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#### of Potential Therapeutic Effects Alfalfa (Medicago sativa, L) Extracts on Oxidative Antioxidative Status in Diabetic Rats

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#### Abstract:

This study discusses the potential therapeutic effects of alfalfa (Medicago sativa, L) ethanolic and aqueous extracts on oxidative / antioxidative status in diabetic rats. Thirty adult male albino rats weighing 150±10g, were used and distributed into two main groups. The first group (5rats) was given a basal diet as control (-), the second main group (25 rats) injected with alloxan to cause diabetes, then distributed into five subgroups. One of them was positive diabetes control. Compared to the various four groups, who consumed a basal diet and administered ethanolic and aqueous extracts of alfalfa at (200 and 400 mg/kg) orally, for 28 days. When the experiment was complete, after treatment with alfalfa extracts, the rats of Alfalfa (Medicago sativa, L) were weighed and slaughtered, blood samples were taken, the serum was Oxidative separated to do the required measurements. Each body weight gain, feed in intake, and feed efficiency ratio were calculated. While blood serum glucose, lipide profile, liver function markers, kidney function levels, oxidative enzymes such as catalase, super oxide dismutase and malondialdehyde were estimated. The obtained results showed that the intake of alfalfa extracts led to a significant improvement in both body weight gain and feed intake, feed efficiency ratio (FER), liver and kidney functions, sugar, and an increase in the percentage of high-density lipoprotein (HDL-c), catalase (CAT), and superoxide dismutase (SOD) at (P<0.05) compared to the positive control group. Therefore, the results recommended the use of alcoholic alfalfa extract at a concentration of 400 mg/kg in our regular diets because the active ingredients in alfalfa are beneficial for diabetics.

Keywords: Plant extract, Oxidative stress, Alloxan, Rats

#### Introduction

It is well recognized that oxidative stress and illnesses brought on by a lack of activity are closely associated. According to (1), oxidative stress is characterized as an imbalance between the formation of reactive oxygen species (free radicals) and antioxidant defenses. According to (2), an excess of free radicals can lead to oxidative damage to biomolecules (lipids, proteins, and DNA) and ultimately many chronic diseases, including atherosclerosis, cancer, diabetes, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, and stroke.

Diabetes mellitus, a hyperglycemia disorder, occurs on by lower insulin production and increased blood sugar ratios or use by body cells, individuals with diabetes who are type 2 exhibit frequent urine (polyuria), frequent drinking (polydipsia), and frequent eating (polyphagia) (3). Blood glucose levels in the body influence the mode of infection or the virus that causes the inflammation. Type 2 hyperglycemia causes macrophages to release the pro-inflammatory cytokine Tumor Necrosis Factor (TNF) and causes elevated levels. TNF in sufferers with kind 2 diabetes has more severe insulin resistance, which in turn causes oxidative stress and subsequent disease consequences (4). It is managed to keep people to prevent complications or death. International Diabetes Foundation (IDF) indicated that the diabetes incidence among adults between the ages of 20 and 79 years in the countries of the world is expected during the next three years (5). As a result of the significant number of patients with diabetes, it is essential to prevent and control the condition, everyone can prevent and manage diabetes mellitus by eating plants high in polyphenols. As a more effective treatment for diabetes mellitus and its chronic problems, polyphenols and particularly flavonoids can be recommended (6).

One of the most essential legume forage plants in the world, alfalfa (Medicago sativa, L.) is particularly utilized as silage, hay, and pasture to feed cattle. Due to its excellent nutritional content, which includes healthy vitamins (i.e., B, C, D, and E), a significant amount of protein, and other vital minerals, this plant, also known as "lucerne" in Europe and other countries, and its sprouts can be used as a staple crop for humans as well as animals (7). Alfalfa represents among those with the greatest valuable and high types of protein sources, and this is not limited to animal nutrition only, but extends as a good and important source of nutritional compounds for humans in developing countries (8). It is preferable to use sprouts over seeds in their raw form because sprouts are rich in biologically active substances important to the body (9). Due to its chemically active components that act as antioxidants such as flavonoids, alkaloids and phytoestrogens, alfalfa leaves or any part of it is adopted in the treatment of many diseases such as cancer, menopausal symptoms, ulcers, kidney disease, diabetes, osteoporosis, coronary artery disease, anemia and high blood pressure, rheumatoid, neurodegenerative, and other diseases (10 and 11). Alfalfa is used in all its forms, such as the use of buds in salads, the use of leaves as herbal powder, or in the form of capsules and tablets. It is available in all food stores (12). Data proved that alfalfa leaves reduce blood sugar perfectly due to the role of the active compounds in their leaves. It is taken the kind that is of oral capsules three times a day as an alternative to insulin (13). As a result, this research aims to examine the therapeutic medicinal benefits of alfalfa ethanolic and aqueous extracts on oxidative / antioxidative status in diabetic rats.

