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Antidiabetic activity of callus extract of *cleome droserifolia* in rats

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- ✓ *Cleome droserifolia*
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Abstract

Herbal medicines are promising choice over synthetic drugs, *Cleome droserifolia* medicinal herb belongs to Family Cleomaceae. The dried herb of *C. droserifolia*, locally named in Egypt Samwah and used as hypoglycemic plant by herbalists. *In vitro* cultures could be a possible alternative to analyze crude extract for pharmacological activities rather than collecting and destroying possibly endangered plants and obtaining important phytochemicals *in vitro*. In this study, callus cultures were produced using hypocotyl and root explants of *C. droserifolia* using different combinations of growth regulators, The maximum of callus percentage was observed with root explants cultured on MS media supplemented with 0.5 mg/L a-naphthaleneacetic acid NAA + 1 mg/L benzyl adenine BA and methanol extract of callus was evaluated for their antidiabetic potential. Diabetes mellitus was induced by injection of single intrapretoneal dose of streptozotocin STZ (60 mg/Kg bw), The crude methanolic extract prepared from the callus of *cleome droserifolia* hypocotyl from media 5 contains 1 mg/L BA (200 mg/kg) was orally administered to STZ-induced diabetic rats once daily for 4 weeks, results demonstrated that, restored serum glucose levels along with improved insulin levels of diabetic rats. Decreased serum aspartate aminotransferase (AST) and alanine aminotransferases (ALT) activites and malondialdehyde (MDA) level, while increament of total Antioxidant Capacity (TAC) , glutathione (GSH), serum and tissue protein, thyroid hormones T3 and T4 levels were also increased after administration of plant callus extract. Furthermore, HPLC chromatograms of crude methanolic callus extract showed the presence of two flavonoids (quercetin and apigenin) which may explain the effect of antidiabetic and antioxidant properties.

1. Introduction

Nowadays herbal drugs play a vital role in the treatment of diabetes and its complications. One of these hypoglycemic plants is Cleome droserifolia (C.) which is a flowering plant belong to family Cleomaceae [1-2] known as Samwah, Afein, Reeh-El Bard in traditional language [3] and it grows in rocky and rough habitats in different regions in the deserts especially North Sinai, Red Sea region and Gebel Elba][4,5]. *C.droserifolia* (C.) were found to contain several classes of compounds; such as volatile oil, gluosinolates with sulfur aglycones, flavonoids, sesquiterpenes, terpenoids, alkaloids and sterols which have antioxidant and hepatoprotective activity [5-7]. Cleome species have a great history of medicinal uses as, rubefacients, stomachics and in scabies treatment, inflammation and rheumatic fever [8]. Also are used in different disorders such as fever, diarrhea, inflammation, bronchitis, liver diseases, malaria fever and skin diseases [9-12]. Leaves and stems were been decocted for the treatment of diabetes by the Bedouins of the southern Sinai [5] and were famous as an antihyperglycemic agent [13,14].

Diabetes mellitus(DM) is a serious metabolic health disease , which is related to troubles in carbohydrate, protein and fat metabolism [15]. The disease is characterized by hyperglycemia or increased blood glucose,

caused by a defect in insulin production, insulin action or both [16]. Diabetes is considered as one of the most important diseases worldwide. Researchers strengthen the evidence that the complications associated to DM are linked with oxidative stress in which due to high levels of oxygen free radicals and a significant reduction in antioxidant defences [17].

The techniques of callus culture and cell suspension culture have been used for studying biosynthesis of secondary metabolites to increase the synthesis of natural compounds or new compounds. This is better than the *in vivo* study of the whole crop plant as the production can be more reliable, simpler, and the isolation of the phytochemicals is efficient, as compared to extraction from complex whole plants [18]. There are many literatures on the tissue culture of Cleome species as [19-24].This study is considered as the first report on the use of tissue culture technique for demonstration of the in vivo antidiabetic activity of the callus extract of C. droserifolia in STZ-diabetic rats.

2. Material and Methods

2.1. *Plant material*

Mature seeds of *C. droserifolia* were collected from their growing habitats in south Sinai, Saint Catherine, Egypt. The *in vitro* raised seedlings reaching about 4-6 cm in height after 15 days of germination were used as a source of starting plant materials. Seeds were sterilized by immersion in 70% ethanol for 30 sec then rinsed three times with sterile distilled water and immersed in 10% NaOCl (Clorox) solution for 5 min followed by washing three times with sterile distilled water. Then, they were cultured on MS medium containing 3% sucrose and 4.4 g/l of basal MS salts without any supplementation of growth regulators and kept under dark condition for 48 hours for germination procedures . All culture were incubated in temperature controlled growth room at 27 ± 1 C° at 16 hrs daily light system using cooling white fluorescent light (2000 Lux).

2.2. *Callus induction*

Hypocotyl and roots explants excised from the *in vitro* raised seedlings were used as the source material for callus induction and cultured on full strength of basal solidified MS medium supplemented with different combinations of growth regulators as follow:

Media 1 (M1) = 1.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) + 1.5 mg/l α- naphthalene acetic acid (NAA), M2= (2.5 mg/l 2,4-D), M3= (0.5 mg/l NAA + 1 mg/l Benzyl adenine (BA), M4= (0.5 mg/l 2,4-D + 1 mg/l BA), M5= (1 mg/l BA). All cultures were incubated in the growth chamber at 27±1 ºC under light conditions of 16 h per day photoperiod at light intensity 2000 Lux, produced from cooling white fluorescent lamps. After 4 weeks of culturing, percentage of callus formation, fresh (FW) and dry (DW) weights (g/ jar) were calculated. The evaluation of the type and concentration of growth regulators that give highest and healthy callus proliferation from different explants were also detected.

2.3. *Preparation of callus extract*

Dry callus was air dried and grounded in the laboratory. The obtained powder was extracted three times at room temperature with 70% methanol/water for an interval of six days[25].The aqueous methanol extract was subjected to High performance liquid chromatography (HPLC) analysis to identify the best media and explant that contain the highest amounts of quercetin and apigenin that will be then subjected to biological investigation.

2.4. *Qualitative and quantitative determination of flavonoids using HPLC technique*

The crude filtered extracts were concentrated under reduced pressure in a rotary evaporator to give a residue which dissolved in methanol.

HPLC was used to determine the flavonoids contents (Quercetin and Apigenin) quantitavely in the total crude extract of each callus.

As follows [26]; an Agilent HPLC 1200 series equipped with diode array detector (Agilent Technologies, Waldbronn, Germany). Chromatographic separations were performed using a water column C18. The binary mobile phase consisted of (A) acetonitrile and (B) 0.1% acidified water with formic acid. The elution profile was: 0-1 min 100% B (isocratic), 1-30 min 100-70% B (linear gradient), 30-35 min 70-20% B (linear gradient). The flow rate was 0.3 ml/min and the injection volume was 5 µl. Chromatograms were recorded at 278 nm. This analysis enabled the characterization of flavonoids on the basis of their retention time and UV spectra. The retention time of the isolated compounds were compared with those of standard samples obtained from Phytochemistry and plant systematic Department, National Research Center.

