glycerol to glycerol-3-phosphate. Glycerol-3-phosphate oxidase enzyme (supplied in the kit components) digests formed glycerol-3-phosphate forming hydrogen peroxide. The generated hydrogen peroxide interacts with chromogenic in presence of peroxidase supplied in the kit components, leading to production of a colored compound to be quantified at OD 600 nm using the plate reader (Mindray, China), which is proportionate with the original triglyceride concentration.

Results

The results confirmed that rats treated with alloxan showed significantly (P < 0.05) higher levels of FSG over the three time points, reaching up to 260 ± 1.15 mg/dL at day 30 when compared to the control group, 90.5 ± 0.86 at day 30 of experimentation. In contrast, rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) reduced FSG over the three time points, falling to 102 ± 1.3 mg/dL at day 30 when compared to the alloxan group 260 ± 1.2 mg/dL on day 30 of experimentation. Moreover, FSG harmonized with the safflower dose used, since rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) reduced FSG over the three time points, falling to 102 ± 1.3 mg/dL at day 30 when compared to safflower extract (100 mg/kg/day) group 120 ± 1.3 mg/dL at day 30 (Fig. 1).

The results confirmed that rats treated with alloxan showed significantly (P < 0.05) low levels of serum insulin over the three time points, measured at 1.64 ± 0.09 U/mL at day 30 when compared to the control group, 2.90 ± 0.05 U/mL at day 30 of experimentation. In contrast, rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) higher serum insulin over the three time points, increasing to 2.77 ± 0.08 U/mL at day 30 when compared to the alloxan group 1.64 ± 0.09 U/mL at day 30 of experimentation. Moreover, serum insulin synchronized with the safflower dose used, since rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) elevated serum insulin over the three time points, increasing to 2.77 ± 0.08 U/mL at day 30 when compared to the safflower extract (100 mg/kg/day) group, 2.10 ± 0.13 U/mL at day 30 (Fig. 2).

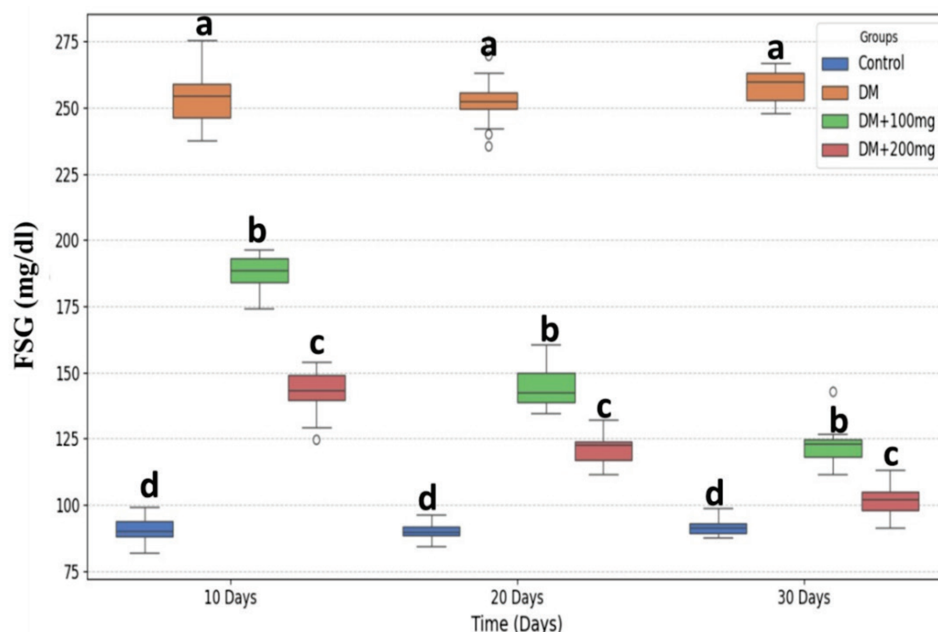

Fig. 1. Serum fasting serum glucose (FSG) of the studied groups over a month of safflower treatment at three time points 10, 20, and 30 days: box limits indicate the range of the central 50% of the data, with a central line marking the median value; the boxplots represents mean ± SD; letters are used to describe significant differences among groups using one-way ANOVA of the same time points followed by Bonferroni tests; different letters express significant difference at P < 0.05; DM group – diabetes mellitus group, DM+100S group – diabetes mellitus group was given alloxan 120 mg/kg/day and safflower extract 100 mg/kg/day, DM+200S group – diabetes mellitus group was given alloxan 120 mg/kg/day and safflower extract 200 mg/kg/day

