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# Studying the Effect of Flavonoids Extracted from *Haloxylon salicornium* and Zygophilium *coccinium* Plants on Burn Healing in Diabetic White Rabbits

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## ABSTRACT

**Background:** The use of plant extracts is a successful way to treat many diseases. Desert plants are rich in many antioxidant compounds, so they have been used since ancient times in folk medicine. The most important of these compounds are flavonoids that contain in their composition an aromatic ring and an oxidative hydroxyl group, which resists the free radical compounds formed in affected tissue and neutralize the tissue to reach the state of recovery.

**Objectives:** The study aims to find an effective treatment to prevent tissue damage in burns or wounds in diabetic patients.

**Materials and methods:** Two plants were used in this study; *Haloxylon salicornicum* and *Zygophyllum coccineum* (after proving their antioxidant activity by DPPH assay, with different concentrations for both plants). Flavonoids were extracted from them, and they were used to treat burns in laboratory rabbits with diabetes. The incidence of infection was confirmed by histological and hematological studies. Then, we studied of the effect of using the treatment on some blood variables, hormones and antioxidants during the treatment period for the stability of their effectiveness for treatment.

**Results:** The study proved the effectiveness of these compounds by accelerating the treatment period despite diabetes and getting fast and effective results.

**Conclusion:** Flavonoids of *H. coccineum* and ***Z. coccineum*** proved a high effectiveness of treatment compared to chemotherapy and accelerated the healing period of the wound.

**Keywords:** Burn, Diabetes mellitus (DM), Flavonoids, *Haloxylon salicornium*, *Zygophilium coccinium*.

## INTRODUCTION

Hyperglycemia caused by abnormalities in insulin secretion, action, or both characterizes the group of metabolic illnesses known as diabetes. The eyes, kidneys, nerves, heart, and blood arteries are particularly vulnerable to long-term damage, dysfunction, and failure caused by the chronic hyperglycemia of diabetes [(1)]. Diabetes symptoms can also show up on the skin. Chronic cutaneous infections are caused by the skin receiving more blood glucose. Pruritus and other signs of skin disease are brought on by bacterial and fungal infections, which are increased by hyperglycemia (HG). Long-term HG induces an excess of reactive oxygen species (ROS), which causes oxidative stress in DM and is mostly associated by a depleted antioxidant defense system [(2)]. As a result, the body's antioxidant defenses can no longer resist the increasing ROS generation. Increased blood glycolipid and glycoprotein as well as blood pressure levels linked to DM are accompanied by oxidative stress. Enzymes such as glutathione peroxidase, catalase, and superoxide dismutase are part of the protective antioxidant system that fight off the negative consequences of oxidative stress [(3)].

Inflammation, proliferation, angiogenesis, apoptosis, decreased chemotaxis and matrix formation, decreased bacterial resistance, and degeneration of the antioxidant protection system are only a few of the many mechanisms that contribute to the complicated wound-healing cascade's impairment in DM. Each of these causes wound healing to fail. Additionally, people with uncontrolled diabetes often have peripheral vascular disease in their legs due to atherosclerosis, which can eventually result in foot amputation [(4)].

Diabetes-related burns and wounds are treated with a variety of medications, although some of them are ineffective, leading to tissue damage and occasionally severing organs. Therefore, in addition to their therapeutic effects, medicinal herbs rich in antioxidant active ingredients have been used to neutralize the free radicals produced in the injured tissue. Microorganisms in the damaged area are killed by this substance [(3)]. Any plant that has elements that can be used for therapeutic reasons in one or more of its organs is considered a medicinal plant. Traditional medicine has always included plants as a key component because of the chemicals present in different plant tissues that have particular physiological effects on humans. There are several phytochemicals that can be isolated from plants and used as extracts or as pure chemicals possess a significant potential function in clinical pathology and are useful in the treatment of several disorders [(5)] .

It has been demonstrated that diabetes mellitus (DM) and platelet dysfunction are both directly correlated with a number of hematological alterations affecting the red blood cells (RBCs), white blood cells (WBCs) and coagulation factors [(6)].

Increased levels of triglycerides (TG), total cholesterol and low-density lipoprotein (LDL) as well as



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HG's all have detrimental impact on the progression of cardiovascular disorders. The development of cardiac disease in diabetes patients may be influenced by cholesterol [7]. Long-term hyperglycemia can cause burn injuries or delay the recovery from burns. The experimental plants were chosen due to their high antioxidant content and their use in folk medicine for the treatment of burns. *H. salicornicum* is a member of the Amaranthaceae family of desert plants. In traditional medicine, this plant was used successfully as a diuretic, ulcer-preventative, hypoglycemic, and anti-microbial. The plant's aerial parts contained alkaloids, cardiac glycosides, anthraquinones, flavonoids, saponins, coumarins, sterols, tannins, volatile oils, and volatile bases, according to a qualitative phytochemical examination [8].

*Z. coccineum* is a small perennial herb that grows in sandy and salinous environments close to the sea. It has succulent leaves and slightly pale blooms. The soil chemical composition of Zygophyllum's habitats determines its growth and distribution [9]. Previous investigations have identified *Z. coccineum* as a therapeutic herb [10]. Folk medicine treats gout, rheumatism, asthma, and hypertension with the leaves, fruits, and stems. Additionally, the plant is employed as a diuretic, an antihistaminic, a local anesthetic, and an anti-diabetic drug.

The class of polyphenolic chemicals known as flavonoids is ubiquitous in the plant kingdom and serves a range of purposes, including coloring and UV protection. Many studies have used flavonoids' antioxidant properties to scavenge ROS [11]. Flavonoids are essential in a wide range of nutraceutical, pharmacological, medical, and cosmetic uses because they are linked to a wide range of health-promoting benefits. This is because they possess strong anti-oxidative, anti-inflammatory, anti-mutagenic, anti-microbial, anti-carcinogenic, vascular, and other therapeutic qualities, as well as the ability to modify crucial cellular enzyme processes [11]. Therefore, it was extracted in this research and used to treat burns associated with diabetes to show its efficacy in treatment compared to the drugs used for this purpose.

## MATERIALS AND METHODS

### Animals:

Experimental forty albino healthy male rabbits weighting between 750- 900 gm and 2-3months in age, were taken from the animal house of Veterinary Medicine College, AlMuthanna University. They were housed in iron cages used in this research. They were kept in standard, separate cages, given full access to tap water, and fed continuously with standard pellets. The reason this model was chosen to fulfill the requirements of this study is that it has several desirable properties. The stainless-steel caging (40 x 60 x 80 cm) had slotted floors and was used to cage animals individually. The cage racks and pans were cleaned once a week and three times a week respectively. All cages were kept in temperature-controlled spaces (20 to 22 ℃) with minimal lighting. The animals were separated for two weeks before the experiment to allow for acclimatization. All animals were kept in fasting state for 21 hours before starting the experiments.

### Study design:

Animals randomly distributed into seven groups with six rabbits each. All animals of the first six groups were induced with diabetes, and after the onset of the infection and symptoms, and on the fourteenth day after the injury, a second-degree burn occurred on a part of the skin in the back area: group one ( G1) for *Haloxylon salicornicum* flavonoid (H.F) ointment treatment, group two (G2) for *Zygophyllum coccineum* flavonoid (Z.F) ointment treatment, group three (G3) for Haloxylon salicornicum crude (H.C) ointment treatment, group four (G4) for Zygophyllum coccineum crude ointment treatment, group five (G5) for chemotherapy (hamazine 1%) ointment treatment, group six (G6) infected animals without treatment (negative control group), group seven (G7) healthy animals (positive control group).

### Diabetes mellitus induction in rabbit:

Diabetes was induced in rabbit by a single injection of alloxan monohydrate (150mg/kg BW) dissolved in normal saline. The injection was performed over a period of 1 min (intraperitoneal). After this process, rabbit became in diabetic state. Solution of alloxan was used immediately after preparation. Additionally, an oral solution of 20% glucose in tap water was provided *ad libitum* for 2 days after alloxan induction then was given to rabbit to counter the shock of blood sugar. After 72 hours of injection, animals with the blood glucose level of more than 200 mg/dl were selected for the study [12]. Rabbits were weighed weekly throughout the study and the weights were recorded. There appeared to be a negative relationship between the highest blood glucose levels observed and body weight gain.