2.5. *In vivo antidiabetic activity*

2.5.1. *Animals and experimental design Ethics*

Anesthetic procedures and handling with animals complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt and faculty of science -Cairo university performed to ensure that the animals do not suffer at any stage of the experiment (Approval no: 11146) .

Male Wistar albino rats (140-160g) were selected for this study. They were obtained from the Animal House, National Research Center, Egypt. Animals were kept for 2 weeks to accommodate to laboratory conditions and were allowed free access to standard diet and water all over the period of the experiment. The standard diet was supplied by El-Nasr pharmaceutical company. It composed of 72.20% carbohydrates, 3.40% fats, 19.80% proteins, 3.60% cellulose, 0.50% vitamins, minerals and 0.50% salts.

Based on the HPLC results we chose the methanol extract from the hypocotyl callus of *C. droserifolia* from media 5 as it contain the highest concentrations of querectin for treatment of rats.

2.5.2.Diabetes induction

Animals were fasted overnight and diabetes induced by using the method of [27]. In brief, (STZ) was dissolved in 0.1M sodium citrate buffer, pH 4.4, and injected intraperitoneally at a single dose of 60 mg/kg b.w.2 hours, following STZ injection, rats were given 5% glucose solution to avoid hypoglycemic shock. After three days of diabetic induction, rats were fasted overnight and the blood glucose level was estimated. Rats with blood glucose level>300 mg/dL were considered diabetic.

Sixty rats were divided into six groups (10 rats each).

Group 1: Rats received a single oral dose of 0.5 ml of 1M citrate buffer and served as control group.

Group 2: Rats treated with a daily oral dose of the callus extract (200 mg/kg b.wt) for four weeks.[28]

Group 3**:** (protective group) rats treated with a daily oral dose of the callus extract (200 mg/kg b.wt) firstly for two weeks then injected with STZ and finally treated with the callus extract for two weeks.[28]

Group 4: Diabetic rats were left untreated

Group 5**:** Diabetic rats were treated with daily oral dose of callus extract (200 mg/kg b.wt) for four weeks.

Group 6: Diabetic rats received glibenclamide at a daily oral dose of (5 mg/kg b.wt) [29]**.** Treatments were carried throughout a period of 4 weeks after diabetes induction.

2.5.3. Samples preparation

Serum samples: Blood was collected from each animal by puncture of the sub-lingual vein in clean and dry test tube, left 10 min to clot and centrifuged at 3000 rpm at 4^{o}C for serum separation. The separated serum was stored at -80^{o}C for further biochemical analysis .

Liver tissue was homogenized in normal physiological saline solution (0.9N Nacl) (1:9 w/v). The homogenate was centrifuged at 4^{o}C for 5 min at 3000 rpm and the supernatant was used for estimation of liver function and marker enzymes and the antioxidant parameters.

2.5.4.Determination of the effect of callus extract on Diabetes markers:

2.5.4.1. *Serum insulin level*: was measured by a quantitative enzyme-linked immunosorbent assay (ELISA) technique [30].

2.5.4.2. *Serum glucagon level*: was measured by a quantitative enzyme-linked immunosorbent assay (ELISA) technique [31]

2.5.4.3. *Serum Glucose level*: was determined by enzymatic colorimetric method [32].

2.5.5. Determination of the effect of callus extract on oxidative stress markers:

2.5.5.1. *Total Antioxidant Capacity (TAC)*: liver tissue of TAC was determined by colorimetric method [33].

2.5.5.2. *Glutathione (GSH) level*: liver tissue of GSH was determined by colorimetric method [34]

2.5.5.3. *Malondialdehyde (MDA) level*: liver tissue of MDA was determined by colorimetric method [35].

2.5.6. *Determination of the effect of callus extract on Liver function:*

2.5.6.1. *Total protein*: was determined in both serum and tissue by colorimetric method [36]

2.5.6.2. *Aspartate and alanine aminotransferases (AST &ALT)*: AST and ALT enzymes activity were estimated in serum by the method of Reitman and Frankel [37].

2.5.7.Determination of the effect of callus extract on thyroid function:
2.5.7.1. *Serum T3 and T4 level*s :were measured by a quantitative enzyme-linked immunosorbent assay (ELISA) technique [38 and 39] respectively.

2.5.8. *Histopathological Analysis:*
Samples were taken from the Liver and pancrease and fixed in 10% formalin. Paraffin embedded samples were prepared for sectioning at 4μm thickness. Slides were stained with hematoxylin and eosin and examined by light microscope according to the method of Bancroft and Stevens [40]**.**

2.6. *Statistical Analysis*:
All data were expressed as mean±SD of ten rats in each group. Statistical analysis was carried out by one-way analysis of variance (ANOVA), Costat Software Computer Program [41]**.**

3. Results and discussion

3.1. *Callus induction*

The proliferation of calli at wounded edges of explants was initiated during the first two weeks. The obtained results indicated that the callusing formation percentage was affected by different combinations between auxins and cytokinins (different concentrations of BA / NAA and by the explant types and indicated that M3 medium supplemented with 0.5 mg/l NAA + 1 mg/l BA showed the highest mean of callus formation; The maximum of callus percentage was observed with root explants while MS media supplemented with 1 mg/l BA (M5) showed the highest mean of embryogenic callus as shown in Figure 1. Based on the previous mentioned results, M3 and M5 media were found to be the best media among all tested media, giving healthy and rapidly growing callus proliferation for all type of explants and the frequencies of callus formation varied from 66.66% to 100% and for embryogenic callus varied from 0 to 88.33 as shown in (Figures 1,2,3).The frequencies of callus formation and embryogenic callus differed significantly among different combinations of growth regulators and different explants.