## **Materials and methods**

## Materials:

## Plant material

Alfalfa leaves were purchased from the local farm in Shebin El-Kom City, Egypt.

## Rats

There were 30 male albino rats brought from the Medicine Insects Research Institute in Doki, Cairo, Egypt, weighing 150+10g.

## Chemicals

From the Company for Bio Diagnostics in Cairo, Egypt, the analysis kits were purchased. Alloxan was purchased from the El-Gomhoria Company for Trading Drugs, Chemicals, and Medical Equipment in Cairo, Egypt.

#### Methods

## Preparation of aqueous alfalfa leaves extract

To prevent potential damage to their bioactive components, alfalfa leaves were dried well for three days in an airy place free of direct sunlight before being milled in mixed with a high-speed mixer (Molunix, Al-Araby Company, Benha, Egypt) and served as powder.

We prepare a glass container, and for every Alfalfa leaf powder weighing 1 g, it is dissolved in 20 ml distilled water for 48 hours, with stirring from time to time, at room temperature, then filtering through three layers of filter paper, to ensure that the extract is free of broken fibers, then put the filter in Petri dishes in dried at 50 °C for a few hours and then kept at 4 °C until use (14).

## Preparation of ethanolic alfalfa leaves extract

Five gram of dried Alfalfa leaves powdered dissolved it in 100 ml of ethanol alcohol at a concentration of 80% with good stirring from time to time for 24 hours in room air. A rotary evaporator can also be used at 40°C to efficiently remove alcoholic solvents by evaporation, then filtering the extract through filter paper, then leaving it in room air to dry somewhat, then keeping the alcoholic extract at 4°C until use (15).

#### Basal diet

The following is the basal diet made using the formula provided by AIN (16) as follow: Protein 10%, cellulose 5%, minerals, 10% corn oil and vitamin blend, 1%, choline chloride 0.2%, methionine 0.3%, and corn starch 69.5%. Also, Campbell (17) advised the vitamin mixture component that was utilized, while Hegested et al., (18) recommended the salt mix component that was used.

Inducing damage for pancreatic beta cells in rats According to (19), alloxan injection at a dose (150 mg /kg body weight) caused chronic damage to pancreatic beta cells in normal healthy rats.

## Experimental design

The Science Research Ethics Committee of the Faculty of Home Economics accepted the research protocol #18-SREC-09-2020.

In this experiment, 30 adult male white albino rats, "Sprague Dawley" strain, 10 weeks old, weighing (150±10g), were used. For adaptation, all rats were fed a basal diet (casein diet) for

7 days. After this adaptation period, rats were divided into 6 groups, 5 rats per each as follows:

Group (1): Rats have been given a standard diet and served as a control negative group.

Group (2): Rats have been given a standard diet and served as control positive group.

Group (3): Rats have been given a standard diet and treated with aqueous alfalfa extract 200mg/kg of rat body weight, orally.

Group (4): Rats have been given a standard diet and treated with aqueous alfalfa extract 400mg/kg of rat body weight, orally.

Group (5): Rats have been given a standard diet and treated with ethanolic alfalfa extract 200mg/kg of rat body weight, orally.

Group (6): Rats have been given a standard diet and treated with ethanolic alfalfa extract 400mg/kg of rat body weight, orally.

#### **Collection of blood samples**

The rats were slaughtered at the conclusion of the experiment following a 12-hour fast. Samples of blood were taken from the portal vein using dry, sterile centrifuge tubes. The blood was centrifuged at 3000 r.p.m for 10 minutes to obtain the serum. For analysis, serum was kept at -20°C. The kidneys and liver were removed at the same time, cleaned with saline solution, dried using filter paper, weighted, and preserved in 10% formalin solution for histological testing (20).