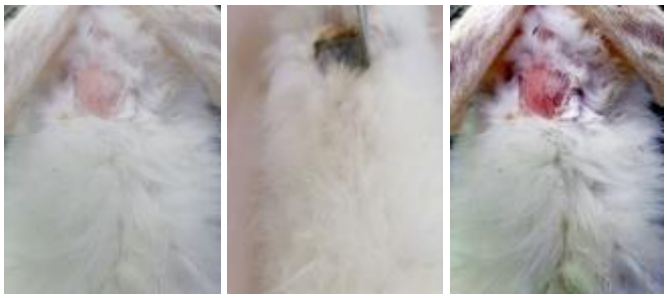
### Measurement of blood glucose concentration:

Glucose concentration was measured before administration and subsequently at 24, 48, and 72 hours after administration from the vein of the ear. Baseline non-fasting blood glucose levels were normal for all animals (115 ± 13mg/dl). After the administration of alloxan there was a characteristic response in blood glucose level concentrations. Blood glucose of rabbit was determined with on-call-plus glucometer using strip method a drop of blood was dropped at one end of the

strip on the glucometer. After ten second the reading was taken [13].

**Surgical technique of burn:**

After two weeks of infecting the animals with diabetes and after verifying the disease and the appearance of symptoms such as loss of appetite, loss of weight, frequent urine, excessive drinking of water, high blood sugar and low level of insulin in the blood, the surgical operation was performed under sterile conditions. The surgical procedures were made under general anesthesia using a mixture of ketamine hydrochloride (10mg/Kg) and xylazine (3mg/Kg) administered intramuscularly (IM) [14] as shown in figure (1). Thermal injuries were made with a solid aluminum piece 2cm in length and width and of 50 g mass. The temperature was previously heated in boiling water until it reached 100 °C as measured with a thermometer. For 15 seconds, the aluminum piece is kept in touch with the animal's dorsal proximal region of skin. Analgesia with sodium dipyrone (40mg/kg) was provided intramuscularly immediately following the procedure and was maintained for three days straight through oral administration of sodium dipyrone (200 mg/kg) in the animals' drinking water [15].



a b c

**Figure (1):** Clinical evolution observed in the experimental model of deep second-degree thermal burns in male albino rabbit. (a) Animal's skin after shaving. (b) Thermal lesion obtained with heated aluminum piece (c) Injured skin.

**Histological analysis:**

A histological examination was carried out. Animals randomly selected underwent anesthesia combination of 10% ketamine (90mg/kg) and 3% xylazine (10mg/kg), intramuscularly [14]. By administering large intraperitoneal dosages of sodium pentobarbital (100mg/kg), euthanasia was accomplished [16]. To demonstrate the presence of a second-degree burn, a piece of one of the animals' afflicted skins underwent histological testing. For histological studies, tissue samples were promptly fixed by immersion in buffered 10% formalin, processed in paraffin blocks, sectioned, and stained with hematoxylin and eosin (HE). In histological investigation, the development of skin healing following thermal stress was assessed using a binocular optical microscope (Zeiss-Axiostar model) and comparative descriptive analysis of the experimental groups. Independent pathologist with experience in examining burn wound specimens conducted the histological analysis in the following ways:

1) granular tissue, which is characterized by the presence of fibroblasts, myofibroblasts, and neovascularization, 2) inflammatory response, which is indicated by the presence of polymorphonuclear leukocytes (PNM) and 3) fibrosis, which is indicated by the density of collagen fibers determined by the intensity of blue color seen under optical microscopy due to staining with hematoxylin and eosin (HE) [16].

**Clinical evaluation:**

Based on the following criteria, the clinical course of burn-related skin lesions was assessed over the course of 24 days: blistering, swelling, redness, crust, bleeding, secretion, granulation tissue, and scar tissue. A caliper was used to measure the wound retraction 3, 7, 10, and 14 days following the burn induction. Wound contraction was measured as a percentage decrease in the size of the initial wound.

% size wound contraction on day X = [(area on day 0 – open area on day X)/area on day 0] × 100 [17].

**Blood sampling:**

Following disinfection with 70% alcohol, 3cc of blood was drawn from the heart and injected into syringes with a volume of 3mL. The blood was then divided into two parts; one placed in an EDTA tube for CBC testing and the other in a gel tube. This procedure was left to sit at room temperature for 20 minutes, after which the serum was extracted using a centrifuge at 2500 cycles per minute for 15 minutes [18]. Serum was used to measure biochemical tests that were measured with Chemistry Analyzer Smart-150, hormones measured by ELISA and antioxidants by spectrophotometer.

**Alcoholic extract preparation of *H. salicornicum* and *Z. coccinium*:**

Fresh plant of *H. salicornicum* and *Z. coccinium* was collected as the aerial parts of plant in March from southern of Sammawa, Iraq. Powder of the aerial parts of plants (50gm) was macerated with 80% ethanol (250 ml) with stirrer by a magnetic bar for 24 hours at room temperature and filtered by using filter paper. The clear filtrate was concentrated using a rotary evaporator at 40°C [19].

**Isolation of flavonoids from *H. salicornicum* and *Z. coccinium***

A 50gm powder made from plant aerial parts was combined with 250ml of 80% methanol and stirred at room temperature for 24 hours. After filtering the

methanolic extract and removing the precipitate, 25ml of 1% lead acetate was added to the filtrate. The mixture was filtered, and the Buchner funnel was used to collect the filtrate. Following the addition of 25ml acetone and 30ml concentrated hydrochloric acid to the precipitate, the mixture underwent filtration, and the filtrate was then evaporated [20, 21].

**Detection of flavonoids in plants extract:**

The presence of flavonoids was determined using the alcoholic potassium hydroxide reagent (5N), which works by adding 1ml of reagent to 1ml of extract to produce a yellow precipitate[19].

**Separation by high performance liquid chromatography (HPLC):**

The distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase is the foundation of the HPLC separation principle (packing material of the column). Depending on the analyte's chemical components. In analytical chemistry and biochemistry, it is used to separate mixtures of chemicals to identify, measure, or purify the mixture's constituent components [22]. It was used to separate the most important active compounds and the most quantitative ones found in the extracts of the two plants.

**Antioxidant activity using DPPH radical scavenging assay:**

One common test for evaluating the antioxidant activity of plant extracts is the DPPH assay. With higher extract concentration ratios, DPPH radical scavenging activity tends to produce better results. In this test, a DPPH compound is employed as a stable free radical with a dark purple color. When the antioxidant chemicals are returned by giving it an electron or a hydrogen root, the free radical's color changes to yellow [19] , to become a stable molecule as follows:

(DPPH•) + HO-R-OH (DPPH): H + HO-R-O•

The reduction in optical absorbance at 517nm brought on by antioxidants yields information on the DPPH radical's return power. Some chemicals, such as phenolic compounds or flavonoids, may have strong free radical scavenging activities depending on the availability of hydroxyl groups in the plant [23]. 0.1 mM DPPH in methanol solution was prepared, and 0.5 mL of this solution was combined with 2.5 mL of the methanol extract at various doses (1.25-100 mg/mL). 30 minutes were spent at 25°C in the dark incubating the reaction solution. With a UV spectrophotometer, the absorbance was measured at 517 nm. A positive control was ascorbic acid. Radical scavenging activity was expressed as the inhibition percentage of free radicals by the sample and was calculated using the following formula:

% Inhibition of DPPH = [(Ac-As) / Ac] x 100

Where Ac is the absorbance of the control (DPPH + methanol) and as the absorbance in the presence of the DPPH + extract [24].