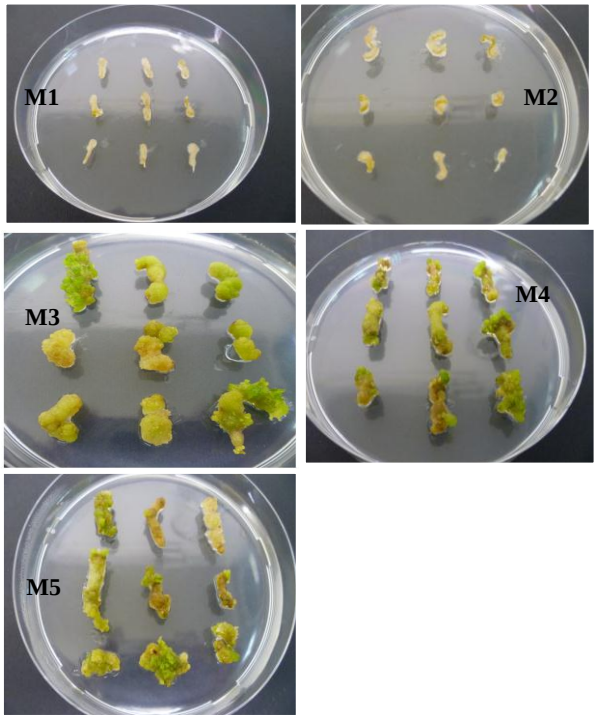

Figure 1: (A) Callus formation (%), (B) Embryogenic callus (%) of hypocotyl and root explants after four weeks of culturing on MS media supplemented with different combinations of growth regulators as the following: M1= (1.5 mg/l 2,4D + 1.5 mg/l NAA), M2= (2.5 mg/l 2,4D), M3= (0.5 mg/l NAA + 1 mg/l BA), M4= (0.5 mg/l 2,4D + 1 mg/l BA). M5= (1 mg/l BA).

These results are in agreement with Sirangi, [22] who observed that the highest yield of callus formation (90%) were obtained from explants cultured on MS medium supplemented with 1.0 mg/ml IAA and 2.0 mg/l BAP of *Cleome rutidosperma* DC **.**

Also our results is in agreement with Other report by Qin et al.[19] who found that leaves explant gave the best results of callus formation percentage than stem explant in *cleome spinosa* when cultured on MS medium supplemented with 6.0 mg/L BAP and 0.02 mg/L NAA had the highest callus induction rate of 93.3%. On the other hand, Anburaj et al.[42] established an effective protocol for callus formation using the leaf explants of the *Cleome viscosa*. and they found that about 81% callusing response was observed in leaf explants cultured on MS basal medium supplemented with 2mg/l indole-3-acetic acid (IAA) and these result is disagreement with our result as the the maximum of callus percentage (100%) was found in root explants cultured on MS media supplemented with 0.5 mg/l NAA + 1 mg/l BA (combination of auxin and cytokinen). Also Ichha et al. [43] showed that Callus induction was 100% at lower concentrations of NAA (0.1mg/l-1.0mg/l) and kinetin or 6-benzylaminopurine (BAP) at the concentrations of 0.5mg/l-2.5mg/l using leaves explants from *Cleome spinosa Jacq.* and *Cleome gyandra Linn.*

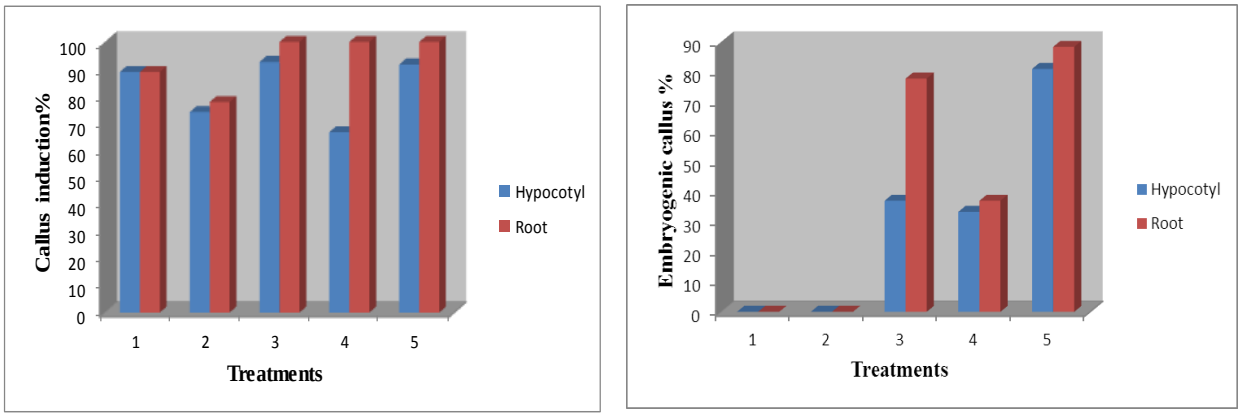

Figure 2: Callus induction from *C. droserifolia* hypocotyl explants after 4 weeks of culturing on MS-medium supplemented with different combinations of growth regulators as the following: M1= (1.5 mg/l 2,4D + 1.5 mg/l NAA), M2= (2.5 mg/l 2,4D), M3= (0.5 mg/l NAA + 1 mg/l BA), M4= (0.5 mg/l 2,4D + 1 mg/l BA). M5= (1 mg/l BA).

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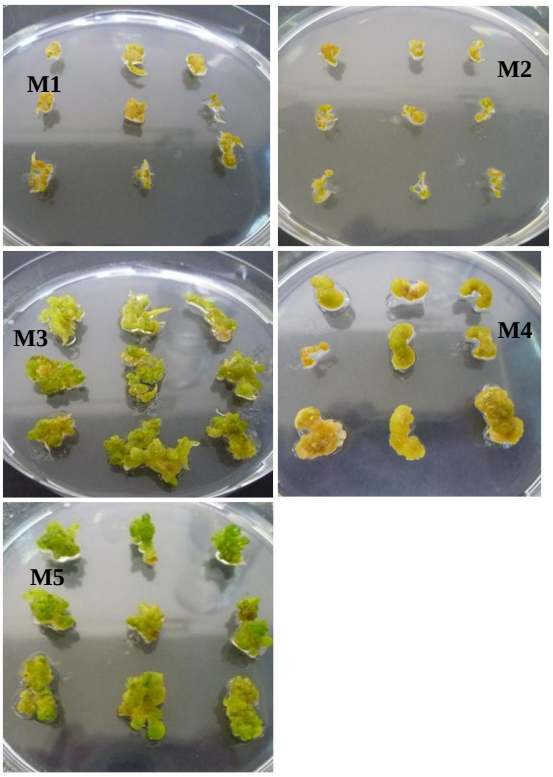


Figure 3: Callus induction from *Cleome droserifolia* root explants after 4 weeks of culturing on MS-medium supplemented with different combinations of growth regulators as the following: M1= (1.5 mg/l 2,4D + 1.5 mg/l NAA), M2= (2.5 mg/l 2,4D), M3= (0.5 mg/l NAA + 1 mg/l BA), M4= (0.5 mg/l 2,4D + 1 mg/l BA). M5= (1 mg/l BA).