#### **Biochemical analysis**

Feed intake (FI), feed efficiency ratio (FER), and percentage of body weight (BWG%) have been calculated in accordance with (21). Serum glucose was estimated using procedure (22). Triglycerides were carried out using the techniques mentioned in (23). Total cholesterol was calculated using the (24) technique. The levels of high-density lipoprotein were determined using the methods of (25). The following equation was used to determine both Very Low-Density Lipoprotein (VLDL) and Low-Density Lipoprotein (LDL).:

LDL-Cholesterol = Total Cholesterol - HDL-c + VLDL.

#### VLDL-c = (TG/5) (26).

The alkaline phosphatase (ALP) concentration was estimated by using the method of (27), alanine aminotransferase (ALT) levels were estimated by using the method of (28), and aspartate aminotransferase transferase (AST) levels were estimated by using the method of (29). While serum creatinine, urea, and uric acid were calculated by using the method of (30, 31 and, 32). Superoxide dismutase (SOD), catalase enzyme (CAT), and malondialdehyde (MDA) levels were measured in accordance with (33), (34) and (35).

#### Statistical analysis:

The data were analyzed using a completely randomized factorial design (36) when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments of ( $P \le 0.05$ ) were considered significant using Costat Program. Biological results were analyzed by One Way ANOVA.

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#### **Results and Discussion**

Table (1) of data displayed the average values of FI, FER, and BWG% of diabetic rats treated with alfalfa leaves extracts by different levels. This table indicates that mean values of FI, FER, and BWG%, in the diabetic rat group (control positive), were 13.9, 0.05 and 0.71, respectively. While at normal rats the mean values were 22.7, 0.1 and 2.5, respectively, showing significant difference, percent of increasing 63.309%, 112.76% and 252.1126% respectively for group (-) in comparison to group (+). Regarding FI, results indicate that alfalfa leaves extract can improve FI value in all groups which are treated with alfalfa leaves extract. For FER (g) and BWG (%), numbers displayed substantial enhancement in diabetic rats fed on alfalfa leaves extracts compared to the group (2). Group (6) showed the best results in FI, FER and BWG% as compared to all groups. These findings are in line with those of (37) who claimed that feeding overweight female rats a formulation of examined herbs and plants powder at a dosage of 10% should help them lose weight and enhance different parameters. Furthermore, (38) they indicated that therapy with alfalfa leaves extract can increase BW and FI. In contrast, (39) showed that at the beginning of the trial, there was no statistically significant difference between the animal weights, but after the experiment, there was a positive control group (+ve) represented the highest weights and the group which treated with alfalfa extract showed the lowest weights.

| Groups  | FI (g)/day   | % of<br>change | FER (g)     | % of<br>change | BWG (%)    | % of<br>change |
|---|--------------|----------------|-------------|----------------|------------|----------------|
| Group (1): Negative control                       | 22.7±0.9a    | 63.31%         | 0.1±0.004a  | 112.76%        | 2.5±0.03a  | 252.113%       |
| Group (2): Positive<br>control                    | 13.9±0.98d   | 0              | 0.05±0.002c | 0              | 0.71±0.09e | 0              |
| Group (3): Aqueous<br>alfalfa extract 200 mg/kg   | 15.5±1.4cd   | 11.51%         | 0.06±0.01c  | 25.53%         | 0.9±0.1d   | 26.76%         |
| Group (4): Aqueous<br>alfalfa extract 400 mg/kg   | 18.3±2.23bc  | 31.65%         | 0.07±0.005b | 48.93%         | 1.4±0.05c  | 97.18%         |
| Group (5): Ethanolic<br>alfalfa extract 200 mg/kg | 20.56±2.23ab | 47.9%          | 0.09±0.009b | 89.36%         | 1.9±0.08b  | 167.61%        |
| Group (6): Ethanolic<br>alfalfa extract 400 mg/kg | 21.13±2.37ab | 52.014%        | 0.11±0.012a | 134.04%        | 2.48±0.06a | 249.29%        |
| LSD   | 3.2          | 0              | 0.014       | 0              | 0.14       | 0              |

Table (1): Influence of alfalfa leaf extracts on FI(g), FER(g), BWG (%) of diabetic rats

Values are represented by means $\pm$ SD; means in similar columns with different superscript letters are significantly different (p $\leq$ 0.05).

