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# Therapeutic Role of Aqueous Extract of Milk Thistle (*Silybum adans L.*) and Burdock (*Arctium lappa*) in Hyperglycemic Rats

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## ABSTRACT

**Bakground:** Diabetes mellitus (DM), the third killer of the mankind health along with cancer, cardiovascular and cerebrovascular diseases, is one of the most challenging diseases facing health care professionals today **Objective:** Therapeutic role of acques extract of Milk thistle (Silybum adans, L.) and Burdock (Arctium lappa) and their mixture in hyperglycemic rats. **Material and methods:** Fourty mature male albino rats weighing 180-200g then divided into 8 equal groups; one group was kept as a (C –ve) group, while the other 7 groups were injected s/c by 150 mg/kg body weight alloxan to induce hyperglycemia. Hyperglycemic rats were disparted into seven equal groups (n= 5 rats) one of them left as control positive while other eight groups orally fed using two doses (250 and 500 mg/kg of milk thistle, burdock , and mixture of them, respectively). At the end of experimental period (45 days), blood samples were collected for serum separation to determine serum glucose, liver enzymes (ALT, AST, ALP,) total protein, albumin, globulin, total cholesterol, triglycerides, lipoprotein fractions (HDLc, LDLc and VLDLc), kidney function (creatinine, urea and uric acid) and histopathological changes. **Resultes:** Data showed that that **aqueous** extract of milk thistle (Silybum adans, L.) and burdock (Arctium lappa) showed significant decrease in serum glucose and improving liver and kidney status specially the mixture of 500 mg of milk thistle snd burdock . **Conclusion:** According to these results, milk thistle and burdock could be used for hyperglycemia and impaired liver and kidney function.

**Keywords:** *Hyperglycemia, milk thistle, burdock , rats, histopathological changes.*

## Introduction

Diabetes mellitus (DM), the third killer of the mankind health along with cancer, cardiovascular and cerebrovascular diseases, is one of the most challenging diseases facing health care professionals today [1].

Diabetes mellitus, commonly referred to as diabetes was first identified as a disease associated with "sweet urine," and excessive muscle loss in the ancient world. Elevated levels of blood glucose lead to spillage of glucose into the urine, hence the term sweet urine. Normally, levels are tightly controlled by insulin, a hormone produced by the pancreas. Insulin lowers the blood glucose level. When the blood glucose elevates insulin is released from the pancrease to normalize the glucose level [2].

*Silymarin*, derived from the Milk thistle plant, *Silybum marianum*, has been used for centuries as a natural remedy for diseases of the liver and biliary tract. As interest in alternative therapy has emerged in the United States, gastroenterologists have encountered increasing numbers of patients taking silymarin with little understanding of its purported properties. Silymarin and its active constituent, silybin, have been reported to work as antioxidants scavenging free radicals and inhibiting lipid peroxidation. Studies also suggested that they protect against genomic injury, increase hepatocyte protein synthesis, decrease the activity of tumor promoters, stabilize mast cells, chelate iron, and slow calcium metabolism [3].

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Traditional milk thistle extract is made from the seeds, which contain approximately 4-6% silymarin. The extract consists of about 65-80% silymarin (a flavonolignan complex) and 20-35% fatty acids, including linoleic acid. Silymarin is a complex mixture of polyphenolic molecules, including seven closely related flavonolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin) and one flavonoid (taxifolin). Silibinin, a semipurified fraction of silymarin, is primarily a mixture of 2 diasteroisomers, silybin A and silybin B, in a roughly 1: 1 ratio. In clinical trials silymarin has typically been administered in amounts ranging from 420–480 mg per day in two to three divided doses. However higher doses have been studied, such as 600 mg daily in the treatment of type II diabetes and 600 or 1200 mg daily in patients chronically infected with hepatitis C virus, an optimal dosage for Milk Thistle preparations has not been established. It is known to help hangovers [4].

Some folk herbalists which consider dried burdock to be a diuretic, diaphoretic, and a blood purifying agent. The seeds of A. lappa are used in traditional Chinese medicine, under the name niupangzi; pinyin: niúpángzi; Some dictionaries list the Chinese as just niúbàng [5].

Lin [6] mentioned that *Arctium lappa* Linne (*A. lappa*) could protect the liver cells from acetaminophen-induced liver damages, perhaps by its antioxidative effect on hepatocytes. Therefore this investigation aimed to study the possible therapeutic effect of acques extract of milk thistle (*Silybum adans* L.) and Burdock (*Arctium lappa*) in healing hyperglycemia using experimental rats.

## Materials and Methods

**Plants:** The tested plants in this investigation were milk thistle (*Silybum adans, L.*) the genus Silybum Adans., a flowering plant of the daisy family (*Asteraceae*) and burdock (*Arctium lappa*) temperate Eurasia , this genus consists of about ten species of upright biennials. One of these plants ( milk thistle "*silymarim*") were obtained as dried material from the local market in Egypt, but the other herb named burdock "*Arctium lappa*" was obtained from China.

**Basal Diet:** The basal diet was prepared according to Reeves [7]. It was consisted of 20% protein(casein), 10% sucrose, 4.7% corn oil, 2% choline chloride, 1% vitamin mixture, 3.5% salt mixture and 5% fiber (cellulose). The remainder was corn starch.

**Hyperglycemia:** Diabetes type II was inducing in normal healthy adult male albino rats by intra peritoneal injection of 60mg/kg dose of Streptozotocin according to the method described by Akbarzadeh [8].