In line with this, the results confirmed that rats treated with alloxan showed significantly (P < 0.05) higher HOMA-IR values over the three time points, increasing to 1.05 ± 0.05 at day 30 when compared to the control group, 0.64 ± 0.01 at day 30 of experimentation. In contrast, rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) reduced HOMA-IR values over the three time points, decreasing to 0.61 ± 0.02 at day 30 when compared to the alloxan group, 0.64 ± 0.01 at day 30 of experimentation (Fig. 3).

Regarding TC, the results confirmed that rats treated with alloxan showed significantly (P < 0.05) higher levels of TC over the three time points, measured at 123.0 ± 1.3 mg/dL at day 30, as compared to the control group, 82.0 ± 1.3 at day 30 of experimentation. In contrast, rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) reduced TC over the three time points, decreasing to 86.4 ± 1.5 mg/dL at day 30 when compared to the alloxan group, 123.0 ± 1.3 mg/dL at day 30 of experimentation (Fig. 4).

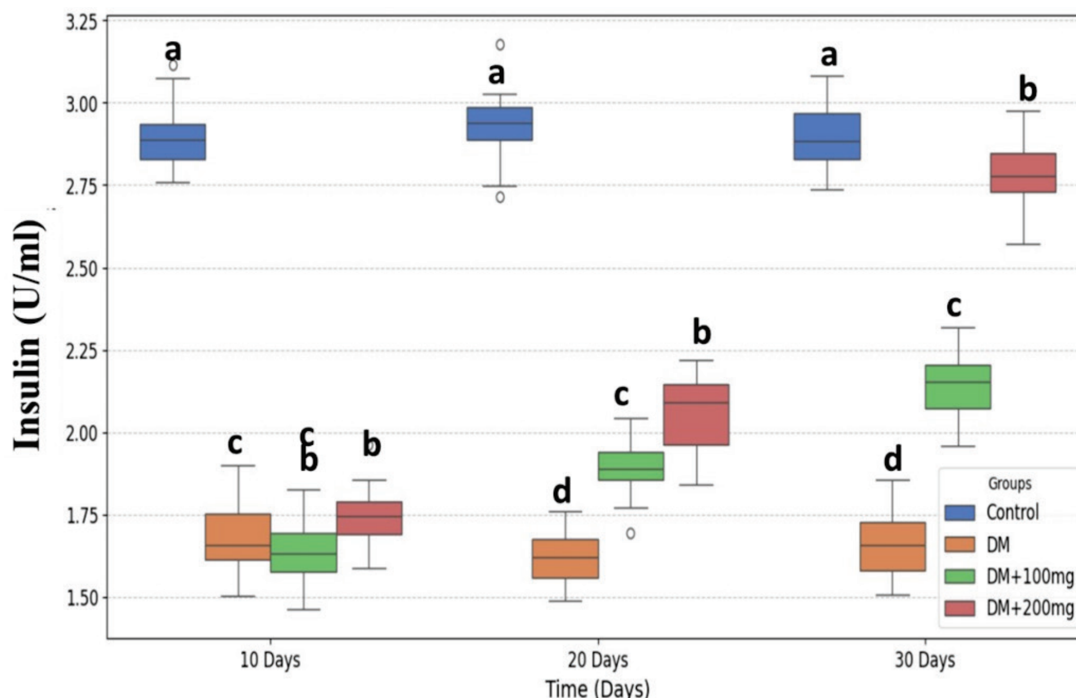

Fig. 2. Serum insulin levels of the studied groups over a month of safflower treatment at three time points 10, 20, and 30 days: see Figure 1