Impact of alfalfa leaf extracts on the serum glucose level of diabetic rats was compiled in Table (2). It could be noticed for serum glucose that the highest mean value was in the control positive group while the group (6) that already fed on 400 mg/kg ethanolic alfalfa extract had the lowest mean value compared to the control positive group. Data also showed that treated groups with aqueous alfalfa leaves extracts showed significant decreases (P≤

0.05) in serum glucose levels. As the amount of alfalfa leaves extracts was increased, serum glucose levels decreased in comparison to the positive control group. The percentage of change in treated groups (3,4,5 and 6) were -3.29, -18.19, -43.54 and 62.12 %, respectively. These findings are consistent with (40), who claimed that alfalfa may be a target for the pancreatic islets since it stimulates insulin secretion. The BRIN-BD11 Pancreatic -Cell Line (Pancreatic Islets From Rat) Produced More Insulin When Exposed To An Aqueous extract of alfalfa leaves (1gm/ml) at a glucose concentration of 16.7mmol. The concentration of the extract depended on the activity of extract.

In addition, (41) showed the significance of alfalfa leaves for diabetics, especially people who have type two diabetes, as it lowers blood glucose for two hours after eating a meal from 344.4 mg/dl to 300.75 mg/dl, in addition to that it helps to raise insulin levels.

In addition, by chemical analysis of the alfalfa plant, it turned out that it contains many effective compounds to protect the body from various diseases, such as diabetes mellitus (42).

Furthermore, (43) mentioned note to that at 2, 4, and 6-hours following extract administration, all the groups treated with plant extracts, in addition to the control group, confirmed an enormous minimize in blood glucose ranges.

|  | 0                           |             |
|--|-----------------------------|-------------|
| Groups   | Serum glucose level (mg/dl) | % of change |
|  | 75.76±3.53 f                | -70.09%     |
| Group (2): Positive control                    | 253.3±3.12 a                | 0           |
| Group (3): Aqueous alfalfa extract 200 mg/kg   | 244.9±2.75 b                | -3.29%      |
| Group (4): Aqueous alfalfa extract 400 mg/kg   | 207.2±2.3 c                 | -18.19%     |
| Group (5): Ethanolic alfalfa extract 200 mg/kg | 143±2.45 d                  | -43.54%     |
| Group (6): Ethanolic alfalfa extract 400 mg/kg | 95.93±1.01 e                | -62.127%    |
| LSD  | 4.7                         | 0           |

#### Table (2): Influence of alfalfa leaf extracts on serum glucose level of diabetic rats.

Values are expressed as means $\pm$ SD; means in similar column with different superscript letters are significantly different (p $\leq$  0.05).

Data in Table (3) showed the impact of alfalfa leaf extracts on oxidative enzymes (CAT, SOD and MDA) in diabetic rats. Table (3) revealed that diabetic rat groups which were without treatment showed that the enzymatic antioxidant CAT and SOD were 4.5 and 2.6 (U/mg tissue), respectively. In the negative control group, the previously listed enzymes levels were 24.03 and 10.46 (U/mg tissue), respectively. Regarding SOD and CAT there were significant differences between each group treated with alfalfa leaves extracts and positive control group. In the positive control category, there were notable declines in enzymatic antioxidants. % of change for CAT, SOD and MDA in group (-) were 434, 302.3 and -43.75 %, respectively as compared to group (+). Group (6) showed the highest result increase in CAT and SOD as compared to all treated groups. As for MDA, there was a significant decrease in the groups treated with alfalfa extracts when compared to the positive control group. There are numerous examples of studies that numerous plants, including alfalfa, are excellent sources of antioxidants, which play a protective and curative role for diseases resulting from

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oxidative stress, such as liver and kidney poisoning in experimental animals (44). It was found that treatment dose (500 mg/kg) of alfalfa extract led to the reduction of (MDA) to the lowest concentration in the group treated with the extract (45).