**Rats:** Forty male albino rats weighing 180-200 g. (B.Wt.) were obtained from Laboratory of Animal Colony, Helwan, Egypt. The animals were allocated in plastic cages with metallic strainless covers and kept under strict hygienic measures. Rats were fed the basal diet for 7 days before the beginning of the experiment for adaptation. Diets were presented to rats in a special non-scattering feeding cups to avoid loss of food and contamination. Water was provided *ad libitum* via a narrow mouth bottle with a metalic tube tightly fixed at its mouth by a piece of rubber tube. Animals were subjected to a 12 hours light and 12 hours dark schedule and kept for 7 days before the start of the experiment for acclimatization.

**Preparation of plant extracts:**

1- Milk thistle and Burdock were ground using porcelain grinder to pass through sieve mesh pores of 1mm diameter.

2- 25g sample of milk thistle + 500ml distilled water and 10g sample of burdock + 500ml distilled water were kept in 2 conical flasks provided with glass condensers, then heated for one hour at 70 °C.

3- The mixture was cooled and filtered. The filtrate poured in different petri dishes and dried in a fan oven at 70 °C tell dried as a film then crushed and the dried powder has been soluble in distilled water and put in dark bottles to prevent oxidation then saved until the experiment [9].

**Grouping and feeding of rats:**

Rats were fed for one week on basal diet before starting the experiment, then divided into two main groups, the first group (number of rats 5) was fed on the basal diet only as a negative control (C –ve ) normal rats for 45 days. The rats of second main group (number of rats 35) were injected s/c by streptozotocin to induce hyperglycemia .

**Rats were disparted into eight groups (n= 5 rats) as follow:-**

**Group (1)**: Fed on the basal diet only as a negative control (C –ve).

**Group (2)**: kept without any treatment as a positive control (C +ve group) and fed on basal diet for 45 days.

**Group (3)**: Fed on basal diet plus oral feeding of milk thistle extract at a single dose of 250mg/kg B.Wt.

**Group (4):** Fed on basal diet plus oral feeding of milk thistle extract at a single dose of 500mg/kg B.Wt.

**Group (5)**: Fed on basal diet plus oral feeding of burdock extract at a single dose of 250mg/kg B.Wt.

**Group (6)**: Fed on basal diet plus oral feeding of burdock extract at a single dose of 500mg/kg B.Wt.

**Group (7)**: Fed on basal diet plus oral feeding of mix of extracts (milk thistle and burdock ) at a single dose of 250mg/kg B.Wt.

**Group (8)**: Fed on basal diet plus oral feeding of mix of extracts (milk thistle and burdock ) at a single dose of 500mg / kg B.Wt.

**Induction of hyperglycemia in rats:**

Diabetes type II was inducing in normal healthy adult male albino rats by Intra peritoneal injection of 60mg/kg dose of streptozotocin according to the method described by Akbarzadeh [8].

**Blood sampling:**
At the end of the experiment period (45 days) rats were sacrificed by ether an anesthesia. Blood samples were obtained by retro-orbital method in a clean dry centrifuge tube. They were left to clot by standing at room temperature for 20 minutes, and then centrifuged at 1500 r.p.m for 15 minutes. Serum samples were collected by a dry clean syringe, poured in Wisserman tubes and then kept frozen in a refrigerator at -10°C till biochemical analysis. The specimens were fixed in 10% neutral buffered formalin, processed, embedded in paraffin wax, sectioned at 5 µm and stained with Haematoxylin and Eosin (H&E) for light microscopic examination and histopathological investigation according to the method described by Drury and Wallington [10].

**Biological evaluation**
At the end of the experiment period (8 weeks) rats were sacrificed by Diethyl Ether an anesthesia. Blood samples were obtained by retro-orbital method in a clean dry centrifuge tube. They were left to clot by standing at 25 °C for 20 minutes, and then centrifuged at 3000 rpm for 15 minutes. Serum samples were collected by a dry clean syringe, poured in Wisserman tubes and then kept frozen in a refrigerator at -10 °C till biochemical analysis.

During the experiment, all rats were weighed once a week and the consumed diets were recorded everyday (daily food intake). At the end of the experiment, biological evaluation of the experimental diets was carried out by determination of body weight gain (BWG) and feed efficiency ratio (FER) according to Chapman [11] Food intake was also calculated daily.

**Biochemical analysis**
At the end of experimental period, blood samples were collected for serum separation to determine the following parameters: Serum glucose according to Trinder [12] liver enzymes (ALT, AST) according to Reitman and Frankel [13] and ALP according to Roy [14], total protein, albumin and globulin according to Doumas *et al.*, (1973), total cholesterol according to Allain [15], triglycerides according to Jacobs and Van-Denmark [16], lipoprotein fractions (HDLc, LDLc and VLDLc) according to Gordon and Amer [17], creatinine according to Henry [18], urea according to Pattn and Crouch, [19] and uric acid according to Schultz [20].

**Statistical analysis:** The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, statistical soft-ware, SAS Institute , Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups, according to Snedecor and Cochran [21].

## Results

**1. Effect on body weight gain, food intake and feed efficiency ratio**
Data listed in Table (1) show the effect of orally fed with aqueous extract of milk thistle seed and Burdock root on BWG, feed intake (FI) and feed efficiency ratio) in hyperglycemic rats.

It could be noticed for hyperglycemic rats(control +ve) group that body weight gain was 13.710.70 compared to 28.182.64 g/ 45days for (control -ve)group. These results showed that there was significant decrease in BWG for (control +ve) group as compared to (control -ve) group.

All hyperglycemic rats and orally fed with milk thistle and burdock showed a significant increase in body weight gain as compared to(control +ve). Hyperglycemic rats and orally fed with mix. of milk thistle seed and burdock root (500 mg/kg) showed the highest significant increase in body weight gain as compared to all groups.