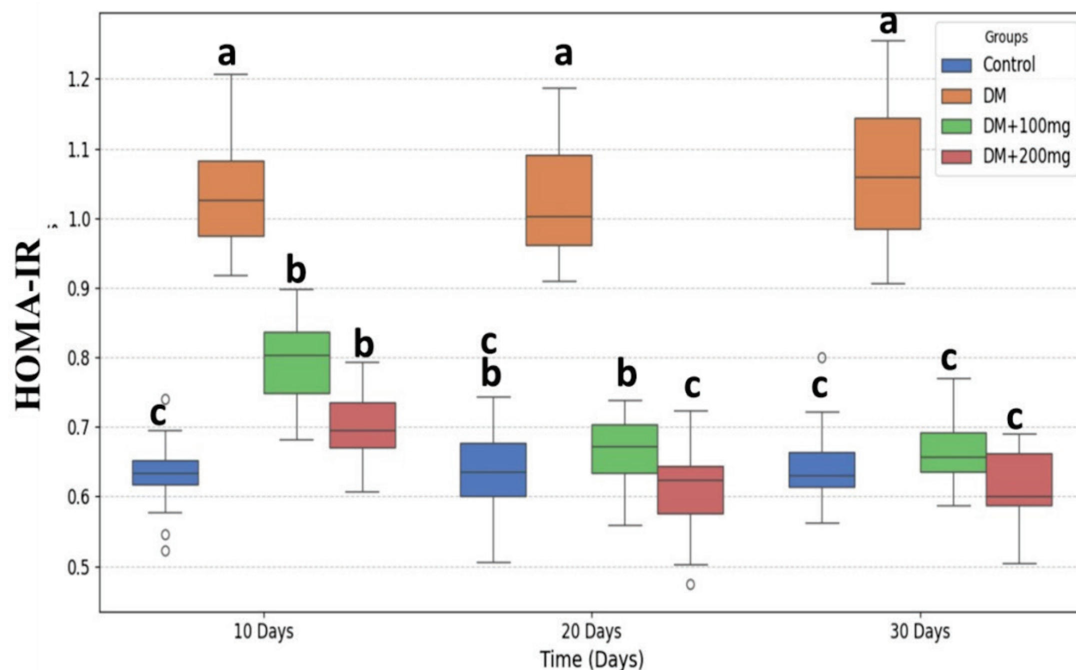

Fig. 3. Homeostatic model assessment for insulin resistance (HOMA-IR) values of the studied groups over a month of safflower treatment at three time points 10, 20, and 30 days: see Figure 1

The results confirmed that rats treated with alloxan showed significantly (P < 0.05) higher levels of TG over the three time points increasing to 117.0 ± 2.5 mg/dL at day 30 when compared to the control group, 55.4 ± 2.3 at day 30 of experimentation. Moreover, TG harmonized with the safflower dose used, since rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) reduced TG over the three time points, falling to 56.3 ± 2.0 mg/dL at day 30 when compared to the safflower extract (100 mg/kg/day) group, 67.3 ± 2.6 mg/dL at day 30 (Fig. 5).

Regarding liver enzymes, the results confirmed that rats treated with alloxan showed significantly (P < 0.05) higher levels of AST over the three time points, increasing to 122.0 ± 1.3 U/L at day 30 when compared to the control group, 66.0 ± 1.3 at day 30 of experimentation. In contrast, rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) reduced AST over the three time points, falling to 102.0 ± 1.5 U/L at day 30 when compared to the alloxan group, 122.0 ± 1.3 U/L at day 30 of experimentation. Moreover, AST harmonized with the safflower dose used, since rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) reduced AST over the three time points, decreasing to 102.0 ± 1.5 U/L at day 30 when compared to the safflower extract (100 mg/kg/day) group 115.0 ± 1.5 U/L at day 30 (Fig. 6).