**The use of extract concentrate for the treatment of burns**

A direct concentration of the extract was applied to the shaved skin of the animal, causing redness and swelling of the skin. The extract was mixed with the neutral ointment at a concentration of 50% (1g each) and applied to the healthy skin, but it also caused redness. Then use a concentration of 25% also cause redness of the skin. It was concluded that a concentration of 15% for crude, 10% for flavonoids did not cause any damage to the skin. Therefore, these concentrations were applied to treat induced burns of the skin. The concentrations were made according to the density law, noting that the density of the ointment is approximately 1.4 gm/ml, and then it was mixed using a sonicators for 40 seconds to ensure that it was mixed homogeneously.

**Ethical approval:**

The project was approved by The Local Ethics Committee at Dhi Qar University in accordance with University Order No. (5489) on 05/26/2021.

**RESULTS**

After induction of diabetes in laboratory animals and to confirm the injury, the burn was induced and the following results were obtained:

**Diabetic result:**

The concentration of glucose increased significantly after injection of alloxan until glucose reached approximately 250mg/dl in blood of experimental animals.

**Histological results:**

Obvious histological changes occurred in the composition of liver of infected rabbits at day 14 showed hepatocyte fatty degeneration with congested blood vessels and perivascular inflammatory cell accumulation. Pancreas of diabetic group at day 14 showed severe damage of islets of Langerhans and inflammatory cell infiltration and it was histologically confirmed that a second-degree burn occurred after burn exhibited greater damage area of desquamated epidermis to continue working with the same technique with the rest of the animals as shown in the sections below in figure (2).

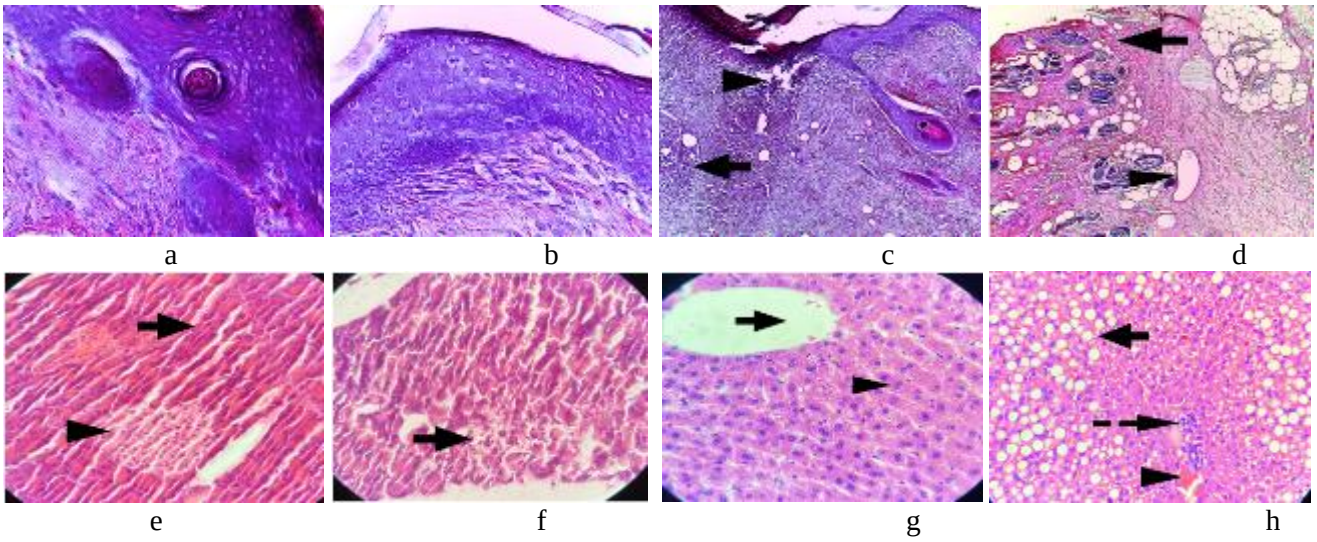



**Fig. (2):** Photomicroscopy results: (a) Normal skin (H & E stain X40). (b) Normal skin (H & E stain X40). (c) Photomicrograph of the skin of a rabbit with second-degree burn at day 3 after burn exhibited coagulation necrosis that was identified in the epidermis (arrowhead) and inflammatory cell infiltration (arrow) of the epidermis and dermis (H & E stain X40). (d) Photomicrograph of the skin of a rabbit with second degree burn at day 3 after burn exhibited greater damage area of desquamated epidermis (arrow) (H & E stain X100). (e) Photomicrograph of the pancreas of control group at day 14 showed normal histological appearance exocrine (arrow) and islets of Langerhans (arrowhead) (H & E stain X40). (f) Photomicrograph of the pancreas of diabetic group at day 14 showed severe damage and inflammatory cell infiltration (arrow) (H & E stain X40) of islets of Langerhans. (g) Photomicrograph of the liver of control group at day 14 showed normal histological appearance central vein (arrow) and cord like hepatocyte arrangement (arrowhead) (H & E stain X40). (h) Photomicrographof the liver of infected rabbits at day 14 showed hepatocyte fatty degeneration (arrow) with congested blood vessels ( arrowhead) and perivascular inflammatory cell accumulation (dotted arrow) (H & E stain X400).

**Clinical examinations**

Clinical examinations of the animals showed significant variations among the groups of animals. According to table (1**)** and figure (3**),** the animals of G7 showed clinical healing approximately at the twenty fourth day postoperative, while those of the treated groups showed clinical skin healing and return to the normal shape for all animals of the group approximately within eight days in G1and G2, within twelve days postoperative in G3, G4 and G5 and in G6 were about thirteen or fourteen days. The significant difference between the trial groups at the time of recovery was: P-Value = 0.031 between G1 & G6, P-Value = 0.040 between G2 & G6, P-Value = 0.031 between G3 & G6, P-Value = 0.041 between G4 & G6 and P-Value = 0.5 between G5 & G6.

**Table (1):** Wound areas measurement of different groups of rabbits. Values were given as mean ± SD for groups of six rabbits each. $p \leq 0.05$, significant differences compared to controls

| Days<br>group | 3 | 7 | 10 | 14 |
|---|---|---|---|---|
| G1 | 2.5±0.64 | 0.8±0.7 | 0 | 0 |
| G2 | 2.7±0.09 | 0.8±0.006 | 0 | 0 |
| G3 | 2.3±1.07 | 1.4±0.2 | 0.5±0.01 | 0 |
| G4 | 2.6±0.13 | 1.1±0.07 | 0.9±0.08 | 0 |
| G5 | 2.6±1.09 | 1.4±0.05 | 1.1±0.034 | 0 |
| G6 | 3.7±0.04 | 3.2±0.7 | 2.9±0.056 | 2.2±0.432 |

