

# Therapeutic Effect of *Zygophyllum cornutum* on Metabolic Disturbances, Oxidative Stress in Heart Tissue and Histological Changes in Myocardium of Streptozotocin-induced Diabetic Rats

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**Abstract:** The present study depicts the therapeutic effect of *Zygophyllum cornutum* methanolic extract (ZCME) on metabolic disturbances, oxidative stress in heart and myocardium histological changes in streptozotocin (STZ)-induced diabetic rats. Three days after diabetes induction, ZCME was administered orally for six weeks (700 mg/kg bw/day). Serum glucose and lipid profile were evaluated. Reduced glutathione (GSH), catalase (CAT) and thiobarbituric acid reactive substances (TBARS) were measured in heart tissue. The results showed increased levels of blood glucose, total cholesterol (TC), LDL cholesterol (LDL-C) and triglyceride (TG) in the diabetic rats. On the other hand, the level of HDL cholesterol (HDL-C) decreased. Compared to the control normal rats, the level of TBARS in heart tissue was markedly increased while GSH and CAT were significantly modified in the diabetic rats. Oral administration of ZCME has normalized serum glucose and lipid profile. TBARS were significantly reduced in heart while CAT and GSH were markedly normalized. Myocardium sections showed the absence of histological changes observed in the diabetic rats. The study suggests that *Zygophyllum cornutum* may provide a useful therapeutic option in the reversal of metabolic disturbances and oxidative stress-induced cardiac dysfunction in diabetes mellitus.

**Key words:** Diabetes mellitus, myocardium, oxidative stress, *Zygophyllum cornutum* coss.

## 1. Introduction

Diabetes mellitus is one of the most costly burdensome chronic diseases of our time and is a condition that is increasing in epidemic population in the whole world. Diabetes mellitus is regarded as a group of metabolic diseases characterized by an elevated blood glucose level resulting from defects in insulin secretion, insulin action or both [1]. Hyperglycemia, resulting from uncontrolled glucose regulation, is widely recognized as the causal link between diabetes, oxidative stress and diabetic complications [2]. Elevated

glucose levels may cause a wide range of metabolic disturbances in vascular cells and organ tissues of diabetic patients [3]. More research is directed towards medicinal plants that are considered as an important source of many herbal substances with antidiabetic and antioxidant activities. The present study depicts the therapeutic effect of *Zygophyllum cornutum* on metabolic disturbances and oxidative stress in heart as one of the target organs in diabetes mellitus.

## 2. Materials and Methods

### 2.1 Preparation of *Zygophyllum cornutum* Methanolic Extract (ZCME)

The preparation method of the methanolic extract

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involved submitting the grounded plant (100 g) to extraction with 500 mL of methanol (70%) for 10 days followed by filtration through Whatman no 1 filter paper. After the extraction, the solvent was evaporated and then the methanolic extract was stored at -20 °C.

## 2.2 Animals

Male Wistar rats weighing 130-170 g and housed in clean cages under appropriate conditions were used. The animals were injected with streptozotocin (55 mg/kg, i.p). Three days after injection, the rats with fasting blood glucose higher than 180 mg/dL were used for the experiment.

## 2.3 Drug Administration and Experimental Design

The extract was administered orally at a dose of 700 mg/kg body wt/10 mL. The rats were divided into five groups. Six rats were used in each group:

Group 1: Normal Control (NC) rats received 10 mL/kg NaCl solution (0.9%).

Group 2: Normal Treated (NT) rats received 700 mg/10 mL/kg ZCME.

Group 3: Diabetic Control (DC) rats received 10 mL/kg NaCl solution (0.9%).

Group 4: Diabetic Treated (DT) rats received 700 mg/10 mL/kg ZCME.

Group 5: Diabetic rats received 5 units/kg of insulin (DIS).

## 2.4 Biochemical Assay

Serum glucose and lipid profile parameters were determined using specific kits. Oxidative stress parameters are measured by specific methods: malonyl dialdéhyd (MDA) [4], catalase activity (CAT) [5] and reduced glutathione (GSH) [6].

## 2.5 Histological Analysis

The histological study of the myocardium was performed by the eosine & hematoxyline method according to an applied protocol in the laboratory of anatomopathology at CHU Constantine, Algeria.