Concerning FI and FER, there were significant decrease for control +ve when compared to (control -ve), also, mix group at adose of 500 mg/kg showed the highest significant increase when compared to both control +ve and all groups. These findings agreed with **Girish** [22] who supported the use of these active phytochemicals of silymarin against toxic liver injury, which may act by preventing the lipid peroxidation and augmenting the antioxidant defense system or regeneration of hepatocytes. These active phytochemicals may be developed as drugs for the treatment of liver diseases.

**Table 1: Effect of oral feeding with aqueous extract of milk thistle and burdock on BWG, FI and FER in hyperglycemic rats.**

| Parameters / Groups | BWG g/28d | FI g/28d | FER |
|---|---|---|---|
| Control -ve | 28.18 ± 2.64 [a] | 9.70 ± 2.95 [a] | 0.065 ± 0.92 [a] |
| Control +ve | 13.71 ± 0.70 [g] | 7.19 ± 1.83 [d] | 0.042 ± 0.07 [c] |
| Milk thistle . 250 mg/kg | 18.99 ± 3.46 [e] | 7.77 ± 1.56 [c] | 0.054 ± 0.26 [bc] |
| Milk thistle . 500 mg/ kg | 22.13 ± 0.72 [c] | 8.23 ± 1.95 [b] | 0.059 ± 0.77 [b] |
| Burdock .250 mg/ kg | 15.20 ± 2.78 [f] | 7.71 ± 1.53 [cd] | 0.044 ± 0.11 [c] |
| Burdock .500 mg/ kg | 20.87 ± 2.17 [d] | 8.44 ± 1.06 [b] | 0.055 ± 0.16 [bc] |
| Mix. 250 mg/kg | 25.12 ± 1.83 [b] | 9.11 ± 2.24 [ab] | 0.061 ± 0.66 [ab] |
| Mix. 500 mg/kg | 27.93 ± 2.19 [a] | 9.63 ± 2.43 [a] | 0.64 ± 0.64 [a] |

*Values denote arithmetic means ± standard deviation of the means.*
*Means with different letters (a, b, c, d) in the same column differ significantly at p≤0.05.*
*Using one way ANOVA test, while those with similar letters are non-significant.*

**Table 2: Effect of oral feeding with aqueous extract of milk thistle seed and burdock root on glucose in hyperglycemic rats.**

| Parameters<br>Groups | Glucose<br>mg/dl |
|---|---|
| Control –ve | 57.93 ± 2.18 g |
| Control +ve | 185.19 ± 3.85 a |
| Milk thistle . 250 mg/kg | 77.30 ± 2.37 c |
| Milk thistle . 500 mg/ kg | 67.80 ± 3.02 e |
| Burdock .250 mg/ kg | 85.50 ± 2.98 b |
| Burdock .500 mg/ kg | 71.60 ± 1.15 d |
| Mix 250 mg/kg | 63.09 ± 2.13 f |
| Mix 500 mg/kg | 59.31 ± 0.79 g |

*Values denote arithmetic means ± standard deviation of the means.*
*Means with different letters (a, b,c,d) in the same column different significantly at p ≤ 0.05.*
*Using one way ANOVA test, while those with similar letters are non-significant.*

**2. Biochemical analysis**

Data from tables (2-7) show the effect of oral feeding with milk thistle, burdock and mix of them at two doses (250 or 500 mg/ kg b. wt.) on blood glucose liver enzymes (AST, ALT and ALP), total protein(T.Protein), albumin(Alb.) & globulin (Glob.), serum lipid profile (total cholesterol, triglycerides, total lipids), serum levels of lipoprotein fractions (HDLc, LDLc and VLDLc), and kidney functions (Creatinine, urea and uric acid) in hyperglycemic rats.

**A. Effect on serum glucose**

Effect of oral feeding with milk thistle and burdock on serum glucose in rats with hyperglycemia is illustrated in Table 2.

It could be observed for hyperglycemic rats(control +ve) group that serum glucose was 185.193.85 compared to 57.932.18 mg/ dl for (control -ve)group. These results showed that there was significant increase in serum glucose for (control +ve) group as compared to (control -ve) group.

All hyperglycemic rats and orally fed with milk thistle and burdock showed significant decrease in serum glucose when compared to(control +ve). Hyperglycemic rats and orally fed with mix. of milk thistle seed and burdock root (500 mg/ kg) showed the highest significant decrease in serum glucose as compared to all groups and no significant differences as compared to control –ve group.

Our results confirmed by Akbarzadeh [8] who found that streptozotocin makes pancreas swell and at last causes degeneration in Langerhans islet beta cells and induces experimental diabetes. It also changes normal metabolism in diabetic rats in comparison with normal rats. Consumption of water and food, volume of urine, serum glucose increases in diabetic animals in comparison with normal rats but the levels of serum insulin, C-peptide and body weight decreases. In this respect, Lapinina and Sisoeva [23] mentioned that burdock extracts have demonstrated hypoglycemic activity in rats. Moreover, Bever and Zahnd [24] who found that burdock have been speculated to lower blood glucose levels in humans**.** Meanwhile, [4] mentioned that milk thistle extract is made from the seeds, contains approximately 4-6% silymarin. The extract consists of about 65-80% silymarin (a flavonolignan complex) and 20-35% fatty acids, including linoleic acid. Silymarin is a complex mixture of polyphenolic molecules, including seven closely related flavonolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin) and one flavonoid (taxifolin). Silibinin, a semipurified fraction of silymarin, is primarily a mixture of 2 diasteroisomers, silybin A and silybin B, in a roughly 1: 1 ratio. In clinical trials silymarin has typically been administered in amounts ranging from 420–480 mg per day in two to three divided doses. However higher doses have been studied, such as 600 mg daily in the treatment of type II diabetes and 600 or 1200 mg daily in patients chronically infected with hepatitis C virus.