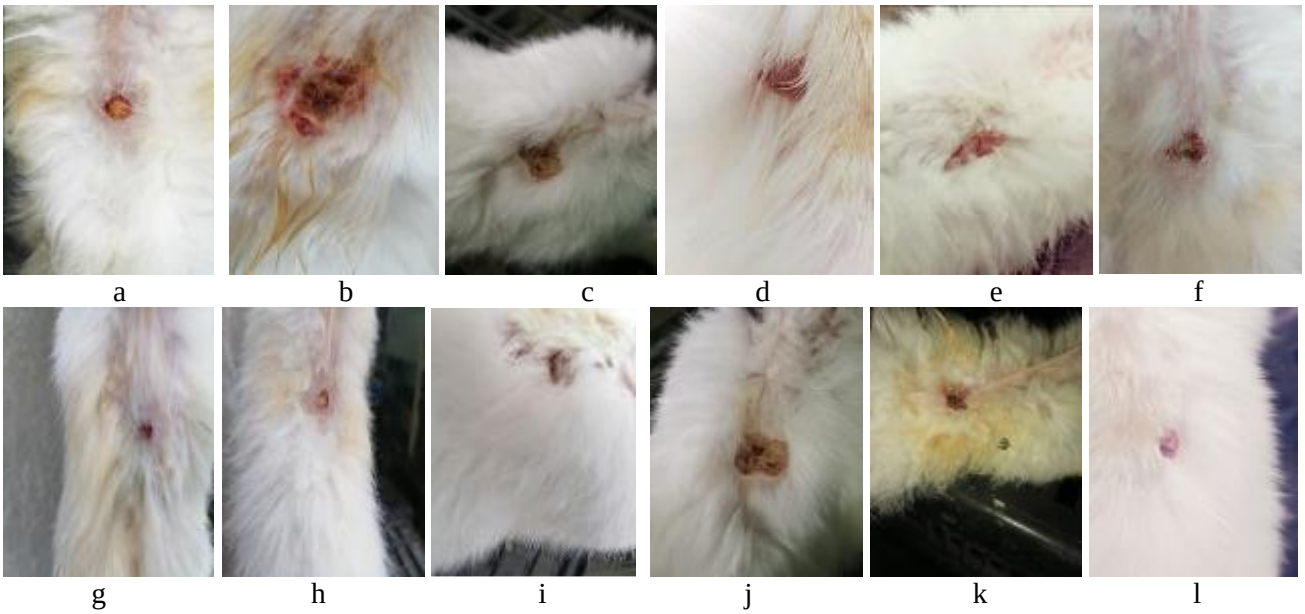



**Figure (3):** Clinical evolution of injured tissue on: (a)5$^{th}$ day after burn induction in G1, (b) 5$^{th}$ day after burn induction in G2, (c) 5$^{th}$ day after burn induction in G3, (d) 5$^{th}$ day after burn induction in G4, (e) 5$^{th}$ day after burn induction in G5, (f) 5$^{th}$ day after burn induction in G6, (g) 8$^{th}$ day after burn induction in G1, (h) 8$^{th}$ day after burn induction in G2, (i) 10$^{th}$ day after burn induction in G3, (j) 10$^{th}$ day after burn induction in G4, (k) 10$^{th}$ day after burn induction in G5, and (l) 10$^{th}$ day after burn induction in G6.

**Hematological Results**

After the animals were infected with diabetes some changes appeared in the normal ratios of some blood parameters in the infected animals compared to the uninfected animals as evidence of the achievement of infection and the emergence of complications. Blood glucose was persistently elevated in diabetic rabbits groups (to about 250mg/dl) compared to control healthy group (G13) (about 110mg/dl) and serum insulin values were significantly lower until reached about 0.95 ng/ml while the normal value in control healthy group was about 6.15 ng/ml. High serum levels of alanine aminotransferase (ALT) (180 U/L while in control group about 90U/L), cholesterol (173mg/dl while in control group about 45mg/dl), Triglycerides (TG) (185 mg/dl while in control group about 81 mg/dl), Low-density lipoprotein (LDL) (182 mg/dl while in control group about 103 mg/dl) and very low-density lipoprotein (VLDL) (29.3 mg/dl while in control group about 16.2 mg/dl) were observed in diabetic rabbits. There were low significant differences in High-density lipoprotein (HDL) level (9 mg/dl while in control group about 25 mg/dl), alkaline phosphatase (ALP) (158 U/L while in control group about 385 U/L) and aspartate aminotransferase (AST) (2 U/L while in control group about 15 U/L). There were low significant differences in HDL level. This is shown in figures (4), (5), (6), (7), (8), (9), (10), (11) and (12).

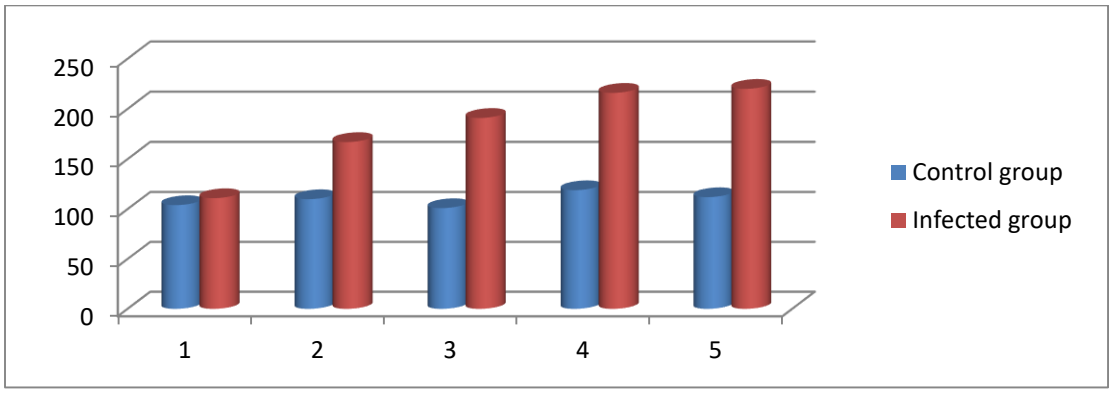



**Figure (4):** The difference between the control group and the infected group in glucose averages (mg/dl) at a significant difference P Value = 0.024.

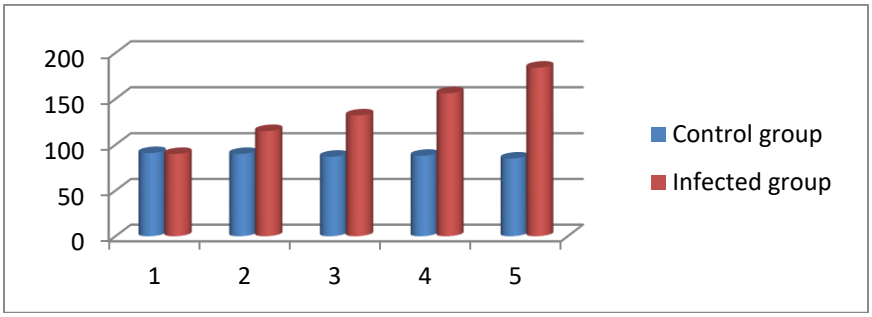



**Figure (5):** The difference between the control group and the infected group in Alanine aminotransferase (ALT) averages (U/L) at a significant difference P Value = 0.044.

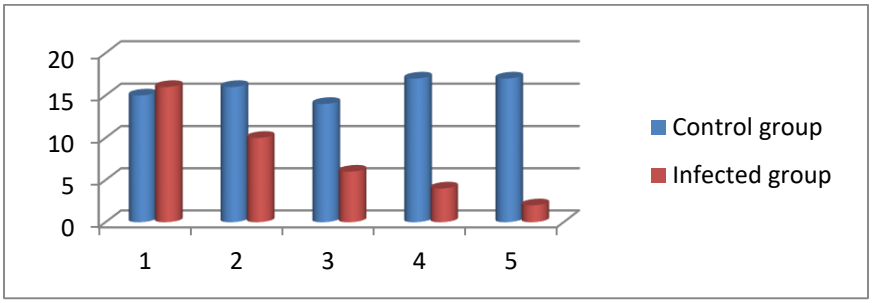



**Fig. (6):** The difference between the control group and the infected group in AST averages (U/L) at a significant difference P Value = 0.032.

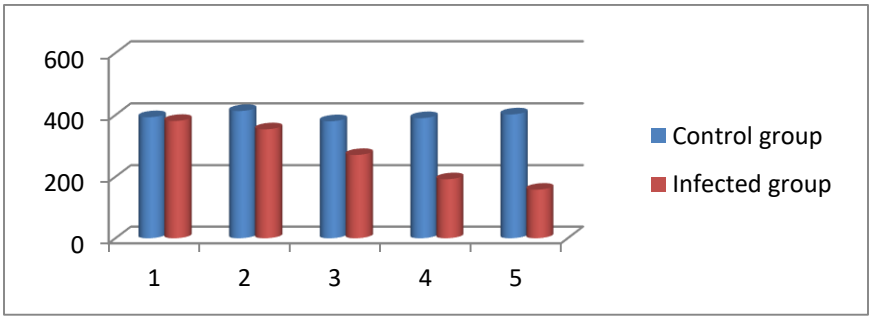



**Fig. (7):** The difference between control group and infected group in ALP averages (U/L) at a significant difference P Value = 0.047.