3.2. *Effect of some growth regulators on production of flavonoids using HPLC*

The present study concluded that the highest value of quercetin contents was 0.0817 g/ 100 g dw which was found in high amount in hypocotyl calli explants culturing on M5 (1 mg/l BA) as compared with other explants and other subjected media, while the highest value of apigenin was 0.0339 g/ 100 g dw on (1.5 mg/l 2,4D + 1.5 mg/l NAA) M1 was recorded with root calli as compared with other type of explants further M1 gave the best results as compared with other subjected media as shown in (Figure 4) and these results are in agreement with El Naggar et al.[28] who identified from the HPLC chromatograms the presence of quercetin which may explain at least in part some of the antidiabetic and antioxidative properties observed in this study. And On the other hand Ezzat and Abdel Motaal [44] were isolated from ethanolic fraction EtFr extract of the aerial parts of Cleome droserifolia three flavonol glycosides, one of them was quercetin-3`-methoxy-3-O-(4``-acetylrhamnoside)-7-O-α-rhamnoside (F2) (quercetin derivative). The pharmacological profile of quercetin has been well demonstrated [45]..

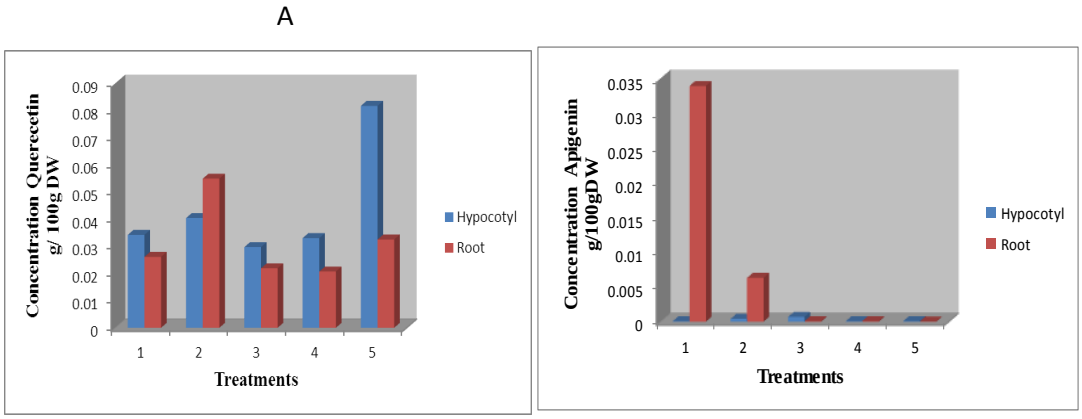


Figure 4: Effect of some growth regulators on production of Querecetin (A) and Apigenin (B) contents (g /100g DW) derived from calli cultures of hypocotyl and root explants of *Cleome droserifolia*

Indeed, the mechanism of quercetin to protect oxidative stress in cells such as (subsequent membrane degradation and lipid peroxidation) is related to chelating activities and free radical scavenging. We selected the crude extract derived from hypocotyl calli of M5 as it contain higher concentration of quercetin as it was the major bioactive compound found in comparison with apigenin for the treatment of rats

3.3. *Antidiabetic activity evaluation*

In our study, we used STZ to induce an *in vivo* hyperglycemia in rats. Several studies have been reported that the effect of STZ and alloxan induction of hyperglycemia in different experimental animals. These reports showed that these compounds have cytotoxicity on B-cells in the pancreas and the induction of permanent or chronic diabetes mellitus in these animals. In which a common mechanisms such as production of oxygen free radicals, changing of internal scavengers of these free radicals, the increment of DNA strand breakdowns, the inhibition of nicotinamide adenine dinucleatide (NAD) value and ultimately suppression of B-cell functions or scavenging activity [46,47].

Our results demonstrated that a significant decrease in serum insulin levels and serum glucagon accompanied with a significant increase in blood glucose levels in diabetic rats in compared with the control group, this finding in consistent with the results of Sambandam and El-Shenawy [48,49] on rats STZ-induced diabetic.

Single daily oral administration of *C. droserifolia* callus extract for 4 weeks significantly decreased the serum glucose of diabetic rats and caused a significant increase in the glucagon and insulin levels as shown in (Figure 5,6,7). These results are in agreement with El-Sherif, El-Shenawy and Abdel-Nabi and Anon [10,50-51] who demonstrated that the cleome decreased the blood glucose levels in hyperglycemic rats who showed that *C. droserifolia* boiling water and ethanolic extract markedly elevate serum insulin in STZ and alloxan-induced diabetic animals, which play an important role in treatment of hyperglycemia in these animals El-Shenawy and Abdel-Nabi , El-Seifi et al. and Rawi et al. [50,52-53] .

Abdel Motaal et al.[14] reported that the antihyperglycemic activity of *C. droserifolia* was attributed to significant insulin like effect in peripheral tissue ,These results may be due to that these herbs enhance insulin

secretion, increase glucose uptake by muscle tissues and suppress glucose absorption from intestine and production from liver [54].

The studied plant extract hypoglycemic potency may be attributed to the presence of certain compounds such as flavonoids which have insulin mimetic functions, so its reduced blood glucose in animals [55]**.** Another approach, flavonoid extracts suppress a-glucosidase activity and may inhibit the non-Na^+dependent smooth diffusion of monosaccharides in epithelial cells of intestine [56,57]

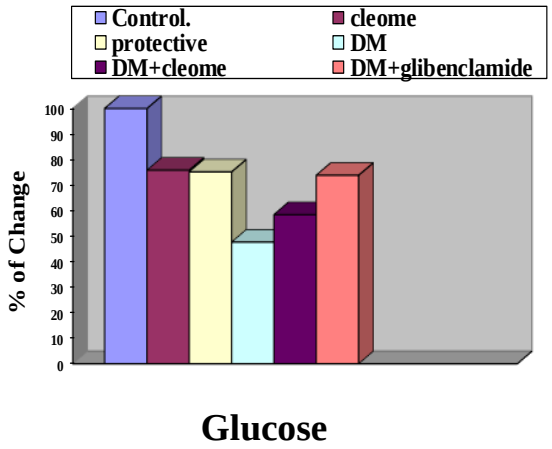


Figure 5 : percentage change in glucose levels of diabetic rats after treatment with callus extract of *cleome droserifolia*.as compared to control group

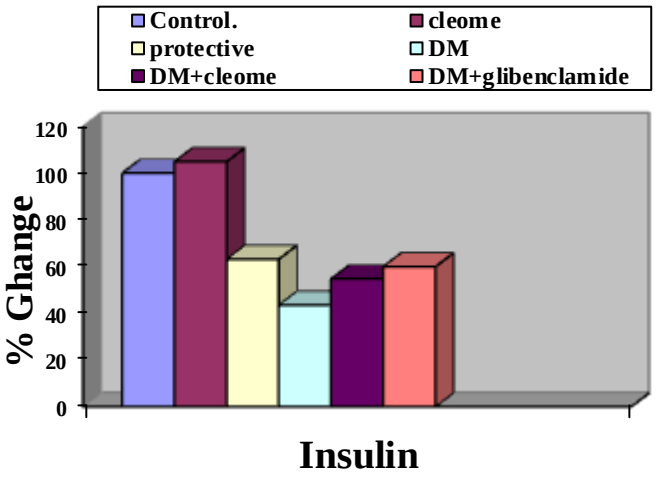


Figure 6 : percentage change in serum insulin levels of diabetic rats after treatment with callus extract of *cleome droserifolia*. as compared to control group