## 2.6 Statistical Analysis

The results were expressed as means  $\pm$  SD (standard deviation) of six rats per group and the statistical significance was evaluated by one way analysis of variance (ANOVA) followed by Tukey test using the INSTAT2 MS-DOS system. Mean values were considered statistically significant when  $P < 0.05$ .

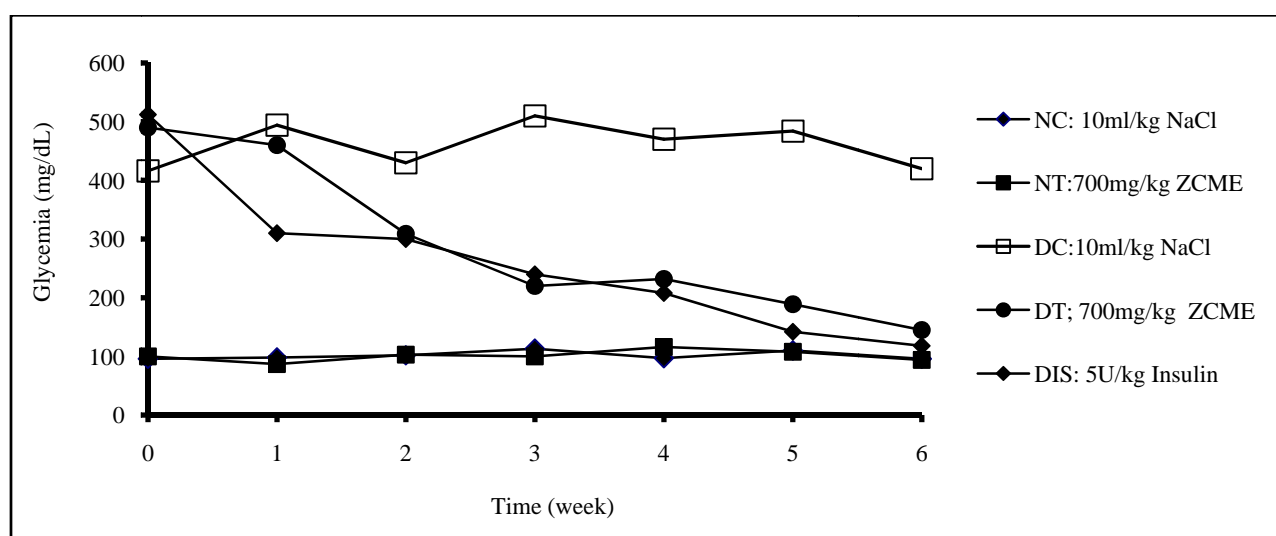
# 3. Results and Discussion

## 3.1 Glycemia and Lipid Profile

After the experimental period (6 weeks), the results showed increased levels of blood glucose, total cholesterol (TC), LDL cholesterol (LDL-C) and triglyceride (TG) in the diabetic rats. On the other hand, the level of HDL cholesterol (HDL-C) decreased in this group. However, oral administration of ZCME improved significantly serum glucose (Fig. 1) and lipid profile (Table 1).

In our study, single intraperitoneal injection of streptozotocin (55 mg/kg body weight) induced diabetes after 3 days. After six weeks of treatment, glycemia in control diabetic rats reached a rate of  $420 \pm 81.05$  mg/dL. In diabetic rats treated with ZCME, a significant decrease of glycemia was noted from the second week and the value recorded after six weeks was  $145 \pm 12.3$  mg/dL. The hypoglycaemic effect of ZCME may be due to polar substances groups [7]. The studied plant is rich in saponins, for which a strong antidiabetic activity is assigned according to the previous [8].

Lipid profile became a useful parameter through its use in the diagnosis of several cardiovascular diseases and control of diabetic patients [9]. The results of our study showed a significant increase in TC (+36.4%), TG (+44.9%) and LDL-C (+53.37%) associated with a remarkable decrease in HDL-C (-15.16%) in diabetic rats. ZCME improved significantly lipid profile. We noted an increase of HDL-C known as good cholesterol [10] because of their beneficial role against cardiovascular complications mainly atherosclerosis [11]. In addition, we noted a decrease of TC, TG and