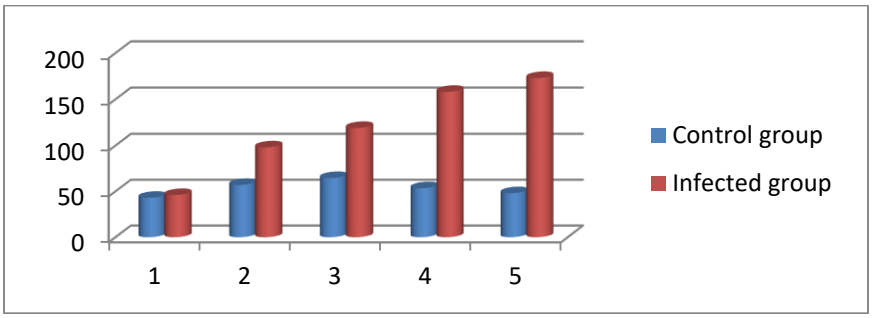



**Figure (8):** The difference between the control group and the infected group in the concentration of Chol (mg/dl) averages at a significant difference P Value = 0.046.

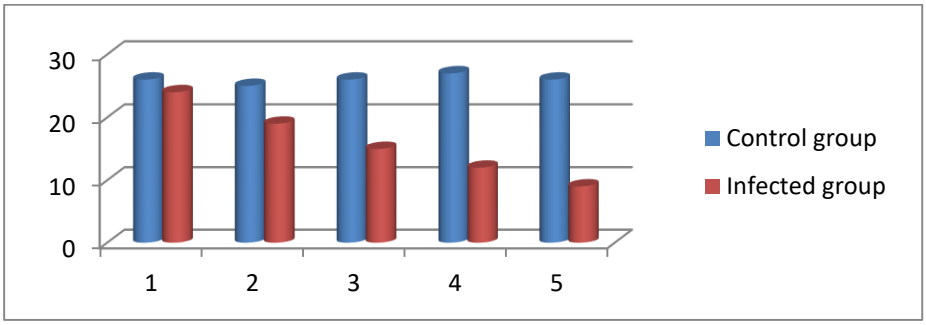



**Fig. (9):** The difference between the control group and the infected group in the concentration of HDL (mg/dl) averages at a significant difference P Value = 0.018

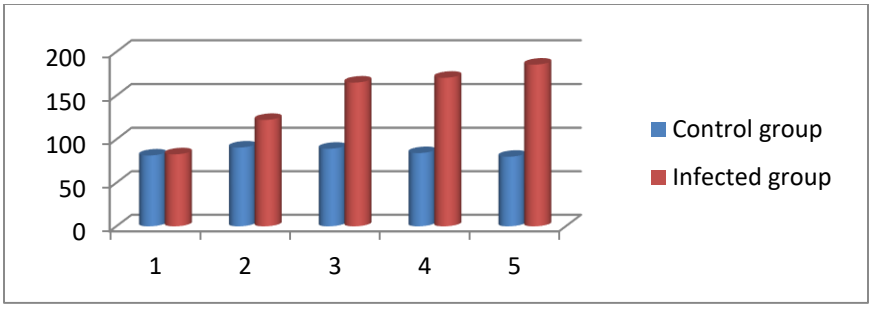



**Fig. (10):** The difference between the control group and the infected group in the concentration of Triglycerides (TG) (mg/dl) averages at a significant difference P Value = 0.034.

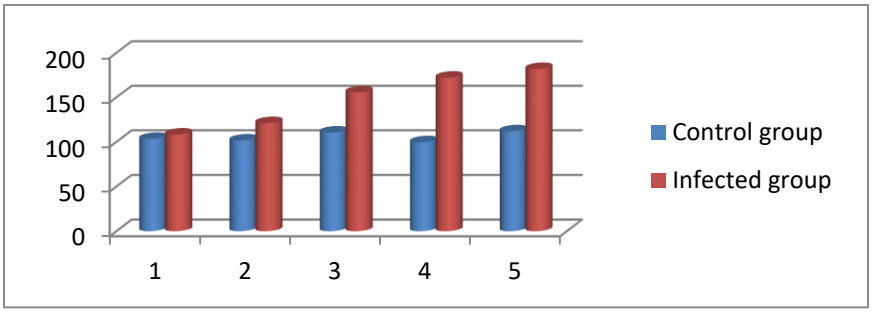



**Fig. (11):** The difference between the control group and the infected group in the concentration of low-density lipoprotein (LDL) (mg/dl) averages at a significant difference P Value = 0.043

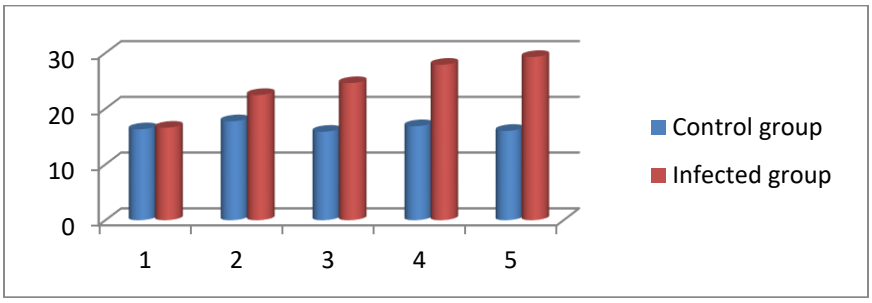



**Fig. (12):** The difference between the control group and the infected group in the concentration of VLDL averages at a significant difference P Value = 0.029.

Random samples were taken from the treatment groups in the experiment during the treatment period and compared with the negative control group for some hematological parameters such as the number of some types of white blood cells, growth hormone, some antioxidants and insulin hormone to compare the effect of the interaction of diabetes, burn and treatment with extracts on some blood parameters and the speed of burn healing. The results showed an increase in the number of white blood cells and the percentage of growth hormone during the treatment period in the animals treated with the extract compared to the control animals, as well as a significant increase in the level of catalase. As for glutathione and insulin hormone, there was no significant change in their percentage during the treatment period. This is shown in figures (13), (14), (15), (16), (17), (18) and (19).

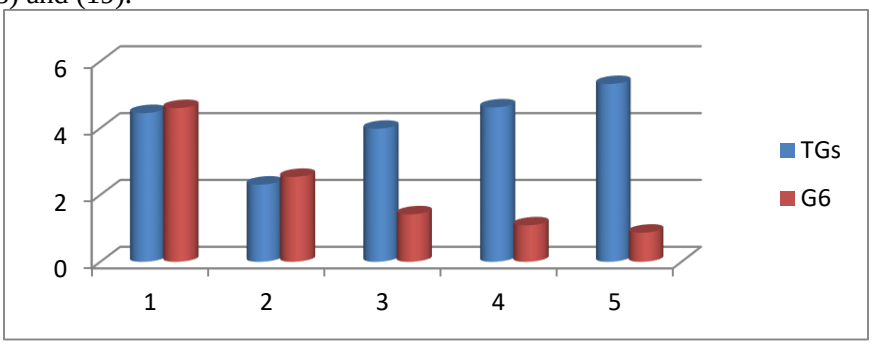



**Fig. (13):** The difference between the control and treated groups (TGs) during the treatment period in the values of growth hormone (ng/ml) averages at a significant difference P Value = 0.040.

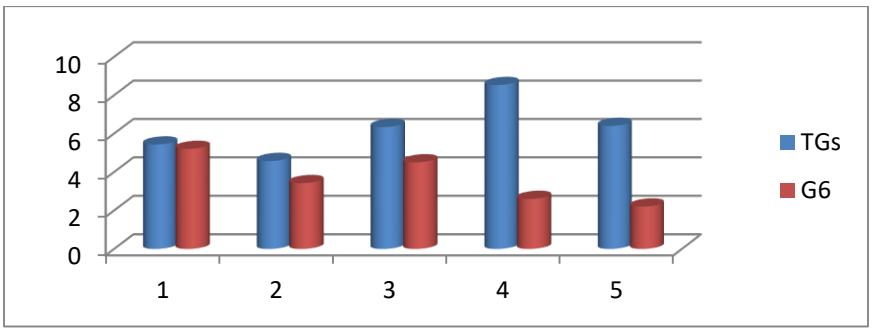



**Fig. (14):** The difference between the control and treated groups (TGs) during the treatment period in the values of WBC (WBC×$10^{3)}$ averages at a significant difference P Value = 0.018.