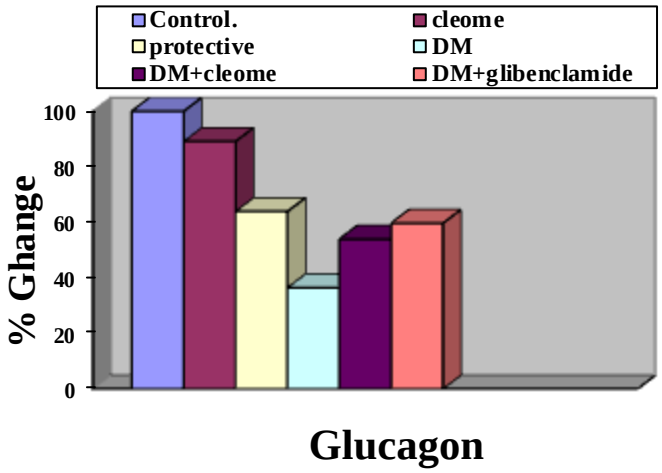


Figure 7: percentage change in glucagon levels of diabetic rats after treatment with callus extract of *cleome droserifolia*. as compared to control group

Diabetic patients with insulin deficiency results suffer from troubles in glucose utilization in which leads to generation high levels of oxygen free radicals and antioxidant capacity were reduced which could play an important role in oxidative stress and diabetic complications etiology. [58,59] **.**The differences in parameters of oxidative stress in diabetes mellitus were reported [60].Three types of oxidative stress parameters were used for evaluation it: lipid peroxidation, plasma total antioxidant and antioxidant defense systems.

In the present study the result showed a significant decrease in GSH content of the liver tissue of diabetic rats accompanied by a marked increase in the level of MDA of diabetic rats which are in agreement with the results of different authors[59-62].

The hepatic GSH decreased could be atributed to lower synthesis or increased GSH degradation by oxidative stress in diabetic animals. Reduced glutathione (GSH) helped the cellular immune system against oxidative

stress by chelating the reactive oxygen free radicals and intermediates, so it is play an important role in managing cellular redox state. The decrease in GSH levels in Diabetes mellitus DM has been recommended as high increase in oxidative stress [63] and the decrease in antioxidants could be considered as the causation of DM [64] .Thus, The decrease in GSH level might represent a direct interaction between GSH and free radicals generated by STZ compound [65] .

The high levels of MDA in diabetic animals may be attributed to hypoinsulinemia that increment the activity of fatty acyl coenzyme A oxidase, which promotes βeta-oxidation of fatty acids, causing lipid peroxidation. Increased lipid peroxidation deteriorates membrane functions by lowering membrane fluidity and changing the function of receptors and membrane attached enzymes [66]. These lipid peroxidation byproducts are more cytotoxic and unstable than reactive oxygen species (ROS) that interact with cellular organelles [67]. Besides that, cellular activities were disturbed by MDA which impairs the signal transduction pathways [68] .

In our study there is a decrease in the total antioxidant status in diabetic rats as compared with the normal control rats which is in consistent with the studies on the patients suffering from any of the two types DM that showed a significant reduction of the plasma total antioxidant status [69]. However, the increase in the total antioxidant capacity of serum diabetic rats after treatment with the callus plant extract could be attributed to absorption of antioxidants and to improvement *in vivo* antioxidant status as described by[46 and 70] as shown in (Figure 10).

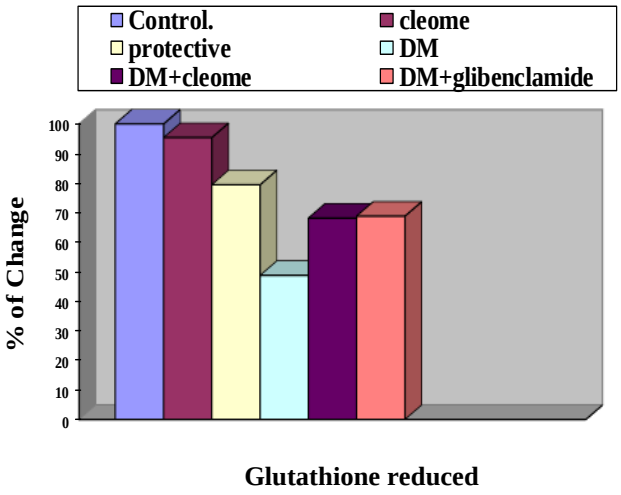


Figure 8: percentage change in glutathione reduced levels of diabetic rats after treatment with callus extract of *C. droserifolia* as compared to control group

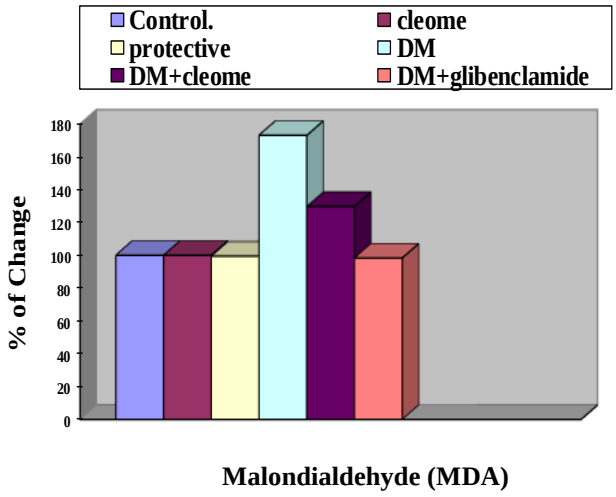


Figure 9: percentage change in malondialdehyde (MDA) levels of diabetic rats after treatment with callus extract of *C. droserifolia* as compared to control group

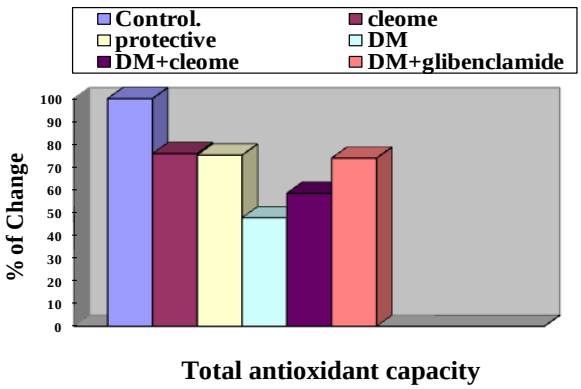


Figure 10: percentage change in total antioxidant capacity levels of diabetic rats after treatment with callus extract of *C. droserifolia*. As compared to control group