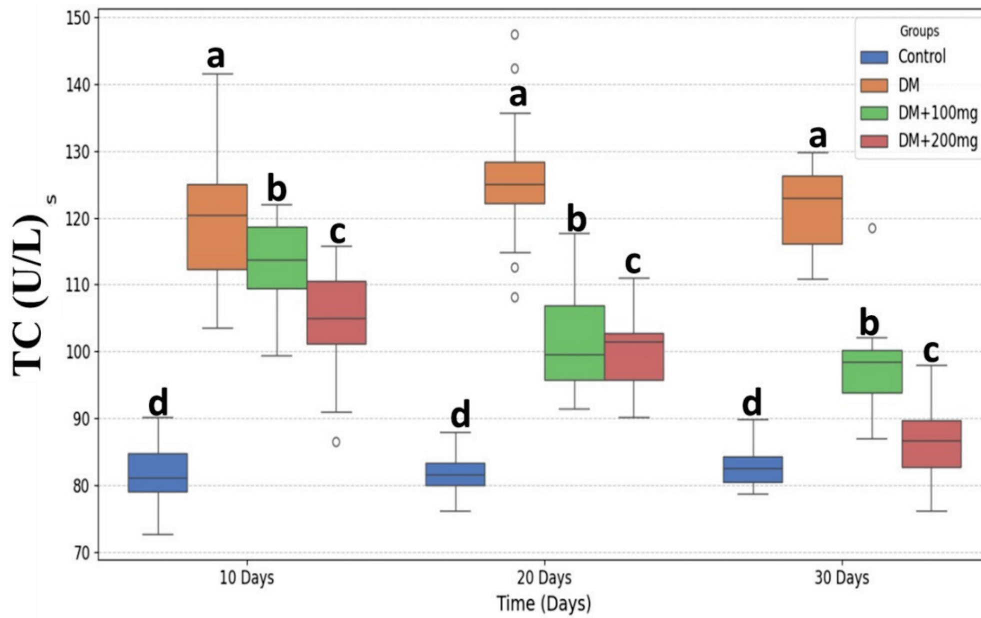

Fig. 4. Serum total cholesterol of the studied groups over a month of safflower treatment at three time points 10, 20, and 30 days: see Figure 1

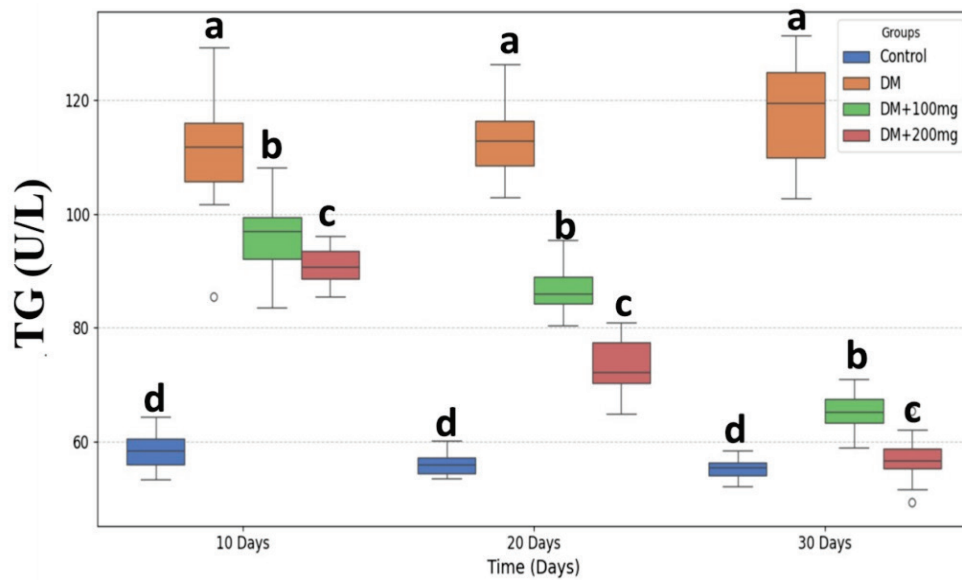

Fig. 5. Serum triglycerides (TG) of the studied groups over a month of safflower treatment at three time points 10, 20, and 30 days: see Figure 1