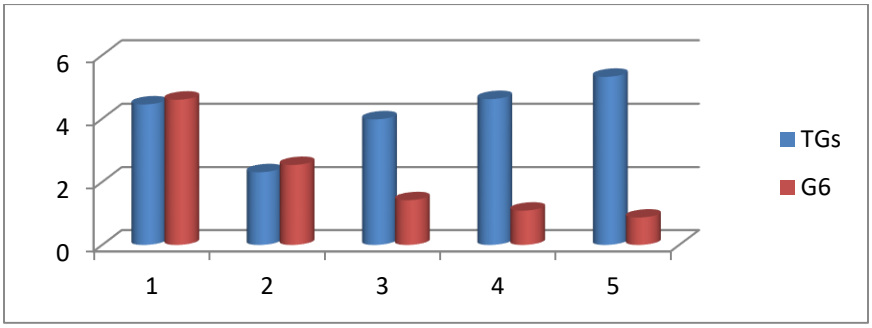


**Fig. (15):** The difference between the control and treatment groups (TGs) during the treatment period in the values of neutrophils cells count averages at a significant difference P Value = 0.049.

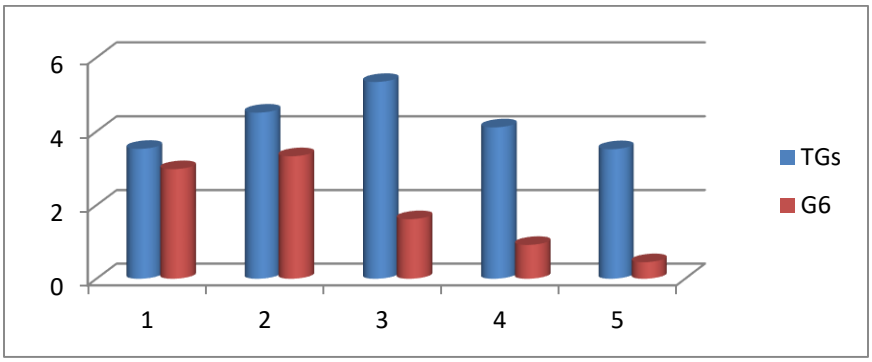



**Fig. (16):** The difference between the control and treatment groups (TGs) during the treatment period in the values of lymphocytes count averages at a significant difference P Value = 0.012.

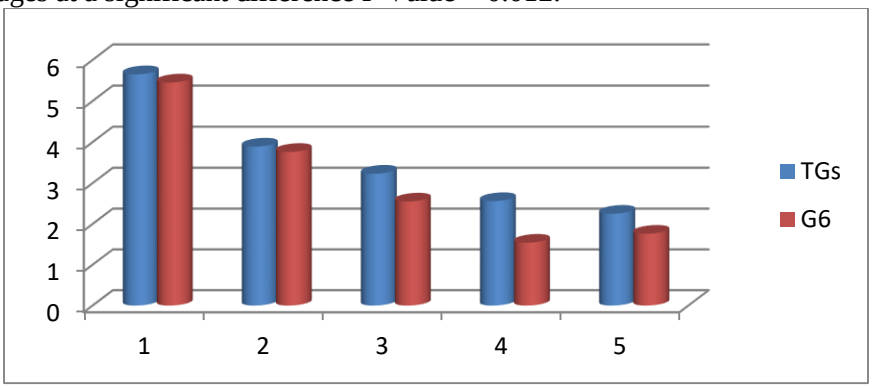



**Fig. (17):** The difference between the control and treatment groups (TGs) during the treatment period in the values of insulin hormone (ng/ml) averages at a non-significant difference P Value = 0.609.

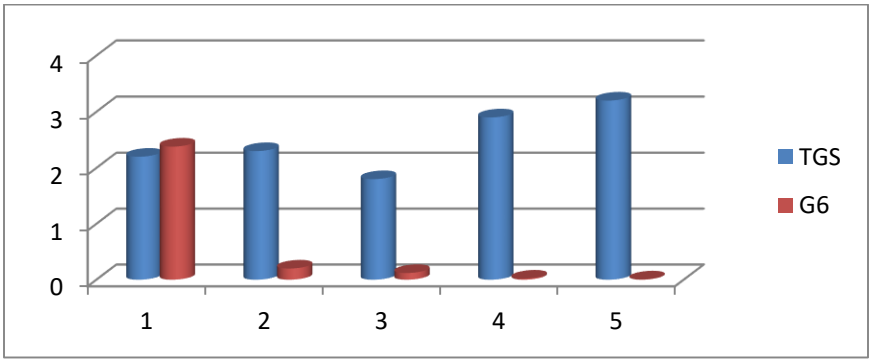



**Fig. (18):** The difference between the control and treatment groups during the treatment period in the values of catalase averages at a significant difference P-Value = 0.010

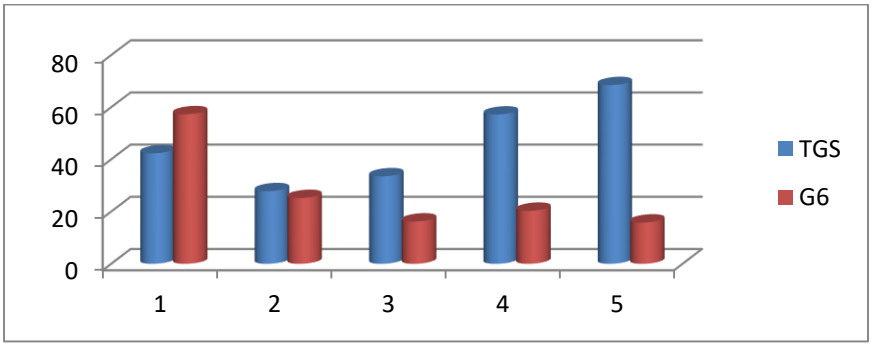


**Fig. (19):** The difference between the control and treatment groups during the treatment period in the values of glutathione averages at a non-significant difference (P-Value = 0.124).

**DPPH results**

With an increased concentration ratio of extracts, the effects of DPPH radical scavenging operation appear to improve. The results shown in table (2) and figure (20) compared to standard ascorbic acid showed the superiority of the flavonoid of *Z. coccinium* at a concentration of 100 µg/ml with a 70.9 % inhibition ratio and the crude isolated 87.19% at a concentration of 100 mg/ml compared to ascorbic acid, which recorded 98.35% at a concentration of 100mg/ml. Whereas, the lowest inhibition rate was at concentration of 1.25 mg/ml.

**Table (2):** The effect of *Zygophilium coccinium*_crude (Z-Crude) extracts and Zygophilium *coccinium* flavonoid (Z-flavonoid) with different concentrations of DPPH radical scavenging

| %Different concentrations of plants | % RSA (radical scavenging activity) at different concentrations (µg/ml) | | |
|---|---|---|---|
| | **Ascorbic acid** | **Z-Flavonoid** | Z-Crude |
| **100** | 98.35 | 70.9 | **87.19** |
| **90** | 98.21 | 70.4 | **86.36** |
| **80** | 97.93 | 69.6 | **85.95** |
| **70** | 97.66 | 66.4 | **84.44** |
| **60** | 97.25 | 66.0 | **83.20** |
| **50** | 96.97 | 63.5 | **83.33** |
| **40** | 96.83 | 61.2 | **80.30** |
| **30** | 96.56 | 59.4 | **78.79** |
| **20** | 96.28 | 57.0 | **74.10** |
| **10** | 95.87 | 52.5 | **70.80** |
| **5** | 95.32 | 12.5 | **66.39** |
| **2.5** | 94.49 | 9.1 | **60.47** |
| **1.25** | 94.08 | 2.1 | **51.10** |