**B. Effect on AST, ALT and ALP**

Effect of oral feeding with milk thistle and burdock on AST, ALT and ALP in rats with hyperglycemia is recorded in Table 3.

**Table 3: Effect of oral feeding with aqueous extract of milk thistle seed and burdock root on AST, ALT and ALP in hyperglycemic rats.**

| Parameters<br>Groups | AST<br>U/L | ALT<br>U/L | ALP<br>U/L |
|---|---|---|---|
| Control –ve | 36.00 ± 1.98 f | 44.00 ± 1.73 f | 119.12 ± 2.03 e |
| Control +ve | 76.00 ± 2.98 a | 65.00 ± 3.00 a | 243.37 ± 2.75 a |
| Milk thistle . 250 mg/kg | 67.30 ± 2.07 bc | 61.50 ± 2.18 bc | 234.57 ± 2.15 b |
| Milk thistle . 500 mg/ kg | 53.80 ± 2.03 d | 53.13 ± 3.04 d | 211.04 ± 2.31 c |
| Burdock .250 mg/ kg | 69.50 ± 1.18 b | 62.50 ± 2.29 b | 236.12 ± 1.76 b |
| Burdock .500 mg/ kg | 61.60 ± 2.31 c | 58.30 ± 2.64 c | 215.43 ± 3.25 c |
| Mix. 250 mg/kg | 41.00 ± 2.12 e | 50.70 ± 2.38 e | 198.17 ± 3.31 d |
| Mix. 500 mg/kg | 34.09 ± 1.73 f | 43.79 ± 2.52 f | 193.74 ± 2.25 d |

*Values denote arithmetic means ± standard deviation of the means.*
*Means with different letters (a, b,c,d) in the same column different significantly at p ≤ 0.05.*
*Using one way ANOVA test, while those with similar letters are non-significant.*

It is clear from table (3) that hyperglycemic rats showed significant increase in AST, ALT and ALP as compared to control -ve group. All hyperglycemic rats and orally fed with mix of *milk thistle* and *burdock at a dose of* 500 mg/kg showed a highest significant decrease in AST and Alt as compared to either control +ve or all treated groups. Meanwhile, the

treated groups which orally fed with mix of *milk thistle* and *burdock at doses of 250* and 500 mg/kg showed a highest significant decrease in ALP as compared to neither control +ve nor all treated groups.

These results supported by Wagoner [25] who demonstrated that silymarin, an extract from milk thistle (*Silybum marianum*), and its purified flavonolignans have been recently shown to inhibit hepatitis C virus (HCV) infection, both in vitro and in vivo. The mechanisms of silymarin's antiviral action appear to include blocking of virus entry and transmission, possibly by targeting the host cell. Moreover, *Jain* [26] investigated the effects of silymarin and naringenin in counteracting arsenic-induced hepatic oxidative stress post exposure. their results point to the antioxidant potential of these flavonoids, which might be of benefit in the clinical recovery of subject exposed to arsenic. These flavonoids can be incorporated into the diet or co-supplemented during chelation treatment, and thus may afford a protective effect against arsenite-induced cytotoxicity.
These data confirmed by Ahmed [27] who examined the possible hepatoprotective effect of aminoguanidine in comparison with silymarin and investigated the possible beneficial effects of the combination of aminoguanidine and silymarin on liver fibrosis. It is concluded that aminoguanidine has protective effect against hepatoxicity via its iNOS inhibition and antioxidant effects. In addition, the combination of AG with silymarin has more potent hepatoprotective effect than each drug alone.

**C. Effect on T. Protein, Albumin and Globulin**

Data illustrated in table (4) show the effect of oral feeding with milk thistle and burdock on total protein (T. Protein), albumin (Alb.) and globulin(Glob.).in hyperglycemic rats.

It is clear from the table that hyperglycemic rats without treatment(control +ve ) showed significant increase in total protein and globulin as compared to control -ve group. Hyperglycemic rats and orally fed with mix of *milk thistle* and *burdock at a dose of* 500 mg/kg showed a highest significant decrease in total protein and globulin as compared to either control +ve or all treated groups. On the other H and, the group of *milk thistle* and *burdock* mix *at a dose of* 500 mg/kg showed a highest significant increase in albumin as compared to either control +ve or all treated groups and showed no significant changes when compared to normal rats.

These results were in agreement with Zick [28] concluded that breast cancer is a major cause of morbidity, mortality, and medical expenditures among women in Canada. Essiac (Resperin Canada Limited, Waterloo, Ontario, Canada), a blend of at least four herbs (burdock root [*Arctium lappa*], Indian rhubarb [*Rheum palmatum*], and the inner bark of slippery elm [Ulmus fulva or U. rubra]), has become one of the more popular herbal remedies for breast-cancer treatment, secondary prevention, improving quality of life, and controlling negative side-effects of conventional breast-cancer treatment.

**D. Effect on Total Lipids, cholesterol and Tri Glycerides**

Data present in table (6) show the effect oral feeding with Milk thistle and burdock on T. Lipids, T. cholesterol and T. Glycerides in hyperglycemic rats.

It is observed that hyperglycemic rats without treatment(control +ve ) showed significant increase in total lipids, total cholesterol and tri glycerides as compared to control -ve group. Hyperglycemic rats and orally fed with mix of *milk thistle* and *burdock at a dose of* 500 mg/kg showed a highest significant decrease in T. Lipids, T. cholesterol and T. Glycerides as compared to either control +ve or all treated groups. These findings were in agreement with those of Muriel and Mourelle [29] who studied the effect of silymarin on liver lipid peroxidation and membrane lipid alterations. Their results indicate that silymarin can protect against the alterations in liver plasma membrane through its antioxidant properties by modifying the plasma membrane phospholipid content. In this concern Lin [30] conducted that *Arctium lappa,* L. (root) has anti-inflammatory and free radical scavenger activity. They suggested that *Arctium lappa* possess free radical scavenging activity.