Our results indicated that the treatment of STZ- induced diabetic rats with callus extract of *C. droserifolia* exhibit a significant elevation in hepatic GSH level and decrease hepatic lipid peroxidation as compared with

the normal control group as shown in(Figures 8,9). This could indicate that the extract of the plant can either decrease the oxidative stress and/or elevate the biosynthesis of GSH which leading to less degradation of GSH.
So, The hypoglycemic consequence of the plant callus extract may be due to, at least in part, to its flavonoid content [71]. Flavonoids commonly contain one or more aromatic hydroxyl groups, which dynamically are responsible for the antioxidant properties and scavenge free radicals . Also, extract enriched with flavonoid prevent α-glucosidase activity and may suppress the non-Na^+dependent facilated diffusion of monosaccharides in epithelial cells of intestinal [72]. Though, from the present result it can be recommended that the *C. droserifolia* callus extract may exert antioxidant activities that keep the tissues from destructive damage of lipid peroxidation [49].
As shown in (Figure 11) it was observed a decrease of serum and tissue proteins (hypoproteinemia) in diabetic group as compared with normal control group which might be related to liver damage. Actually, the reduced in hepatic protein synthesis or amino acid uptake has been informed to be due to liver disease. Treatment with callus extract of *C. droserifolia* significantly increased serum and tissue protein concentration when compared to diabetic rats, which may have been due to the increment in insulin serum level, which led to acceleration in amino acid transport through cells and encourages the protein developing machinery of the cell[50 and 73].

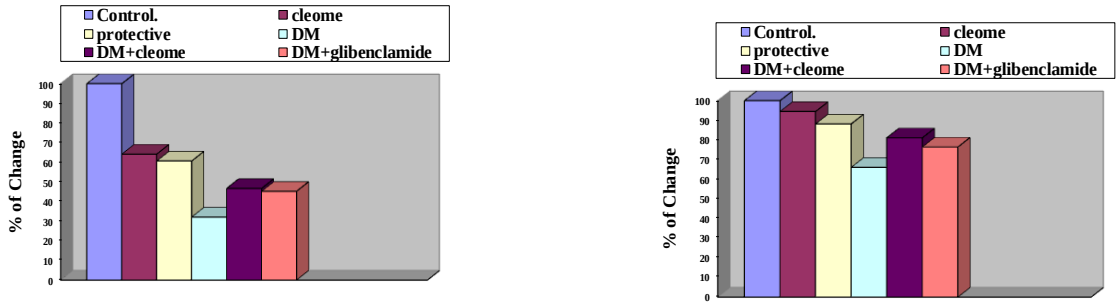
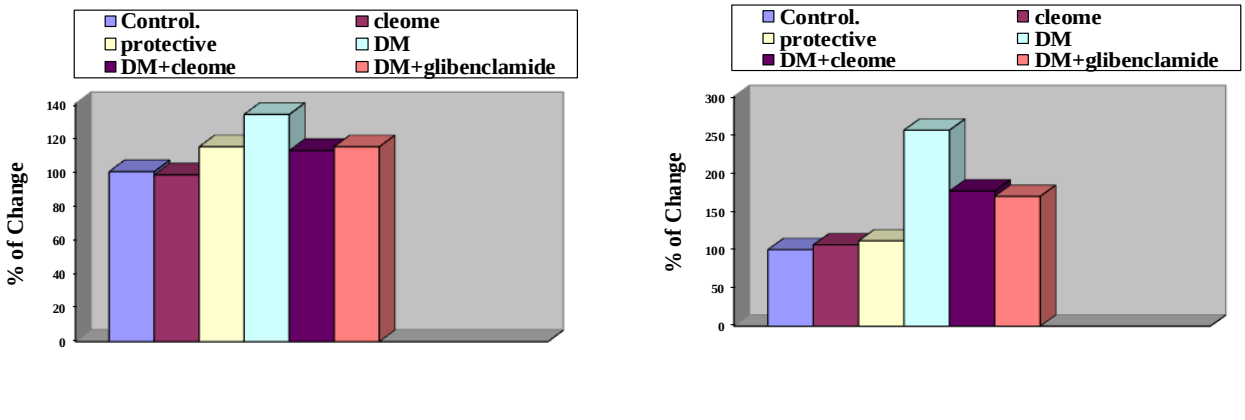


Figure 11: percentage change in tissue protein and serum protein levels of diabetic rats after treatment with callus extract of *C. droserifolia* as compared to control group.

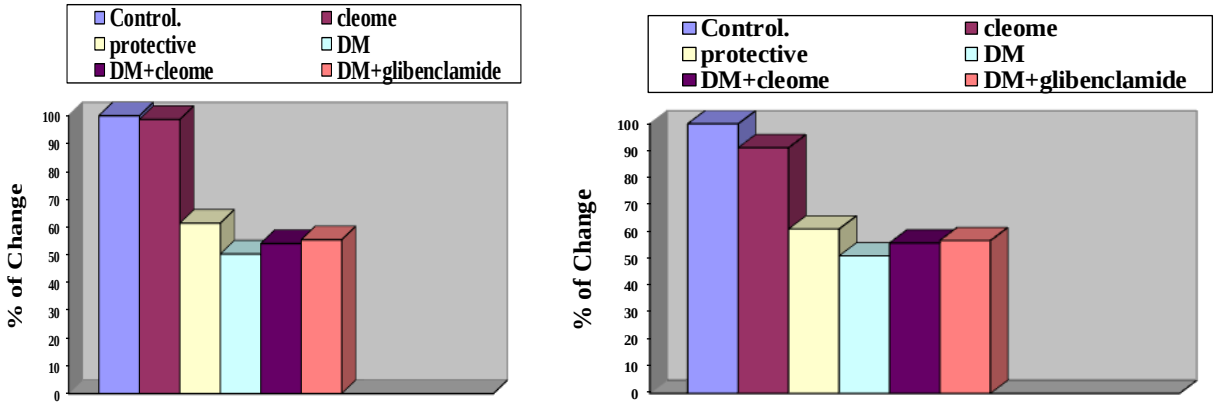
In the our present study there is a considerably increment in the levels of AST and ALT enzymes in serum of diabetic rats when compared with the control group. The increase in the activities of ALT and AST enzymes in diabetic groups reveals a state of hepatocyte injury. This is may be due to the reduction of insulin in the case of diabetic which results in the failure in glucose utilization so the oxygen free radicals generation increased which encouraged liver injury [74] furthermore, daily medication of diabetic rats with callus plant extract significantly reduced the activities of ALT and AST enzymes when compared to diabetic rats as shown in (Figure 12). These result is in agreement with some authors [10,57]. *C. droserifolia* was known to be contain highly amount of phenolic compounds, such as flavonoids (kaempferol-3-gluco-7-rhamnoside, kaempferol-3,7-dirhamnoside, isorharmnetin-3-gluco-7- rhamnoside,) [14,75]. While, in our study it found that the callus extract was rich in querecetin, several reports have indicated that flavonoids help in reducing hyperglycemia.
Flavonoids have long been known to have hepatoprotective, anti-inflammatory, antioxidant activities. They can apply their antioxidant activity by several mechanisms, e.g., by scavenging or overcome free radicals, by chelating metal ions, or by inhibiting enzymatic systems which responsible for free radical generation[76] . So, they can ameliorate the functions of the liver by inhibition the proinflammatory mediators and protection of hepatocytes. The amelioration of liver functions may be due to beta-trophin hormone which primarily formed in the liver and adipose tissues, and lately described as a key stimulator of beta-cell mass expansion in response to obesity and insulin-resistant states, This hormone stimulates beta cells in the pancreas to multiply and produce more insulin [77]**.** Therefore, our results are in agree with earlier studies which found that the *C. droserifolia* had a hepatoprotective effect by inhibiting the liver damage leading to enhancement in the liver functions [51].