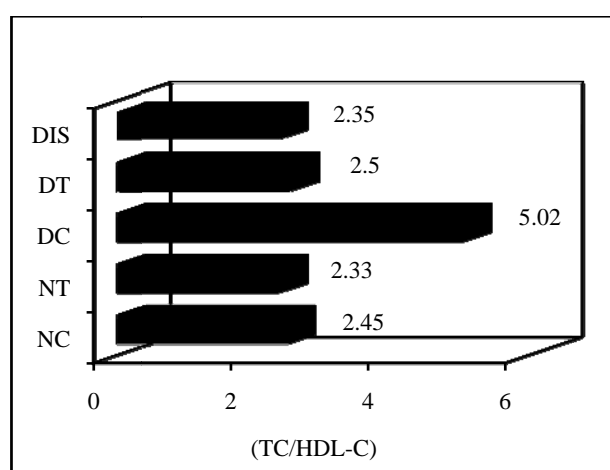


**Fig. 1** Blood glucose evolution in normal control (NC) rats, normal treated (NT) rats, diabetic control (DC) rats, diabetic treated (DT) rats and insulin diabetic treated (DIS) rats during 6 weeks.

**Table 1** Serum lipid profile after 6 weeks treatment.

| Parameters    | Normal control (NC) | Normal treated (NT) | Diabetic control (DC) | Diabetic treated (DT) | Insulin diabetic treated (DIS) |
|---------------|---------------------|---------------------|-----------------------|-----------------------|--------------------------------|
| TC (mg/dL)    | 83.57 ± 2.92        | 81.28 ± 3.57        | 131.43 ± 8.03 ***     | 98.28 ± 4.97 **       | 98.14 ± 2.53 **                |
| TG (mg/dL)    | 49.85 ± 2.74        | 42.14 ± 8.07        | 90.43 ± 5.21 ***      | 45.43 ± 3.62 ***      | 52.14 ± 3.31 **                |
| HDL-C (mg/dL) | 34.14 ± 4.01        | 34.85 ± 2.58        | 28.71 ± 2.49 *        | 39.28 ± 2.6 **        | 41.71 ± 3.05 ***               |
| LDL-C (mg/dL) | 39.45 ± 3.87        | 38 ± 6.15           | 84.63 ± 0.50 ***      | 49.91 ± 6.35 ***      | 46 ± 2.21 ***                  |

Data are expressed as mean ± SD (n = 6). Group 3 (DC) compared to group 1 (NC). Group 4 (DT) and group 5 (DIS) compared to group 3 (DC) (\*)  $0.05 > P > 0.01$ , (\*\*\*)  $P < 0.001$ .



**Fig. 2** Cardio-vascular risk estimation in normal control (NC), normal treated (NT), diabetic control (DC), diabetic treated (DT) and insulin diabetic treated (DIS) rats.

LDL-C called bad cholesterol [10]. High and persistent LDL-C level in blood increase their susceptibility to oxidation by free radicals [12], thereby producing oxidized LDL characterized by their high

pro-atherogenic properties [13]. The improvement of the lipid profile may result from changes in lipoprotein metabolism [14] or by increasing the LDL uptake [15].

The relationship between dyslipidemia and cardiovascular diseases is particularly well established [7, 8]. The cardiovascular risk can be estimated using the Framingham ratio: [Total Cholesterol/HDL-C]. It particularly increases when this ratio is over than 5. Figure 2 shows cardio-vascular risk estimation in the different groups.

Studies have shown that the decrease of lipid levels during diabetes reduces the cardiovascular complications risk [2]. The Framingham study particularly popularized the ratio [TC/HDL-C]: the cardiovascular risk increases particularly when this ratio exceeds 5. In our study, [TC/HDL-C] = 5.02 in diabetic rats, so it is on the edge of danger. Diabetic rats treated with ZCME showed a value close to that of

normal and diabetic rats treated with insulin. This result shows the preventive role of *Zygodium cornutum* against cardiovascular complications associated with diabetes.