**E. Effect on HDL, LDL and VLDL**

Data illustrated in table (7) show the effect of oral feeding with milk thistle and burdock on HDL, LDL and VLDL in hyperglycemic rats.

These data showed that control +ve group showed significant decrease in HDL and significant decrease in LDL and VLDL as compared to control -ve group. Hyperglycemic rats and orally fed with *milk thistle at a dose of* 500 mg/kg showed a highest significant increase in HDL as compared to all groups. Rats and orally fed with *milk thistle and burdock at the same dose of* 500 mg/kg showed a highest significant decrease as compared to control

**Table 4: Effect of oral feeding with aqueous extract of *milk thistle* seed and burdock root on T. Protein, albumin and globulin in hyperglycemic rats.**

| Parameters<br>Groups | T. Prot.<br>g/dl | Alb.<br>g/dl | Glob.<br>g/dl |
|---|---|---|---|
| Control –ve | 7.30 ± 0.53b | 3.80 ± 0.58 a | 3.50 ± 0.29 d |
| Control +ve | 10.10 ± 0.65 a | 2.80 ± 0.08 c | 7.30 ± 0.38 a |
| Milk thistle . 250 mg/kg | 6.70 ± 0.33 d | 3.40 ± 0.77 ab | 3.30 ± 0.54 d |
| Milk thistle . 500 mg/ kg | 6.90 ± 0.42 cd | 3.50 ± 0.80 ab | 3.40 ± 0.51 d |
| Burdock .250 mg/ kg | 8.50 ± 0.17 b | 3.20 ± 0.57 ab | 5.30 ± 0.52 b |
| Burdock .500 mg/ kg | 7.20 ± 0.03 c | 3.40 ± 0.83 b | 3.80 ± 0.29 cd |
| Mix 250 mg/kg | 7.70 ± 0.34 bc | 3.30 ± 0.53 b | 4.40 ± 0.51 c |
| Mix 500 mg/kg | 8.5 ± 0.17 a | 3.7 ± 0.03 a | 4.80 ± 0.19 bc |

*Values denote arithmetic means ± standard deviation of the means.*
*Means with different letters (a, b,c,d) in the same column different significantly at p ≤ 0.05.*
*Using one way ANOVA test, while those with similar letters are non-significant.*

**Table 5: Effect of oral feeding with *aqueous extract of milk thistle* seed and burdock root on T. Lipids, T. cholesterol and T. Glycerides in hyperglycemic rats.**

| Parameters<br>Groups | T. lipids g/dl | T. cholesterol g/dl | T. Glyceride g/dl |
|---|---|---|---|
| Control –ve | 214 ± 3.61 g | 101.1 ± 2.05 f | 73 ± 2.65 e |
| Control +ve | 584 ± 3.46 a | 146 ± 2.64 a | 213 ± 2.57 a |
| Milk thistle . 250 mg/kg | 515.5 ± 3.12 b | 118.8 ± 2.43 c | 98.4 ± 3.07 b |
| Milk thistle . 500 mg/ kg | 500 ± 2.13 c | 139.3 ± 2.04 b | 83.9 ± 2.64 c |
| Burdock .250 mg/ kg | 460 ± 2.11 d | 115 ± 2.65 cd | 97.2 ± 3.19 b |
| Burdock .500 mg/ kg | 335.2 ± 2.03 e | 113.8 ± 2.34 cd | 85.2 ± 3.02 c |
| Mix 250 mg/kg | 528 ± 2.64 b | 132 ± 2.57 bc | 81.4 ± 2.51 cd |
| Mix 500 mg/kg | 259 ± 2.57 f | 109.4 ± 2.33 f | 74.8 ± 3.02 e |

*Values denote arithmetic means ± standard deviation of the means.*
*Means with different letters (a, b,c,d) in the same column different significantly at p ≤ 0.05.*
*Using one way ANOVA test, while those with similar letters are non-significant.*

**Table 6: Effect of oral feeding with aqueous extract of milk thistle seed and burdock root on HDL, LDL and VLDL in hyperglycemic rats.**

| Parameters<br>Groups | HDL Mg/dl | LDL Mg/dl | VLDL Mg/dl |
|---|---|---|---|
| Control -ve | 68.40 ± 1.51 c | 18.20 ± 2.03 d | 14.60 ± 1.51 e |
| Control +ve | 61.50 ± 2.10 d | 41.80 ± 2.89 a | 42.60 ± 2.26 a |
| Milk thistle . 250 mg/kg | 72.60 ± 2.51 b | 26.50 ± 2.18 c | 19.68 ± 2.37 c |
| Milk thistle . 500 mg/ kg | 95.60 ± 2.25 a | 18.70 ± 2.86 d | 25.00 ± 3.46 b |
| Burdock .250 mg/ kg | 67.50 ± 2.50 c | 31.20 ± 3.30 b | 16.24 ± 1.65 d |
| Burdock .500 mg/ kg | 78.20 ± 2.79 b | 17.70 ± 2.56 d | 17.04 ± 1.69 d |
| Mix 250 mg/kg | 77.80 ± 2.43 b | 34.23 ± 2.81 b | 20.08 ± 2.69 c |
| Mix 500 mg/kg | 81.80 ± 2.44 b | 28.43 ± 2.88 cd | 23.96 ± 1.09 bc |

*Values denote arithmetic means ± standard deviation of the means.*
*Means with different letters (a, b,c,d) in the same column different significantly at p ≤ 0.05.*
*Using one way ANOVA test, while those with similar letters are non-significant.*