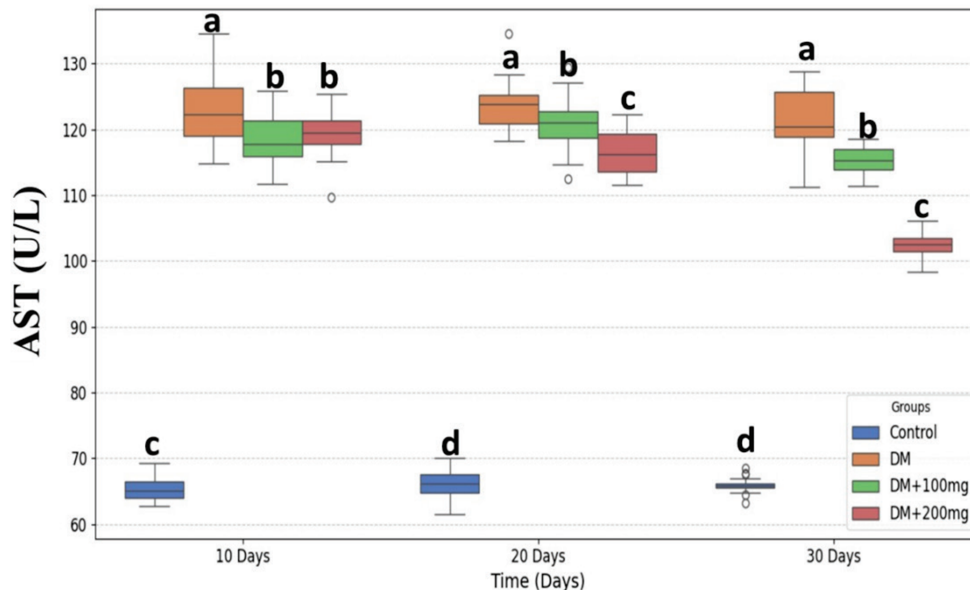

Fig. 6. Serum aspartate transferase (AST) enzyme of the studied groups over a month of safflower treatment at three time points 10, 20, and 30 days: see Figure 1

Moreover, the results confirmed that rats treated with alloxan showed significantly (P < 0.05) higher levels of ALT over the three time points reaching 49.0 ± 1.4 U/L at day 30 when compared to control group, 37.0 ± 0.9 at day 30 of experimentation. In contrast, rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) reduced ALT over the three time points falling to 33.0 ± 1.1 U/L at day 30 when compared to alloxan group 49.0 ± 1.4 U/L at day 30 of experimentation. Moreover, ALT harmonized with the safflower dose used, since rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) reduced ALT over the three time points falling to 33.0 ± 1.1 U/L at day 30 when compared to the safflower extract (100 mg/kg/day) group, 40.0 ± 0.6 U/L at day 30 (Fig. 7).

The results confirmed that rats treated with alloxan showed significantly (P < 0.05) higher levels of ALP over the three time points, reaching 178.0 ± 1.6 U/L at day 30 when compared to the control group, 142.0 ± 0.6 at day 30 of experimentation. In contrast, rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) reduced ALP over the three time points, decreasing to 118.0 ± 1.8 U/L at day 30 when compared to the alloxan group, 178.0 ± 1.6 U/L at day 30 of experimentation. Moreover, ALP harmonized with the safflower dose used, since rats treated with alloxan and safflower extract (200 mg/kg/day) showed significantly (P < 0.05) reduced ALP over the three time points, decreasing to 118.0 ± 1.8 U/L at day 30 when compared to the safflower extract (100 mg/kg/day) group, 154.0 ± 2.8 U/L at day 30 (Fig. 8).