### 3.2 Oxidative Stress Parameters in Heart

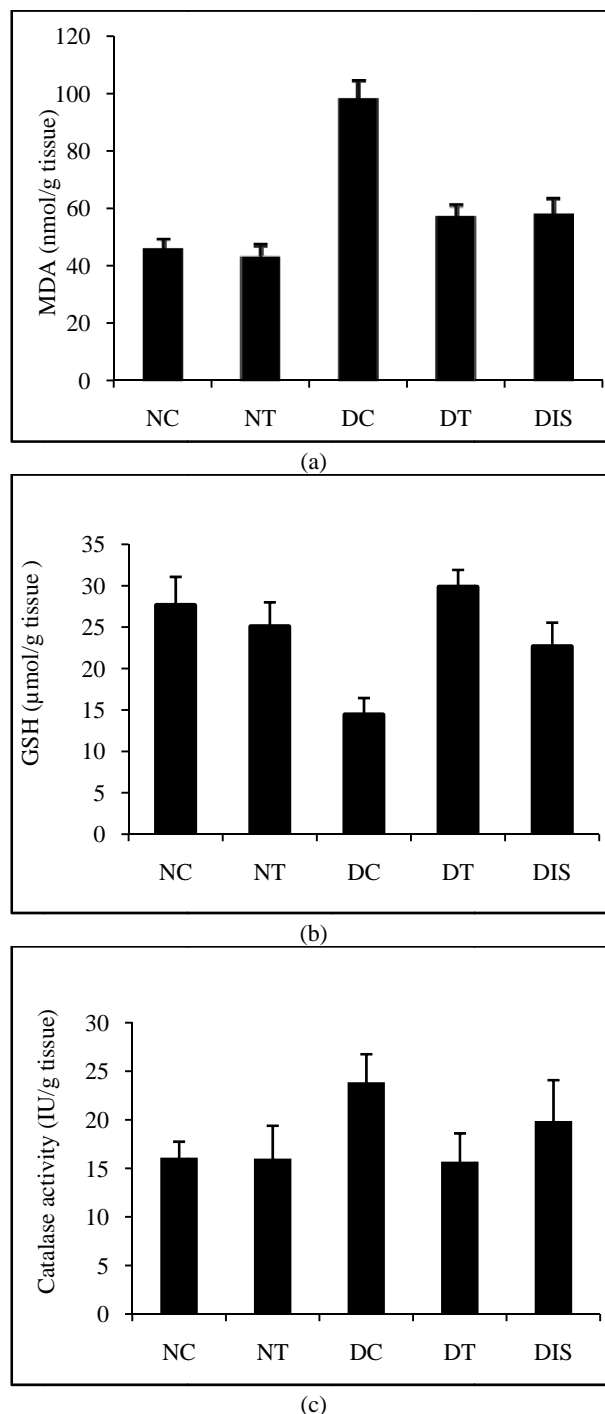
The evaluation of heart oxidative stress markers showed that MDA concentration in heart tissue was markedly increased while GSH and CAT were significantly decreased in diabetic rats compared to control normal rats. After oral administration of ZCME, the MDA concentration was significantly reduced in heart while CAT and GSH were markedly normalized (Fig. 3 (a); (b); (c)).

The evaluation of oxidative stress parameters in the heart showed a significant increase of the TBARS level in diabetic rats. A significant increase in catalase activity was also noted with remarkable depletion of reduced glutathione. Ouali K and his collaborators [15] considered that this increase is a compensatory effect against oxidative stress resulting from the accumulation of endogenous  $H_2O_2$  and GSH depletion. These parameters are positively improved in rats treated with ZCME which is rich in phenolic compounds characterized by antioxidant activity.