Saponins found in alfalfa leaves may protect cells from oxidative damage by preventing H2O2-induced activation of apoptosis. By restoring GSH homeostasis, alfalfa saponins' antioxidant effects were made possible. In addition, by activating the MAPK signaling pathway, alfalfa saponins may improve cell survival rates. As a result, alfalfa saponins may act as a cellular oxidative damage inhibitor or as a prospective therapeutic candidate, offering a fresh method for preventing the cell apoptosis brought on by oxidative stress in monogastric animals (46).

| Croups  | CAT             | % of    | SOD           | % of    | MDA                     | %of      |
|---|-----------------|---------|---------------|---------|-------------------------|----------|
| Groups  | (U/g tissue)    | change  | (U/mg tissue) | change  | (nmol/g tissue)         | change   |
| Group (1): Negative                               | 24.03±2.27      | 121%    | 10 46+0 85    | 202.2%  | 52 12+7 77 <sub>0</sub> | 12 75%   |
| control   | а               | 434/0   | 10.40±0.88    | 302.370 | JJ.1J12.276             | -43.73/0 |
| Group (2): positive control                       | 4.5±0.9d        | 0       | 2.6±0.89e     | 0       | 94.46±1.9a              | 0        |
| Group (3): Aqueous alfalfa<br>extract 200 mg/kg   | 6.8±1.3d        | 51.11%  | 4.16±1.001de  | 60%     | 88.1±0.5b               | -6.73%   |
| Group (4): Aqueous alfalfa<br>extract 400 mg/kg   | 10.6±1.9c       | 135.55% | 5.8±0.9cd     | 123.07% | 85.7±3.17b              | -9.27%   |
| Group (5): Ethanolic<br>alfalfa extract 200 mg/kg | 15.8±2.34b      | 251.11% | 7.4±1.05bc    | 184.61% | 72.5±1.9c               | -23.25%  |
| Group (6): Ethanolic<br>alfalfa extract 400 mg/kg | 21.06±2.03<br>a | 368%    | 8.9±1.07ab    | 242.3%  | 62.13±2.2d              | -34.226% |
| LSD   | 3.32            | 0       | 1.7           | 0       | 3.8                     | 0        |

#### Table (3): Influence of alfalfa leaf extracts on CAT, SOD and MDA of diabetic rats

Values are expressed as means±SD; means in similar column with different superscript letters are significantly different ( $p \le 0.05$ ).

Data presented in Table (4) show the impact of alfalfa leaf extracts on kidney function which includes (uric acid, creatinine, and urea) in diabetic rats. In the positive control group, data indicated that there were significant increases in mean values of uric acid, urea and creatinine, and the mean values were 3.63, 51.9 and 1.33 mg/dl, respectively. While these values were 1.05, 27.6 and 0.31 mg/dl, respectively in the negative control group. The percent of change for uric acid, urea and creatinine were -71.07, -46.82 and -76.15 %, respectively in group (1) as compared to group (2). All groups which were treated with alfalfa extracts showed significant decreases in renal functions compared to the positive control group. According to the findings, the ethanolic alfalfa leaf extract had a stronger impact on improving kidney functioning than the aqueous extract. Alfalfa reduces development of the kidney stones, lowers blood pressure, protects the liver from excess fat, as in the case of fatty liver, and improves the metabolism of sugars against oxidants (47). Additionally, (48) showed that biochanin A in alfalfa protects the kidneys, with the presence of some chemical drugs such as cisplatin, which acts as an anti-inflammatory and anti-ovulatory.