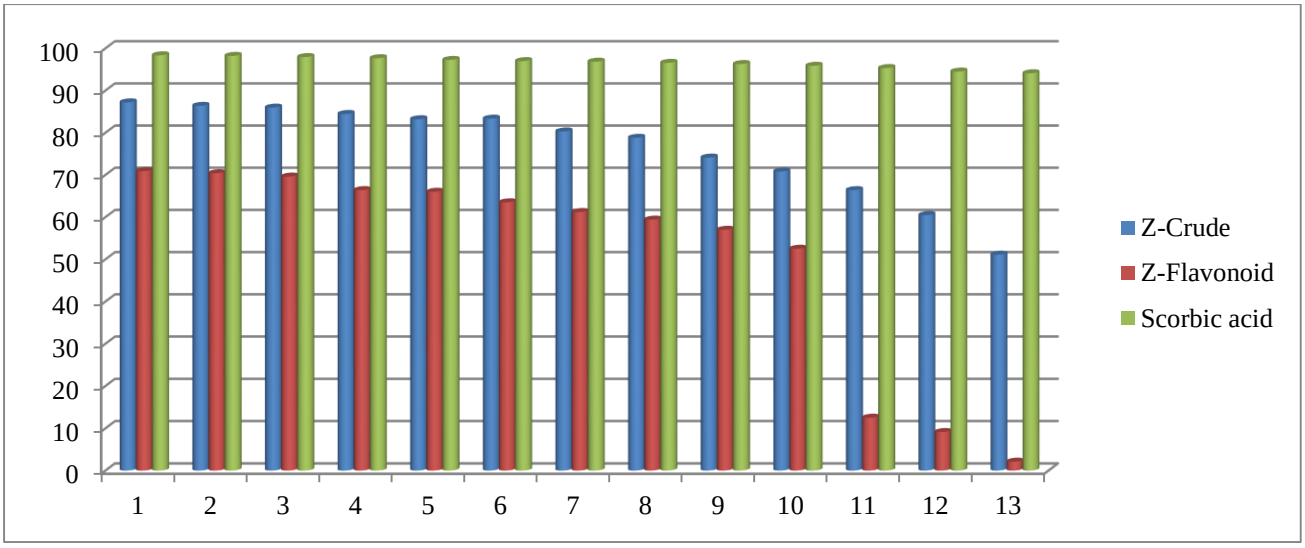



**Fig. (20):** DPPH radical scavenging activity of *Zygophilium coccinium* crude (Z. crude) and *Zygophilium coccinium*_crude (Z. crude) isolated with different concentrations when compared to the standard (ascorbic acid)

The results in table (3) and figure (21) showed the superiority of the flavonoid of *H. salicornium* at a concentration of 100 µg/ml with a 77.27 % inhibition ratio and crude isolated 81.96 % at a concentration of 100 mg/ml compared to ascorbic acid, which recorded 98.35% at a concentration of 100 mg/ml. Whereas, the lowest inhibition rate was at concentration of 1.25 mg/ml.

**Table (3):** The effect of *Haloxylon salicornium* crude (H-Crude) extracts and *Haloxylon salicornium* flavonoid (H-flavonoid) isolated with different concentrations of DPPH radical scavenging

| %Different concentrations of plants | % RSA (radical scavenging activity) at different concentrations (µg/ml) | | |
|---|---|---|---|
| | **Ascorbic acid** | **H-Flavonoid** | H-Crude |
| **100** | 98.35 | 77.27 | **81.96** |
| **90** | 98.21 | 77.13 | **81.54** |
| **80** | 97.93 | 76.86 | **81.27** |
| **70** | 97.66 | 71.07 | **80.58** |
| **60** | 97.25 | 69.01 | **78.65** |
| **50** | 96.97 | 68.18 | **77.96** |
| **40** | 96.83 | 66.25 | **76.58** |
| **30** | 96.56 | 65.84 | **74.93** |
| **20** | 96.28 | 65.01 | **72.59** |
| **10** | 95.87 | 46.42 | **69.01** |
| **5** | 95.32 | 29.20 | **33.75** |
| **2.5** | 94.49 | 9.09 | **30.17** |
| **1.25** | 94.08 | 0.96 | **26.45** |

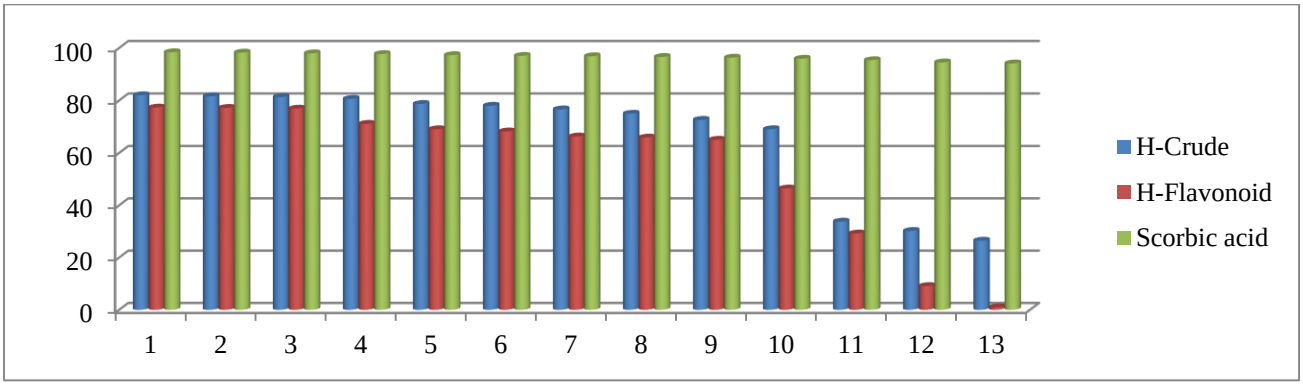



**Fig. (21):** DPPH radical scavenging activity of *Haloxylon salicornium* crude (H. crude) and *Haloxylon salicornium* crude (H. crude) isolated with different concentrations when compared to the standard (ascorbic acid).

**High performance liquid chromatography (HPLC)**

According to the findings of this analysis and HPLC data, flavonoids extract of *Haloxylon saliconium* contained a number of essential compounds, including gallic acid, furlic acid, qurcetien, keamferol, apigenin and rutin as shown in figure (22) but flavonoids extracts of *Zygophylium coccinium* contained a number of essential compounds, including p-cumaric acid, keamferol, qurcetien and gallic acid as shown in (Figure 23).