**Table 7: Effect of oral feeding with *aqueous extract of milk thistle* seed and burdock root on creatinine, urea and uric acid in hyperglycemic rats.**

| Parameters<br>Groups | Creatinine mg/dl | Urea mg/dl | U. Acid mg/dl |
|---|---|---|---|
| Control -ve | 0.60 ± 0.01b | 56.40 ± 1.83 b | 2.10 ± 0.02 bc |
| Control +ve | 1.50 ± 0.3 a | 69.00 ± 2.65 a | 5.40 ± 0.08 a |
| Milk thistle 250 mg/kg | 0.66 ± 0.05 ab | 53.40 ± 2.04 b | 2.20 ± 0.05 bc |
| Milk thistle 500 mg/ kg | 0.86 ± 0.08 ab | 54.20 ± 1.96 b | 3.60 ± 0.09 b |
| Burdock 250 mg/ kg | 0.36 ± 0.03 c | 43.00 ± 2.57 d | 1.90 ± 0.01 c |
| Burdock 500 mg/ kg | 0.58 ± 0.02 b | 48.00 ± 3.00 c | 3.50 ± 0.02 b |
| Mix 250 mg/kg | 0.54 ± 0.02 b | 52.20 ± 2.69 bc | 2.20 ± 0.07 bc |
| Mix 500 mg/kg | 0.38 ± 0.03 c | 55.4 ± 2.65 b | 1.03 ± 0.03 b |

*Values denote arithmetic means ± standard deviation of the means.*
*Means with different letters (a, b,c,d) in the same column different significantly at p ≤ 0.05.*
*Using one way ANOVA test, while those with similar letters are non-significant.*

positive group. Meanwhile, groups of burdock at two doses 250 and 500mg/dl showed the lowest significant decrease in VLDL as compared to neither control positive nor all tested groups. These findings agreed with Flora [3] who demonstrated that *silymarin*, derived from the Milk thistle plant, *Silybum marianum*, has been used for centuries as a natural remedy for diseases of the liver and biliary tract. As interest in alternative therapy has emerged in the United States, gastroenterologists have encountered increasing numbers of patients taking silymarin with little understanding of its purported properties. Silymarin and its active constituent, silybin, have been reported to work as antioxidants scavenging free radicals and inhibiting lipid peroxidation. Studies also suggested that they protect against genomic injury, increase hepatocyte protein synthesis, decrease the activity of tumor promoters, stabilize mast cells, chelate iron, and slow calcium metabolism. Also Cetinkaya [31] investigated the aqueous extract from the roots of *Rumex patientia* L. (Polygonaceae) for its effects on rat liver and erythrocyte antioxidant enzyme systems and lipid peroxidation. Measurements of the GSH-Px, SOD and CAT activities, and MDA levels of liver and erythrocytes in D-1 administered animals showed that there was an increase in GSH-Px and SOD activities when compared to that of controls. No significant decrease was observed in catalase activity and no changes in malondialdehyde levels were observed.

**F. Effect on creatinine, urea and uric acid**

Table (7) show the effect of oral feeding with Milk thistle and burdock on creatinine , urea and uric acid in hyperglycemic rats

Data illustrated in the table showed that hyperglycemic rats without treatment(control +ve ) showed significant increase in creatinine, urea and uric acid as compared to control -ve group. All hyperglycemic rats and orally fed with all doses and mix of *milk thistle* and *burdock* showed significant decrease in creatinine, urea and uric acid when compared to control +ve. Rats orally orally fed with burdock root 250mg/kg and mix 500mg/kg showed the highest significant decrease in creatinine as compared to either control +ve group or control -ve group. Concerning creatinine, there were a significant increase in control +ve group as compared to control –ve group. All groups showed with significant decrease when compared to control +ve group except milk thistle seed 500mg/kg. Regarding uric acid, there were non-significant

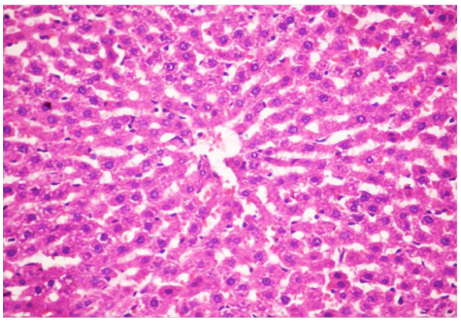

Photo (1): Liver tissue of control -ve rat revealed the normal histological structure of hepatic lobule. (H and E X200).

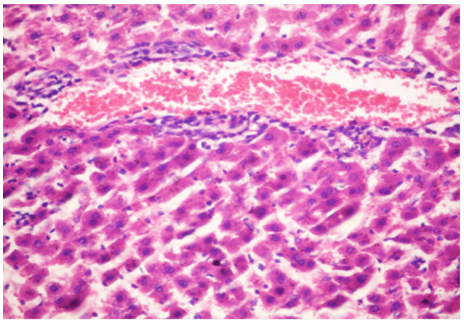

Photo (2): Liver tissue of control +ve rat focal hepatic necrosis associated with leucocytic cells infiltration congestion of hepatoportal blood vessels and portal infiltration with leucocytes as well as necrosis of hepatocytes (H and E X200).

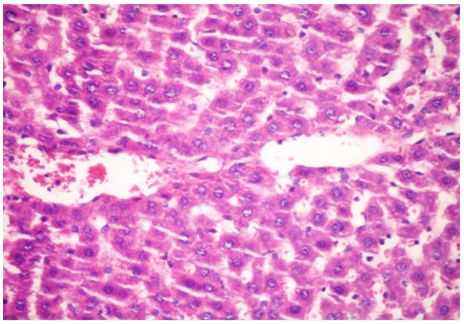

Photo (3): Liver tissue of group 3 revealed normal hepatic lobule was noticed in liver. (H and E X200).