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| Groups  | Uric acid       | % of    | Urea            | % of    | Creatinine      | % of     |
|---|-----------------|---------|-----------------|---------|-----------------|----------|
| Gloups  | (mg/dl)         | change  | (mg/dl)         | change  | (mg/dl)         | change   |
| Group (1): Negative control                       | 1.05±0.3c       | -71.07% | 27.6±2.8d       | -46.82% | 0.31±0.19c      | -76.153% |
| Group (2): Positive control                       | 3.63±0.4a       | 0       | 51.9±3.35a      | 0       | 1.33±0.45a      | 0        |
| Group (3): Aqueous alfalfa<br>extract 200 mg/kg   | 3.26±0.5a       | -10.19% | 44.4±2.3b       | -14.45% | 0.94±0.05b      | -27.69%  |
| Group (4): Aqueous alfalfa<br>extract 400 mg/kg   | 2.73±0.45a<br>b | -24.79% | 39.6±0.8bc      | -23.69% | 0.7±0.1bc       | -46.154% |
| Group (5): Ethanolic alfalfa<br>extract 200 mg/kg | 1.93±0.47b<br>c | -46.83% | 33.9±8.7cd      | -34.68% | 0.58±0.04b<br>c | -55.38%  |
| Group (6): Ethanolic alfalfa<br>extract 400 mg/kg | 1.33±0.8c       | -63.36% | 27.03±1.02<br>d | -47.91% | 0.4±0.15bc      | -67.69%  |
| LSD   | 1.059           | 0       | 7.34            | 0       | 0.38            | 0        |

Table (4): Influence of alfalfa leaf extracts on renal biomarkers of diabetic rats

Values are expressed as means $\pm$ SD; means in similar column with different letters are significantly different (p $\leq$  0.05).

Table (5) shows the impact of alfalfa leaves extracts on triglycerides (T.G) and total cholesterol (T.C) of negative and diabetic groups. Treatment with 200, 400 mg/kg aqueous and ethanolic extracts of alfalfa leaves showed significantly improved in the ranges of TG and TC in contrast to group (2). % of change in treatment groups (3,4,5 and 6) for TC were -16.76, -23.3, -29.78 and -40.49%, TG values were -7.75, -22.9, - 42.16 and -53.16 %, respectively. The best outcome was in group (6) compared with group (2).This result is in the same line with (17) they reported that using both 250 and 500 mg/kg as a dosage of aqueous extract of alfalfa led to a very significant decrease in the levels of total cholesterol and triglycerides. This may be due to the vitamins in alfalfa that play an important role, such as vitamin E, vitamin C, and beta-carotene through their antioxidant and anti-obesity activity (49). In addition, hyperglycemic rats had significant increases in the serum levels of total cholesterol (TC) and triglycerides (T.G). Consumption of plants individual and their mixture at (5%) percent caused significant decreases in serum levels of TC and TG when compared to the positive control group (50).

Table (5): Influence of alfalfa leaves extracts on total cholesterol (T.C) and triglycerides (T.G) of diabetic rats

| Groups   | TC (mg/dl)  | % of change | TG (mg/dl)  | % of change |
|--|-------------|-------------|-------------|-------------|
| Group (1): Negative control                    | 92.76±2.37e | -43.26%     | 57.5±3.8e   | -56.8%      |
| Group (2): Positive control                    | 163.5±2.9a  | 0           | 133.2±2.34a | 0           |
| Group (3): Aqueous alfalfa extract 200 mg/kg   | 136.1±2.9b  | -16.76%     | 122.9±2.9b  | -7.75%      |
| Group (4): Aqueous alfalfa extract 400 mg/kg   | 125.4±3.3c  | -23.302%    | 102.7±3.7c  | -22.91%     |
| Group (5): Ethanolic alfalfa extract 200 mg/kg | 114.8±4.2d  | -29.78%     | 77.06±2.8d  | -42.16%     |
| Group (6): Ethanolic alfalfa extract 400 mg/kg | 97.3±1.5e   | -40.49%     | 62.4±2.3e   | -53.16%     |
| LSD  | 5.34        | 0           | 5.47        | 0           |

Values are displayed as means  $\pm$  SD, means in similar column with different letters are significantly different (p $\leq$  0.05).

Influence of alfalfa leaf extracts on lipid profile (HDL, VLDL and LDL) of diabetic rats are recorded in Table (6). According to LDL and VLDL, Results showed that the positive control group's mean value was much greater than the negative control group's (healthy rats) while HDL had the opposite trend. % of change in group (1) for HDL, LDL and VLDL were 150.9, - 84.49 and -56.76 %, respectively as compared to group (2). Treatment with alfalfa extracts in groups (3, 4, 5, and 6) can raise HDL levels and lower LDL and VLDL levels when compared to the positive control group. The results also showed that the ethanolic alfalfa leaf extract had greater effects on lipid profile improvement than the aqueous extract. Also, (51) demonstrated that alfalfa has an effective effect on lipid levels significantly, with higher HDL levels and lower LDL levels. (52) proved that treatment with alfalfa and its supplements restores the balance of lipids, such as improving lipid peroxidation, increasing the level of HDL, and decreasing the level of LDL, due to the presence of flavonoids and polyphenols in the plant.