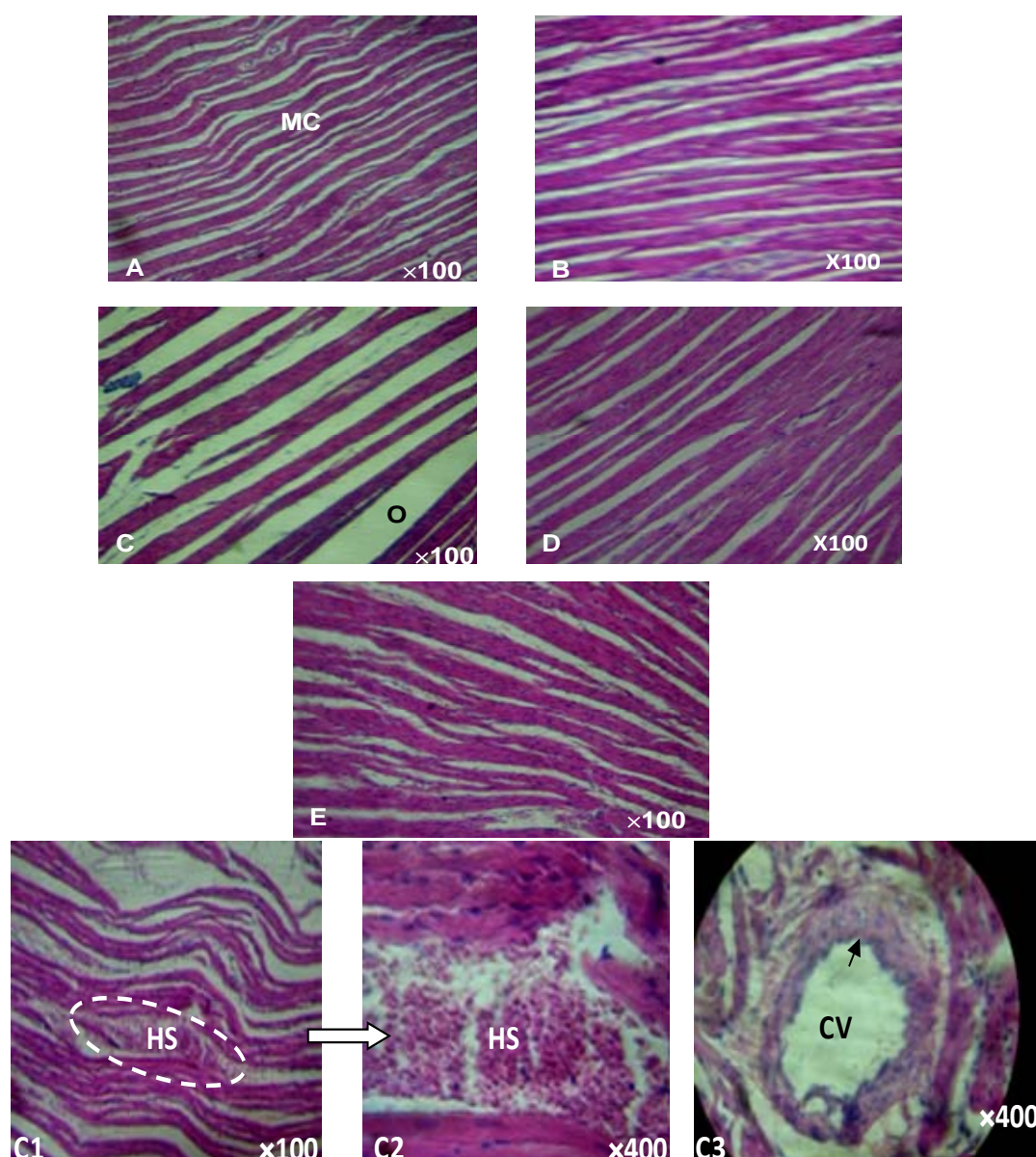
### 3.3 Histological Analysis

In diabetic rats, the histological sections showed Oedemous rearrangements, hemorrhagic suffusion and vascular congestion (Fig. 4 (c); (c1); (c2); (c3)). Myocardium tissue appears very loose compared to normal control rats. In ZCME and insulin diabetic groups, histological sections showed a normal myocardium. Histological examination of the myocardium performed with eosine & hematoxyline coloration showed different aspects of damage in the diabetic group: oedema, vascular congestion and haemorrhagic suffusion. A significant prevention of the oxidative damage was noted in diabetic group treated with ZCME. These results may be due to the hypoglycemic and antioxidant effects made by the plant. Atangwho and his collaborators suggested that

plants flavonoids may exert a protective effect against oxidative stress damage in organs [6].



**Fig. 3** Oxidative stress parameters in heart tissues of normal control (NC), normal treated (NT), diabetic control (DC), diabetic treated (DT), and insulin diabetic treated (DIS) rats. (a) MDA concentration, (b) GSH concentration, (c) catalase activity. Results are expressed as means  $\pm$  SD (n = 6). DC compared to NC. DT and DIS compared to DC. (\*\*)  $0.01 > P > 0.001$ , (\*\*\*)  $P < 0.001$ .



**Fig. 4** Myocardium histology (Hematoxyline & Eosine method). A (normal control rats), B (normal rats treated with ZCME), C [1, 2, 3] (diabetic control rats), D (diabetic rats treated with ZCME), E (diabetic rats treated with insulin). MC: myocardium cells. O: Oedema, VC: vascular congestion, HS: hemorrhagic suffusions.

#### 4. Conclusions

In summary, the present study demonstrated an increase of metabolic disturbances in diabetic rats associated with oxidative stress in the heart which results in myocardium damages. This study clearly showed that *Zygophyllum cornutum* may provide a useful therapeutic option in the reversal of metabolic disturbances and oxidative stress induced cardiac dysfunction in diabetes mellitus.

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