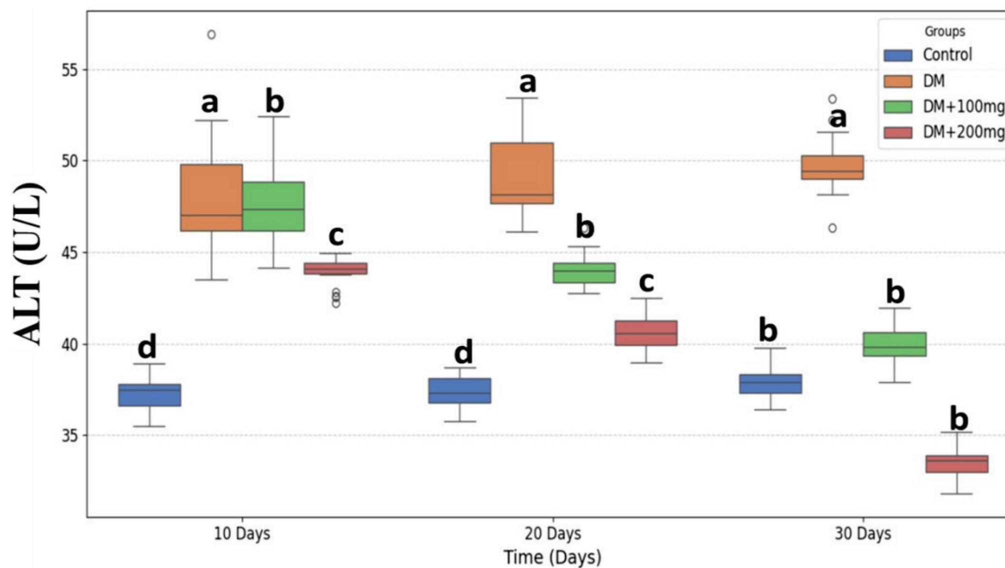

Fig. 7. Serum alanine transaminase (ALT) enzyme of the studied groups over a month of safflower treatment at three time points 10, 20, and 30 days: see Figure 1

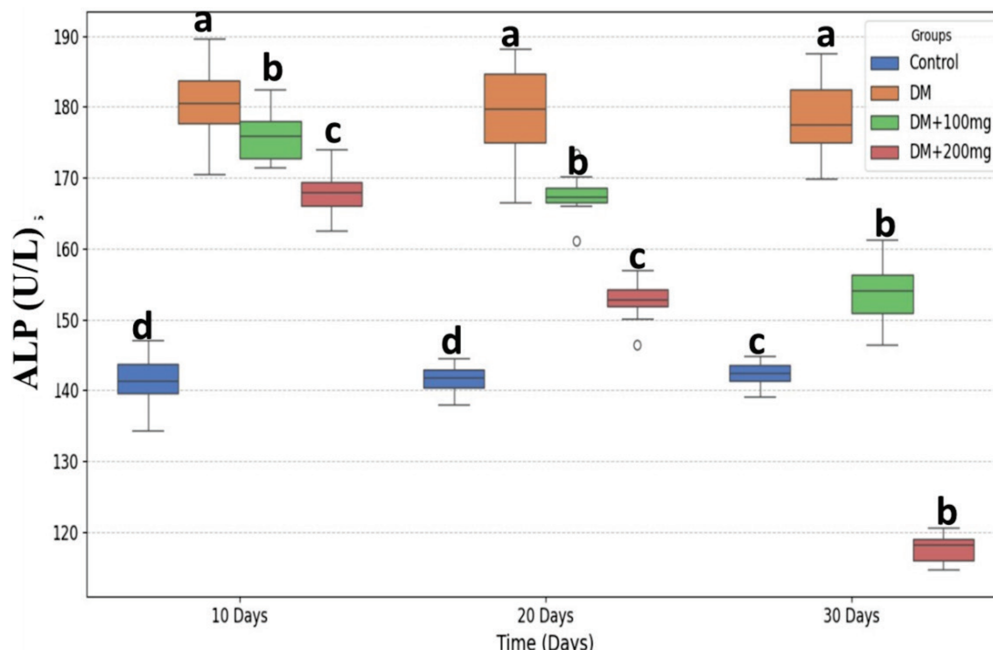

Fig. 8. Serum alkaline phosphatase (ALP) enzyme of the studied groups over a month of safflower treatment at three time points 10, 20, and 30 days: see Figure 1

Discussion

Safflower has been shown to effectively improve glycemic parameters via increasing insulin secretion, improvement of insulin resistance, and reduce glucose levels in rat diabetic models exposed to alloxan. Moreover, safflower has also been shown to moderate the elevated cholesterol and triglyceride levels which are due to alloxan exposure. Alloxan has elevated liver enzymes which have been slightly improved only by high doses of safflower. The composition of the extract is helpful in providing the tissue protection against various injuries. The levels of insulin were increased by safflower, and glucose levels reciprocally reduced together with improved insulin resistance.

This is in line with Asgary et al. (2012), who has reported that safflower reduced glucose and improved insulin levels in alloxan induced laboratory animals (Asgary et al., 2012). The extract of *C. tinctorius* has shown inhibitory impacts on lipopolysaccharides-induced RAW cell line apoptosis alongside inhibition blocking α-glucosidase activity, providing a good template for diabetic patients (Liao et al., 2014). Some chemical compounds were isolated from safflower oil and could be considered as a template for synthesis of new compounds that will inhibit α-glucosidase, including N-(p-coumaroyl)serotonin and N-feruloylserotonin, which offer a stronger inhibitory activity on α-glucosidase compared to acarbose-the standard drug (Takahashi & Miyazawa, 2012).