| Groups  | HDI (mg/dl)  | % of     | IDI (mg/dl)      | % of    | VIDI (mg/dl) | % of    |
|---|--------------|----------|------------------|---------|--------------|---------|
|   | HDE (Hig/ul) | change   | LDL (IIIg/ul)    | change  | VEDE (mg/ui) | change  |
| Group (1): Negative control                       | 64±2.7a      | 150.98%  | 17.26±0.76f      | -84.49% | 11.5±0.7e    | -56.77% |
| Group (2): Positive control                       | 25.5±2.25f   | 0        | 111.35±2.5<br>6a | 0       | 26.6±0.46a   | 0       |
| Group (3): Aqueous alfalfa<br>extract 200 mg/kg   | 30.2±2.57e   | 18.43%   | 81.38±4.6b       | -26.92% | 24.58±0.59b  | -7.59%  |
| Group (4): Aqueous alfalfa<br>extract 400 mg/kg   | 37.6±2.86d   | 47.45%   | 67.19±2.9c       | -39.66% | 20.54±0.75c  | -22.78% |
| Group (5): Ethanolic alfalfa<br>extract 200 mg/kg | 45.8±2.6c    | 79.607%  | 53.58±1.35<br>d  | -51.88% | 15.41±0.57d  | -42.07% |
| Group (6): Ethanolic alfalfa<br>extract 400 mg/kg | 58.6±2.4b    | 129.803% | 26.25±1.8e       | -76.42% | 12.48±0.46e  | -53.08% |
| LSD   | 4.6          | 0        | 4.71             | 0       | 1.09         | 0       |

Table (6): Effect of alfalfa leaves extracts on HDL, LDL and VLDL of diabetic rats

Values are expressed as means $\pm$ SD; means in similar column with different letters are significantly different (p $\leq$  0.05).

Data in Table (7) indicated that rats in the group (2) had increased the mean value of ALT (171.26 U/L) compared to the control negative group (73.03 U/L). The rats which treated with alfalfa leaves extracts (aqueous and ethanolic extract 200, 400mg/kg body weight) When compared to the control positive group, had lower ALT values. The numbers were (163.9, 111.1, 91.4 and 74.6 U/L), respectively. Regard to GOT showed increases in the control positive group compared with the control negative group. Also, rats treated with various amounts of alfalfa extracts (aqueous and ethanolic extract 200, 400mg/kg). ALP's percentage change was -5.18%, -20.008%, -40.016% and-51.58% for groups (3,4,5 and 6) respectively, compared with the control positive group. Group 6 treatment with ethanolic alfalfa extract 400 mg/kg had the best GPT, Got, and ALP (U/L) findings. (12) discovered that alfalfa plant contains many secondary receptors such as flavonoids, alkaloids, coumarin and phytoestrogen, which shows great effectiveness as an antioxidant, so alfalfa decreases liver

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enzymes in blood serum. (41) proved that with different treatment doses of alfalfa extract, it led to an improvement in liver function by decreasing the levels of ALP and ALT in the serum.

| Groups  | ALT(GPT)     | % of    | AST(GOT)   | % of    | ALP         | % of     |
|---|--------------|---------|------------|---------|-------------|----------|
| Gloups  | (U/L)        | change  | (U/L)      | change  | (U/L)       | change   |
| Group (1): Negative control                       | 73.03±2.19e  | -57.36% | 61±1.5e    | -36.13% | 120.8±1.8e  | -52.33%  |
| Group (2): Positive control                       | 171.26±3.13a | 0       | 95.5±2.6a  | 0       | 253.4±3.26a | 0        |
| Group (3): Aqueous alfalfa<br>extract 200 mg/kg   | 163.9±2.9b   | -4.29%  | 88.2±2.3b  | -7.64%  | 240.26±3b   | -5.18%   |
| Group (4): Aqueous alfalfa<br>extract 400 mg/kg   | 111.1±3.2c   | -35.13% | 78.7±2.4c  | -17.59% | 202.7±1.6c  | -20.008% |
| Group (5): Ethanolic alfalfa<br>extract 200 mg/kg | 91.4±2.26d   | -46.63% | 70.4±1.6d  | -26.28% | 152±3.1d    | -40.016% |
| Group (6): Ethanolic alfalfa<br>extract 400 mg/kg | 74.6±1.3e    | -56.44% | 62.9±0.47e | -34.14% | 122.7±2.58e | -51.58%  |
| LSD   | 4.6          | 0       | 3.5        | 0       | 4.7         | 0        |