AST **ALT**

Figure 12: Percentage change in AST and ALT levels of diabetic rats after treatment with callus extract of *C. droserifolia* as compared to control group

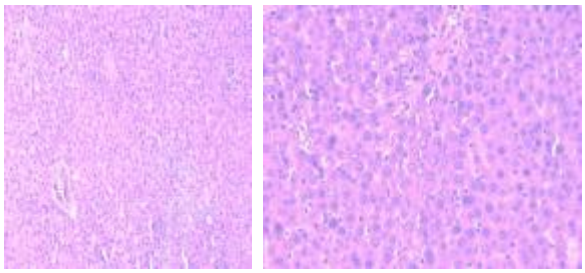
Serum T3 and T4 levels are valued indicators of thyroid function [73] . In diabetic rats, T3 and T4 level reduced when compared to normal control rats . These reductions revealed the significant reduction of T3/T4 ratio in diabetic rats. Many systemic non-thyroid diseases encourage subnormal T3 levels representing reduced microsomal capacity to convert T4 to T3 and the mechanism behind it is the improvement of oxidative states[78] . Consequently, it is reasonable to determine that liver damage could be the mechanism underlying the observed decrease of T3 in diabetic rats. Diabetes inhibited thyroid hormones (T3 and T4) and serum insulin levels that control the basal metabolic rate [48]. The treatment of diabetic rats with callus extract of *C. droserifolia* increase level of T3 and T4 level as compared with diabetic group, as shown in (Figure 13) when insulin returned to its normal level, it was able to restore serum T4 and T3, hepatic conversion of T4 to T3. These observations were in agreement with that reported by El-Shenawy and Abdel-Nabi and Jennings et al. [50,79], which appears to be secondary to its effect as an antioxidant. The effect of *C. droserifolia* in increasing thyroid hormone levels may return to its effects on insulin increase.



T3 **T4**

Figure 13: percentage change in T3 and T4 levels of diabetic rats after treatment with callus extract of *C. droserifolia*. As compared to control group

From the histological point of view, it was found that the liver of control group showed normal morphological appearance, most of the cells contain a central rounded nucleus. The blood sinusoids are present between the cords. The sinusoidal endothelium is formed of endothelial lining cells and the phagocytic kupffer cells, treated group with callus extract of *C. droserifolia* hypocotyl showed preserved lobular hepatic architecture congested central vein and hepatoprotective group with mild sinusoidal dilatation as compared with diabetic rats group which showed preserved (intact) lobular hepatic architecture with enlarged hepatocytes and binucleated hepatocytes, focal necrotic area, as shown in (Figures 14-19) these observations were in harmony with the findings of [10,80].



Figu r e 1 4 : photomicrograph of liver section from control group showed preserved (intact) lobular hepatic architecture and normal morphological appearance, most of the cells contain a central rounded nucleus while some binucleated. The blood sinusoids are present between the cords. The sinusoidal endothelium is formed of endothelial lining cells and the phagocytic kupffer cells (H&E x200 ; x400)

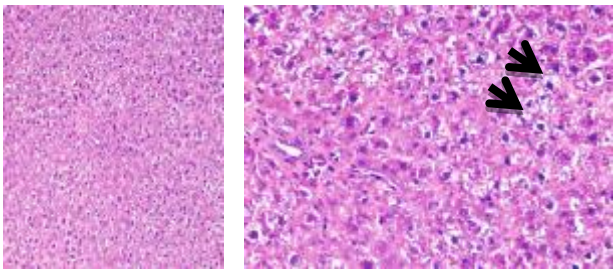


Figure 15: photomicrograph of liver section from normal rats treated with *cleome droserifolia* crude callus extract group showed preserved (intact) lobular hepatic architecture with mild hydropic (black arrow),
(H&E x200 ; x400).

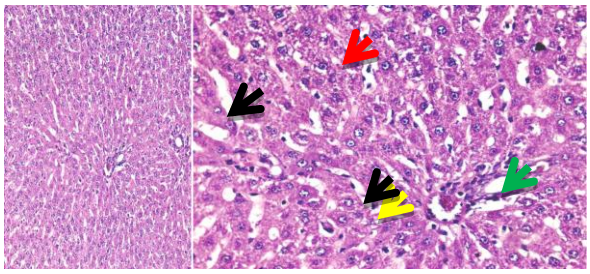


Figure 16 : photomicrograph of liver section from protective group showed preserved (intact) lobular hepatic architecture with severe injury illustrated in mononuclear cell infiltrate extending through hepatic tissue (red arrow), Kupffer cell appeared engulfing debris (yellow arrow) and hyperplasia of bile duct ,mild sinusoidal dilatation and congestion (black arrow) (H&E x200 ; x400)

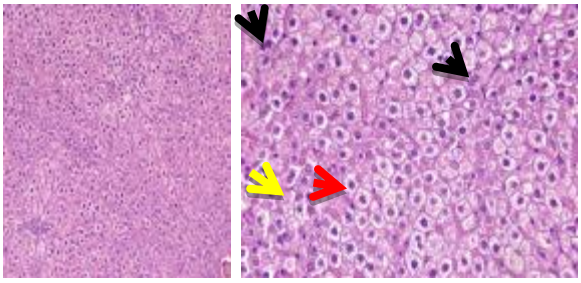


Figure 17: photomicrograph of liver section from diabetic group showed preserved (intact) lobular hepatic architecture with enlarged hepatocytes containing pale stained cytoplasm and darkly stained nuclei (red arrow), binucleated hepatocytes (black arrow), focal necrotic area(yellow arrow) (H&E x200 ; x400)

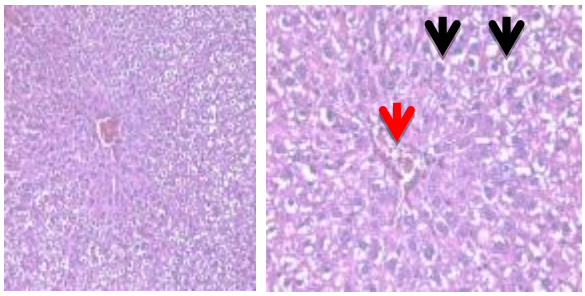


Figure 18 : photomicrograph of liver section from diabetic rats receieved methanol extract from the callus of cleome droserifolia hypocotyl from media 5 showed preserved (intact) lobular hepatic architecture with mild hydropic (black arrow) congested central vein (red arrow) (H&Ex200 ; x400)