Safflower has induced hypolipidemic effects through reducing triglyceride and cholesterol levels. These effects were also reported by Venkatesh et al. (2010) and Arpornsuwan et al. (2010), which confirmed that the alloxan-induced hyperlipidemia was reduced in the safflower treated group compared to the alloxan group without safflower treatment (Arpornsuwan et al., 2010; Venkatesh et al., 2010). This could potentially explain the therapeutic effects of safflower for treatment of cardiovascular diseases (Liu et al., 2014; Yin et al., 2014). This could be explained in term of the capacity of *C. tinctorius* to reduce serum lipid profile and positively enhance cardiovascular function and provide protection for endothelial cells and cardiomyoblasts preventing injury to cardiomyocytes (Huang et al., 2009; Tien et al., 2010; Wan et al., 2011). This was confirmed by *in vitro* inhibition of lipopolysaccharides induced apoptosis of cardiomyoblast using H9c2 cell line (Tien et al., 2010). Moreover, extract of *C. tinctorius* seed has potentially dampened the oxidation of important cardiac biomarkers indicating cardioprotection (Koyama et al., 2008), and the extract has reduced arteriosclerosis in hypertensive patients (Suzuki et al., 2010). Diet- induced hyperlipidemia in rats has shown improvement in lipid parameters following use of safflower extract and more importantly the safflower extract has reduced apoptosis due to ox-LDL in *in vitro* experiments (Arpornsuwan et al., 2010; Meng et al., 2022). The mechanism of reducing cholesterol could be related to increased hepatic mRNA expression of the ATP binding cassette, subfamily A, member 1 (ABCA1) and scavenger receptor class B, in type I (SRBI) Wistar-strain rats which facilitate the increase in HDL expression (Wellington et al., 2003). Moreover, safflower has increased the the expression of ABCA1 in comparable manner to atorvastatin and even better SRB1 expression in diet treated rats (Arpornsuwan et al., 2010).

Safflower has improved liver enzymes of rats following exposure to alloxan. This outcome has been reported in Asgary et al. (2012), who reported that extract of *C. tinctorius* has led to improvement of liver enzymes following rats' exposure to alloxan (Asgary et al., 2012). It has been reported that the extract of *C. tinctorius* content is associated with antioxidant effects and can protect the liver tissue from damaging effects of oxidative stress (Li et al., 2014; Xie et al., 2015). The extract of *C. tinctorius* has been found to protect liver cells from oxidative stress due to its flavonoid content (Li et al., 2014; Xie et al., 2015). The extract has also been reported to protect liver tissue from damage induced by carbon tetrachloride together with liver enzyme preservation (Wu et al., 2013). Alcohol- induced liver damage has been attenuated by safflower indicated by modulated oxidative stress markers, malondialdehyde and total antioxidant capacity (He et al., 2015), alongside attenuated liver enzymes, indicating reduced damage. It has also been found that safflower reduced oxidative stress associated with liver fibrosis in a rat model exposed to carbon tetrachloride (Zhang et al., 2011). Moreover, several damaging and tissue remodeling parameters were reduced by safflower, including α-smooth muscle actin, collagen α type I, matrix metalloproteinase-9, and transforming growth factor (TGF)-β1, alongside reduced apoptosis due to activation of peroxisome proliferator-activated receptor-γ (PPA-γ) (Li et al., 2012; Wang et al., 2013). Furthermore, inflammatory response was reduced efficiently via regulation of cytokine expression via reduced proinflammatory markers and increased anti-inflammatory markers from immune cells and cells in the vicinity of the site of injury (Jiang et al., 2014).

Conclusion

Safflower extract has shown therapeutic activities in the model of rats stressed by alloxan. The study revealed that safflower oil has improved glycemic parameters, lipid parameters, and liver injury parameters in rats after being exposed to damaging effects of alloxan. These findings provide preliminary results, providing a template for isolation of new drugs from the extract to be applied for treatment of diabetes.

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