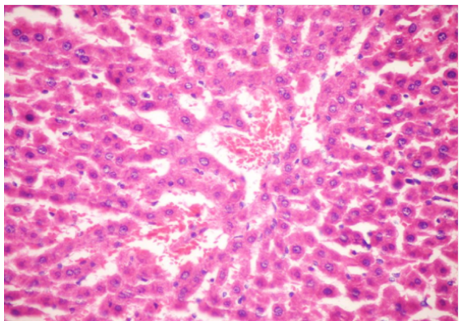

Photo (4): Liver tissue of group 4 revealed dilatation of hepatic sinusoids which was the only change observed. (H and E X200).

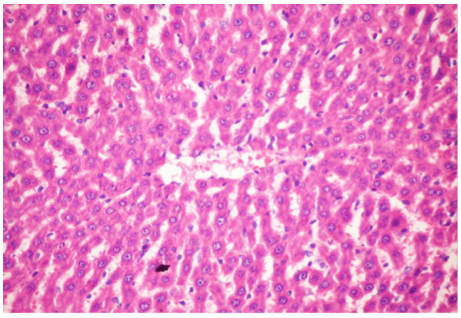

Photo (5): Liver tissue of group 5 revealed dilatation and congestion of central vein as well as binucleated hepatocytes. (H and E X200).

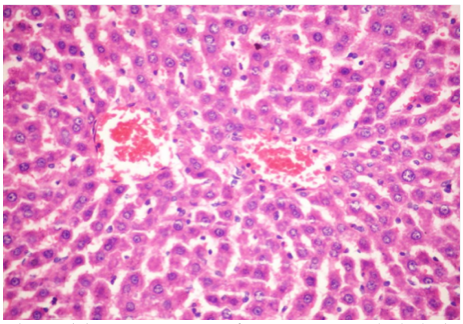

Photo (6): Liver tissue of group 6 revealed slight congestion of central veins whereas, other sections revealed kupffer cells activation and portal infiltration with leucocytes as well as focal hepatic necrosis (H and E X200).

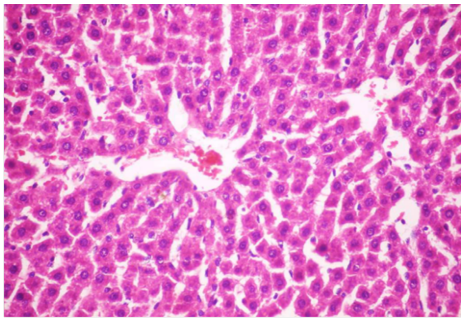

Photo (7): Liver tissue of group 7 revealed slight dilatation of central vein and hepatic sinusoids as well as focal hepatic necrosis associated with leucocytes cells infiltration.(H and E X200).

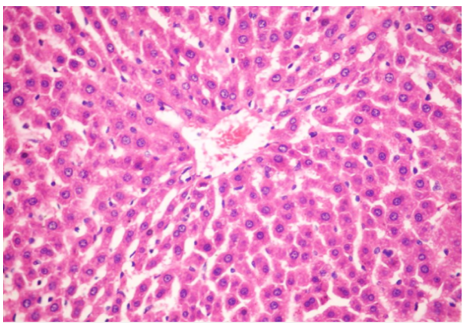

Photo (8): Liver tissue of group 8 revealed no hhistopathological changes. (H and E X200).

differences between control +ve group and control –ve group. Also, there were non-significant changes between all groups when compared to control +ve group. These data agreed with Bown [5] who mentioned some folk herbalists which consider dried burdock to be a diuretic, diaphoretic, and a blood purifying agent. The seeds of A. lappa are used in traditional Chinese medicine, under the name niupangzi; pinyin: niúpángzi; Some dictionaries list the Chinese as just niúbàng. Also Karimi [32] studied the protective effect of methanolic extract of Milk thistle seeds and silymarin against cisplatin-induced renal toxicity. They found that Milk thistle may protect against cisplatin-induced renal toxicity and might serve as a novel combination agent with cisplatin to limit renal injury.

**Histopathological results**

**A. Liver**

Microscopically, liver of rat from group 1 which revealed the normal histological structure of hepatic lobule (photo 1). Mean while, liver of rat from group 2 showed focal hepatic necrosis associated with leucocytic cells infiltration, congestion of hepatoportal blood vessels and portal infiltration with leucocytes as well as necrosis of hepatocytes (photo 2). Apparent normal hepatic lobule was noticed in liver from group 3 (photo 3). Moreover, dilatation of hepatic sinusoids (photo 4) was the only change observed in-group 4. However, liver of rat from group 5 revealed dilatation and congestion of central vein (photo 5) as well as binucleated heptocytes. Some examined sections from group 6 showed slight congestion of central veins whereas, other sections revealed kupffer cells activation and portal infiltration with leucoautes as well as focal hepatic necrosis (photo 6). Moreover, liver of rat from group7 revealed slight dilatation of central vein and hepatic sinusoids as well as focal hepatic necrosis associated with leucocytic cells infiltration (photo 7). Meanwhile, liver rat from groups 8 revealed no histopathological changes (photo 8) the same occur in [33].

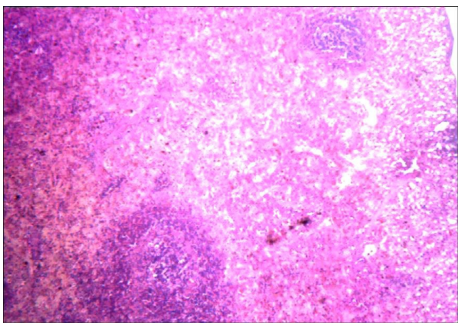

Photo (9): Spleen tissue of control -ve rat fed on basal diet only showed normal splenic histology. (H and E X200).