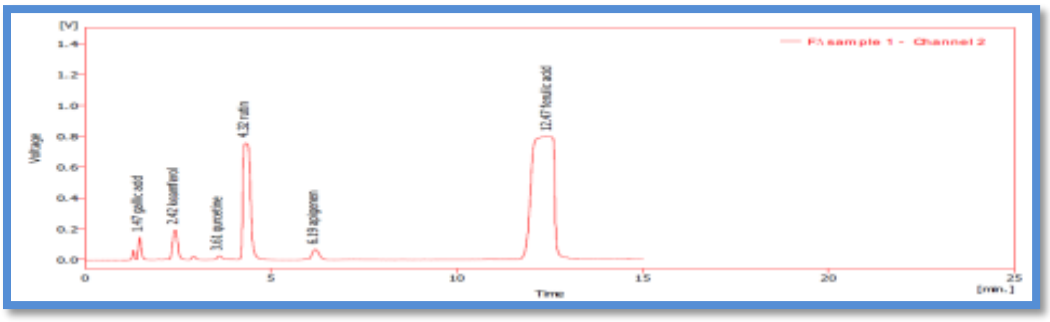



**Fig. (22):** HPLC study of sample flavonoids compounds in *Haloxylon saliconiumium*

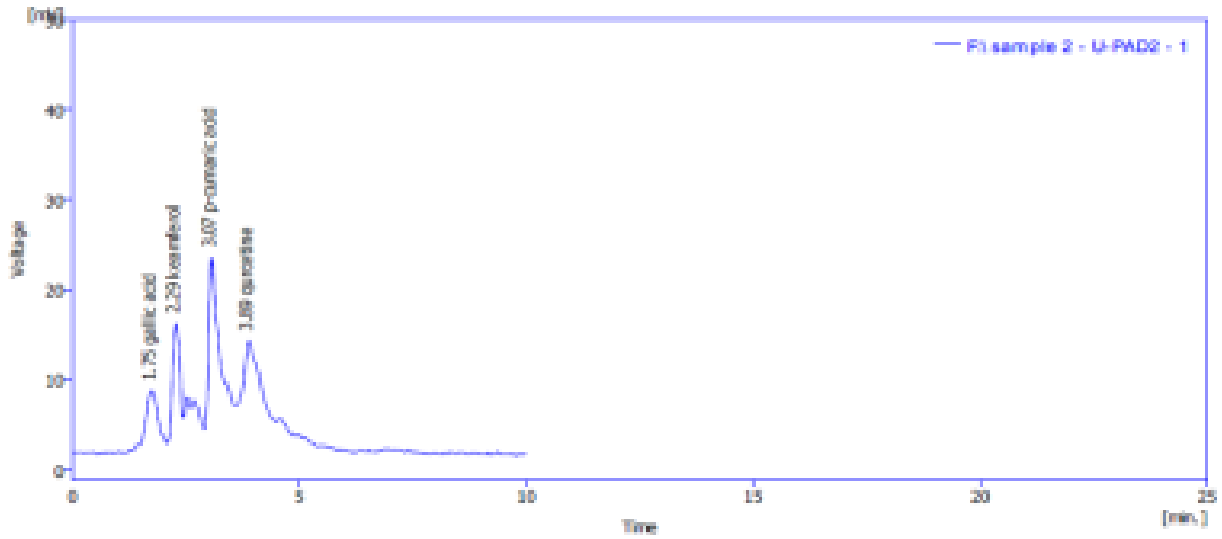



**Figure (23):** HPLC study of sample flavonoids compounds in *Zygophylium coccinium*

## DISCUSSION

In recent years, the use of traditional plant extracts and alternative therapies has helped raise awareness around the world, especially in underdeveloped nations where they are more readily available, effective, and have less adverse effects. The study's findings suggest that the extracts play a role in oxidative action, which works in concert with the antioxidant effects of DPPH root. The link between component concentrations allows for high total phenolic and total flavonoid content to provide important antioxidants. The removal of phenolic and antioxidant potency suggests that these elements are mostly to blame for it has a polar appearance and serves as an antioxidant, making it a powerful antioxidant [25]. This action may also be attributed to the flavonoids found in this extract, as studies have linked their displacement impact to the chemical components of flavonoids [11] and their antioxidant activity increases with concentration [26]. The results showed that the extracts have some oxidative activity and that this activity has a synergistic effect on the antioxidant effect of DPPH radical. The high contents of total phenolic, total flavonoid, and tannin compounds in *H. salicornicum* and *Z. coccineum* alcoholic extracts can contribute to important antioxidants because of the strong relationship between the concentration of the compounds [27]. The phenolic extraction and the effectiveness of antioxidants, which showed that these compounds are mostly to charge as they are effective antioxidants has a polar nature [28].

The beta cells of the pancreatic islets are essentially partially degraded by alloxan, which compromises both the quality and quantity of insulin produced by these cells.

The model uses two separate pathogenic effects, including the selective suppression of insulin production triggered by glucose and the induction of reactive oxygen species (ROS), which encourages the selective necrosis of pancreatic beta cells. Together, these two mechanisms cause cells to enter an insulin-dependent diabetes mellitus or type 1-like state [29]. The former is related to alloxan's specialized suppression of the pancreatic glucose sensor enzyme glucokinase, whilst the latter is more related to alloxan's capacity for redox cycling, which leads to the production of ROS. More significantly, both effects have been connected to the chemical composition and structure of alloxan [30, 31].

According to the histological findings of the infected animals' livers, it is evident that the liver's modifications ranged from fatty liver cell degeneration to steatohepatitis and periportal fibrosis. In this study, diabetic animals' livers were typically found to have lesions that affected all the organ's structures, including the sinusoids and portal areas, hepatocytes, nuclei, and intracytoplasmic organelles [32, 33].

Other lesions included steatohepatitis, periportal fibrosis, micro- and macrovesicular fatty degeneration, and fatty degeneration of the liver's cells. Additionally, hepatocytes' ultrastructure, particularly the mitochondria, rough endoplasmic reticulum (rER), and cell nuclei, underwent alterations. It was unclear, however, whether the observed histological abnormalities in the livers of ALX-induced diabetic rabbits were brought on by the diabetes condition or the drug's harmful effects. This action changed the levels of liver enzymes, which is consistent with **Abood and colleagues** [30] and **Al-Fartosi and colleagues** [34] who showed that ALX changed the typical cellular metabolic pathways, including the inhibition of specific enzymes, which caused liver damage and mortality. Additionally, **Gerard** [35] explained how the beta cells eventually fail due to the pancreas' work overload. Lack of glucose transfer to cells is caused by insulin resistance in the muscle, liver, and fat tissues. Despite hyperinsulinemia, postprandial and fasting hyperglycemia can still happen. An extensive burn may result in systemic inflammation, which then leads to insulin resistance. Additionally, because of insulin resistance and compromised repair, the liver continues to generate glucose [34].

Diabetes and burns caused significant alterations in various blood parameters, which is consistent with numerous research, including them. Hematological indices are crucial markers for assessing differences in blood cell size, quantity, and maturity. They are crucial for the evaluation and treatment of DM patients. It has been demonstrated that DM is closely correlated with a number of hematological abnormalities affecting coagulation factors, white blood cells, and red blood cells (RBCs), among others. RBC, WBC, and platelet dysfunction are other hematological abnormalities seen in DM patients [7, 35].

In the control group of experimental mice with diabetes, wound healing took longer time, which is consistent with what **Gerard** [35] reported that in addition to causing neuropathy (nerve damage), diabetes can also impact how well wounds heal. Uncontrolled blood sugar levels might harm the local nerves and numb the area's senses. This imply why individuals with diabetes have foot damage may not be aware of the injury. Diabetes patients, whether they have type 1 or type 2 are more likely to get a bacterial infection in the wound [35]. Other ways that diabetes may hinder the healing of wounds include a weaker skin barrier, decreased collagen formation, decreased production of growth and healing hormones and decreased production and repair of new blood vessels. Untreated wound infections have the potential to advance to the stage of gangrene. People with diabetes who surgically remove limbs frequently do so due to gangrene.

Sepsis, which happens when an infection spreads into the bloodstream, can occasionally develop in individuals with uncontrolled illnesses. Sepsis may endanger life. Renal involvement is an important concern in this context and this is due to numerous factors. There are changes in the kidney's composition and some blood parameters are linked to it. Diabetes mellitus and chronic liver disease, or both may be linked to kidney damage. Additionally, persistent viral hepatitis in individuals with diabetes mellitus may have a detrimental effect on the development of diabetic nephropathy. Patients with diabetes mellitus might potentially develop renal disease. Insulin resistance that develops as liver disease progresses is assumed to be connected to the pathogenic mechanisms of this so-called "hepatogenous diabetes" [33]. The link between diabetes mellitus and chronic liver disease, however, may be a double-edged sword because some writers contend that diabetes mellitus in cirrhotic patients may hasten the development of fibrosis and result in hepatocellular carcinoma (HCC). [34]

## Conclusion

Medicinal plants are rich in many effective antioxidant compounds that are used to neutralize free radicals formed in damaged or infected tissues in different parts of the body to reach a state of recovery and return to a normal neutral state. Flavonoids are considered the most powerful antioxidants that contain effective hydroxyl groups found in large quantities in desert medicinal plants and in different parts according to the type of plant. They were extracted for the first time in this study from

*Haloxylon salicornium* and Zygophilium *coccinium*. These plants were collected from the Samawa desert (they were used in folk medicine to treat burns). They were used as an ointment to treat burns in diabetic animals, and it proved a high effectiveness of treatment compared to chemotherapy and accelerating the healing period of the wound. Changes from the normal state were recorded in many blood parameters due to the effect of sugar and burning and some of them returned to normal values after the completion of healing, which indicated the therapeutic effectiveness of these extracts and their ability to resist free radicals that destroy tissue.

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