| Table (  | (7): | Influence | of alfalfa | leaf | extracts o  | on diabeti | c rat's | liver | health |
|----------|------|-----------|------------|------|-------------|------------|---------|-------|--------|
| I abit ( | 1.   | Innucieu  | u anana    | ivai | call acts 0 | m utabeti  | c rat s | IIVCI | ncann  |

*Values are expressed as means* $\pm$ *SD; means in similar column with different superscript letters are significantly different (p* $\leq$  0.05).

## Conclusion

The obtained data showed that alfalfa extracts should be included in our diets because it contain active ingredients are beneficial for people with diabetes.

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# التأثيرات العلاجية المحتملة لمستخلصات البرسيم الحجازي على حالة المؤكسدات ومضادات

الأكسدة في الفئران المصابة بمرض السكرى

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الملخص العربي:

تهدف هذه الدراسة إلى استكشاف التأثيرات العلاجية المحتملة لمستخلصات البرسيم الحجازي على الحالة المؤكسدة / المضادة للأكسدة في الفئران المصابة بمرض السكري. تم استخدام ثلاثين من ذكور الفئران البالغ وزنها ١٥٠± ١٠ جرام وتم توزيعها إلى مجموعتس رئيسيتس. يم تغذية المجموعة الأساسية الأولى (٥ فئران) على الغذاء الأساسي كمجموعة ضابطة سالبة أما المجموعة الأساسية الثانية (٢٥فأر) تم حقنها بالألوكسان للإصابة بمرض السكرى، ثم وزعت على خمس مجموعات فرعية. مجموعة منهم تركت كمجموعة ضابطة موجبة بينما تناولت المجموعات الأربع الأخرى نظاما غدّائيا أسَّاسيا وعولجت بمستخلص البرسيم الحجازي المائي بتركيز ٢٠٠، ٤٠٠ مجم / كجم من وزن الفأر والمستخلص الكحولي بتركيز ٢٠٠، ٤٠٠ مجم / كجم من وزن الفأر عن طريق الفم لمدة ٢٨ يومًا. وفي نهاية التجربة وبعد المعاملة بمستخلص البرسيم الحجازي المائي والكحولي تم وزن الفئران وذبحها وأخذ عينات الدم وفصل السيرم لعمل التقديرات المطلوبة. تم حساب المأخوذ من الغذاء ووزن الجسم المكتسب ونسبة الاستفادة من الغذاء بينما تم تقدير مستوى الجلوكوز في الدم وصورة دهون الدم ومؤشرات وظائف الكبد ووظائف الكلي وانزيمات الاكسدة مثل الكتالبر والسوبر أكسيد ديسموتيز والمالونالدهيد. أوضحت النتائج المتحصل عليها أن تناول مستخلصات البرسيم الحجازي أدى إلى تحسن معنوى في كلا مِّن وزن الجسم المكتسب والمأخوذ من الغذاء ونسبة الاستفادة من الغذاء ووظائف الكبد والكلى والسكر وزيادة نسبة البروتين الدهني عالى الكثافة وانزيم الكتاليز والسوبر أكسيد ديسموتيز عند معنوية (P<0.05) مقارنة بألمجموعة الضابطة الموجبة. لذلك أوصت النتائج باسـتخدام مسـتخلص البرسـيم الحجازي الكحولي بتركيز ٤٠٠ مجم / كجم في أنظمتنا الغذائية العادية لأن المكونات النشطة في البرسيم الحجازي مفيدة لمرضى السكر.

الكلمات المفتاحية: مستخلص النباتات، جهد الأكسدة، الألوكسان، الفئران.