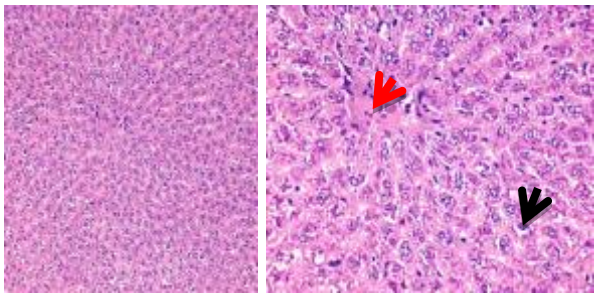


Figure 19 : photomicrograph of liver section from diabetic rats received glibenclamide group showed preserved (intact) lobular hepatic architecture with mild hydropic (black arrow) (H&E x200 ; 400)

In our study it was observed that, Pancreatic sections of diabetic group in which pancreatic islets appeared with irregular cells, not well defined necrosis of cells as compared with control group which showed pancreatic islets regularly and arranged evenly group treated with callus extract of *C. droserifolia* hypocotyl showed pancreatic islets which shaped regularly and arranged evenly, with normal islets of Langerhans and normal acini tissues

and hepatoprotective groups showed pancreatic islets irregular cells with not well defined necrosis of cells compared with diabetic group these observations were in agreement with the findings of some authors [4,47] The regularly shape of pancreatic , with normal islets of Langerhans in the treated group may be regenerated from the duct epithelium of the exocrine portion of the pancreas[81;83] as shown in (Figures 20-25)

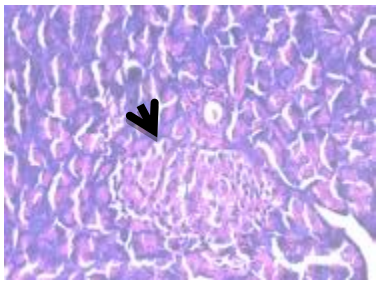


Figure 20: photomicrograph of Pancreatic section from control group showed pancreatic islets were shaped regularly and arranged evenly, with normal islets of Langerhans and normal acini tissues (arrows) (H&E x200)

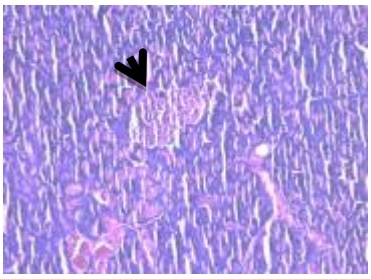


Figure 21: photomicrograph of Pancreatic section from normal rats treated with *cleome droserifolia* crude callus extract group showed pancreatic islets were shaped regularly and arranged evenly, with normal islets of Langerhans and normal acini tissues (arrows) (H&E x200)

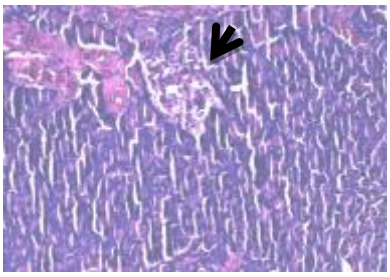


Figure 22: photomicrograph of Pancreatic section from protective group showed pancreatic islets with irregular cells, not well defined necrosis of cells (black arrows) (H&E x200)

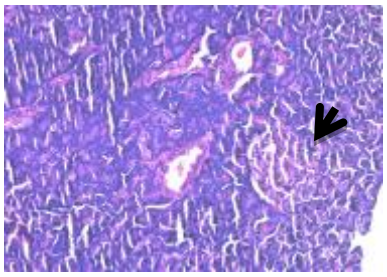


Figure 23: photomicrograph of Pancreatic section from diabetic group showed pancreatic islets with irregular cells, not well defined necrosis of cells (black arrows) (H&E x200)

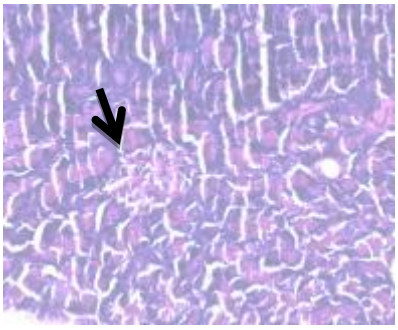


Figure 24: photomicrograph of Pancreatic section from diabetic rats received methanol extract from the callus of *cleome droserifolia* hypocotyl from media 5 on the showed pancreatic islets were shaped regularly and arranged evenly, with normal islets of Langerhans and normal acini tissues (arrows) (H&E x200)

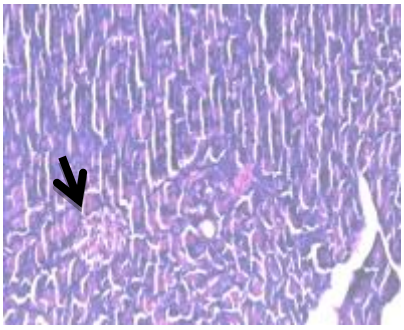


Figure 25: photomicrograph of Pancreatic section from diabetic rats received glibenclamide group showed pancreatic islets were shaped regularly and arranged evenly, with normal islets of Langerhans and nearly normal acini tissues (arrows) (H&E x200)

Conclusion

The present study demonstrated the antihyperglycemic activity of callus extracts derived from *C. droserifolia* on STZ diabetic rats. It could be concluded that the effect of *C. droserifolia* callus extracts as antidiabetic herbal treatment strategy recommended in our study.

Studies of *C. droserifolia* cllus extract showed that formation and regeneration of cells may be occurred *in vitro* and could pushes us for drug development, The reason of these results may be due to the used growth regulators which stimulate and maintain the callus, where these regulators stimulate and increase the production of secondary metabolite in callus, sub culturing may also have a role in somaclonal variation initiation in cells which leads to increasing its productivity of secondary metabolite. The stages of callus induction, maintenance and the optimum conditions for cultures are particular stimulators that led to increasing in production of secondary metabolites in callus of newly synthesized hypocotyls and roots as compared with the mother plant [84,85]

Conclusion, the present findings indicate that the methanol extract of the callus powder of *C. droserifolia* is as potent as the *in vivo* plant parts extract in the management of DM which demonstrated that the herb have a hypoglycemic effects, and are potent in restoring biochemical markers associated with diabetes, thus increase saour belief to our folkloric suggestion that *C. droserifolia* leaves were used in the treatment and/or management of type 2 DM in Egypt .

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