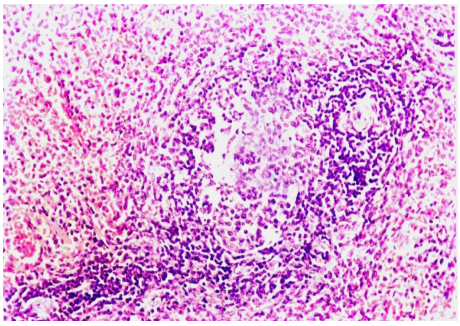

Photo (10): Spleen of control +ve rat fed on basal diet only showed revealed lymphocytic necrosis.(H and E X200).

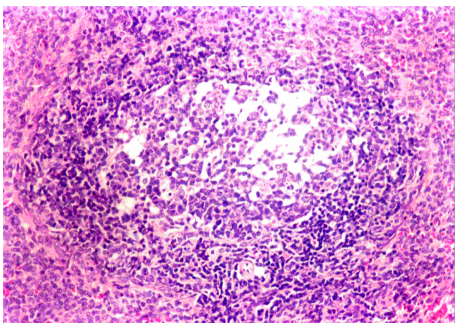

Photo (11): Splenic sections from rat from group 3 (milk thistle extract at a single dose of 250mg/kg B.Wt.) revealed little lymphocytic necrosis. (H and E X200).

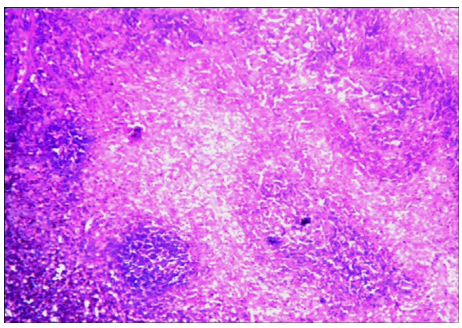

Photo (12): Splenic tissue from rat from group 4 (milk thistle extract at a single dose of 500mg/kg B.Wt.)showed focal lymphocytic necrosis. (H and E X200).

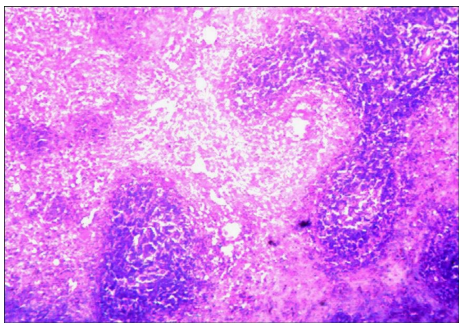

Photo (13): Splenic sections from from rat from group 5 (burdock extract at a single dose of 250mg/kg B.Wt.) revealed focal lymphocytic necrosis (H and E X200).

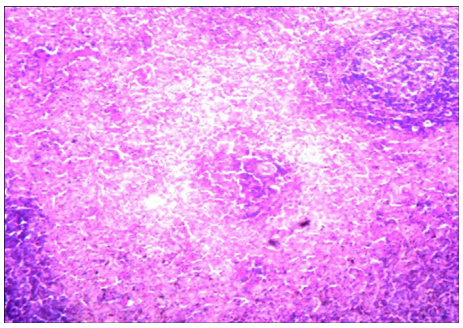

Photo (14): Splenic tissue from rat from group 6 (burdock extract at a single dose of 500mg/kg B.Wt.) showed normal splenic histology (H and E X200).

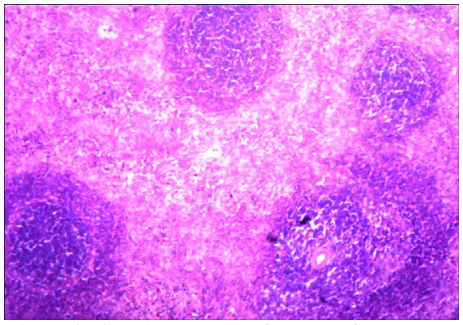

Photo (15): splenic tissue from rat from group 8 (mix of extracts (milk thistle and burdock at a dose of 250mg/kg B.Wt.) showed normal splenic histology. (H and E X200).

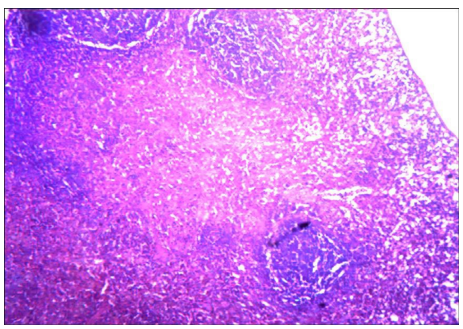

Photo (16): Splenic tissue from rat from group 9 (mix of extracts (milk thistle and burdock at a dose of 250mg/kg B.Wt.) showed normal splenic histology. (H and E X200).

**B. Spleen**

Microscopically, spleen of rat from group 1 which showed normal splenic histology (photo 9). Moreover, spleen of rat from group 2 which revealed lymphocytic necrosis (photo 10). Meanwhile, spleen of rat from group 3 which revealed little lymphocytic necrosis (photo 11). However, spleen of rat from group 4 which showed focal lymphocytic necrosis (photo 12). Also, spleen of rat from group 5 which revealed focal lymphocytic necrosis (photo 13). In the same time, spleen of rat from group 6 which revealed normal splenic histology (photo 14). Moreover, spleen of rat from group 7 which showed normal splenic histology (photo 15). Also, spleen of rat from group 8 which showed normal splenic histology (photo 16). the same occur in [33,34].

## Conclusion

Milk thistle and burdock could be used for hyperglycemia and impaired liver and kidney function by using two doses (250 and 500 mg/kg of milk thistle, burdock , and mixture of them).

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