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## ***Taraxacum officinale* and *Silybum marianum* alone or combined orchestrate experimentally induced hepatic steatosis through lipogenicity, glucose tolerance and oxidant/antioxidant status**

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### **ABSTRACT**

Natural products with a long history of safety can modulate obesity. *Taraxacum officinale*, known as (dandelion) and *Silybum marianum* known as (milk thistle) have garnered attention for their antioxidant and antiobesity activities. The present study was conducted to evaluate the potential role of dandelion and milk thistle alone or combined against high fat diet (HFD) induced steatohepatitis. 60 male albino rats which were equally subdivided into four groups: group I was received only HFD, other groups (II, III, IV) were received dandelion, milk thistle or dandelion/milk thistle combination respectively for 8 weeks alongside HFD. Insulin resistance, glucose tolerance, lipogenesis and antioxidant capacity were evaluated in the liver tissue. HFD fed rats exhibited increased insulin resistance-related biomarkers, H<sub>2</sub>O<sub>2</sub> level, mRNA expression of sterol regulatory element-binding protein-1c (SREBP-1c) as well as fatty acid synthase (FAS) activity with decreased reduced glutathione (GSH) level. Herbal supplementation improved those results with best results were for dandelion/milk thistle combination group. Results were confirmed with histopathological examination. Both dandelion and milk thistle alone or combined improved glucose tolerance and insulin sensitivity, decreased lipogenesis and increased antioxidant capacity with best results obtained in dandelion/milk thistle combination group, implying a potential application in the treatment of hepatic steatosis associated obesity.

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**Keywords:** High fat diet, obesity, dandelion, milk thistle, lipogenesis, insulin resistance.

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### **INTRODUCTION**

The worldwide incidence of obesity has increased dramatically during recent decades (Holvoet, 2012). By 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese according to the World Health Organization projections (Holvoet, 2012). Obesity is associated with a high incidence of steatosis, insulin resistance and chronic inflammation (Ismail, 2011).

Obesity-related non-alcoholic fatty liver disease (NAFLD) has recently been recognized as one of the major causes of chronic liver disorders, estimated to affect at least one-quarter of the general population (Ismail, 2011). NAFLD is characterized firstly by excess liver lipid accumulation with insulin resistance and later hepatic inflammation, leading to nonalcoholic steatohepatitis (NASH) with subsequent hepatic fibrosis or

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cirrhosis (Abenavoli, 2015). One of the major causes of steatohepatitis is the inability of the liver to regulate the changes in lipogenesis which may result from either increased triacylglycerol (TAG) synthesis or decreased fatty acid oxidation, both leading to increased TAG content in the liver (Tacer and Rozman, 2011). Fatty acid synthase (FAS) is one of the key enzymes of lipogenesis (Tacer and Rozman, 2011). Excess lipogenesis with subsequent fat accumulation worsening hepatic insulin resistance via a network of transcription factors, which regulate hepatic lipogenesis and fatty acid oxidation, including sterol regulatory element-binding protein-1c (SREBP-1c) (Berlanga et al., 2014).

The inflammatory response and increased oxidative stress associated obesity involves many components of the classical inflammatory response and increases in inflammatory cytokines with subsequent insulin resistance, dyslipidemia and elevated levels of oxidized low density lipoprotein (ox-LDL) (Iantorno et al., 2014).

*Taraxacum officinale*, known as dandelion, has been used in folk medicine in the treatment of hepatic disorders and several women's diseases such as breast and uterine cancers and has choleric, diuretic, anti-rheumatic and anti-inflammatory properties (Choi et al., 2010). Several flavonoids including caffeic acid, chlorogenic acid, luteolin, and luteolin 7-glucoside have been isolated from the dandelion also it is a rich source of vitamins A, B complex, C, and D, as well as minerals such as iron, potassium and zinc (Choi et al., 2010).

*Silybum marianum* (Milk thistle) has a long and important history in herbal medicine, silymarin is the active extract of milk thistle (Kazasis et al., 2014). It is the most ancient and extensively used medicinal plant for its beneficial effects on liver and other organs; it was primarily recognized for its capacity to enhance bile flow and to remove obstructions from liver and spleen (Kazasis et al., 2014). The present study was designed to explore the beneficial effects of dandelion and milk thistle alone or together on biochemical and

molecular changes associated obesity in order to represent a novel approach for treating steatohepatitis associated obesity.

## MATERIALS AND METHODS

This current work was carried out at Medical Biochemistry Department, in accordance to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) to minimize animal suffering and in accordance to the guidelines of the Ethical Committee of Medical Research, Faculty of Medicine, Tanta University, Egypt.

### Herbal supplementation

Both dandelion and milk thistle whole plant extracts were prepared and supplied in the form of powder by Egyfarma, Egypt.

### Animal modeling and grouping

The present study was carried out on 60 male albino rats, aged 3 months, and weighted 80-120 g. During the study, the animals were kept in wire mesh cages with *ad-libitum* access to water. The room temperature was about 22-24 °C and the animals were exposed to 12:12 hours light dark cycles. All animals were given two weeks to acclimate to laboratory conditions, during that time they were maintained on *ad-libitum* laboratory chow and water. The animals were then divided into four equal groups each of 15 rats as follow: obese group (group I) was fed a HFD (53.15%, 19.68% and 27.17% of energy from fat, protein and carbohydrate, respectively) which served as control group receiving distilled water using a gavage via intubation daily, dandelion treated group (group II) was fed HFD alongside dandelion (dandelion in a dose of 300 mg/kg/day orally dissolved in distilled water using a gavage via intubation) (Nnamdi Chinaka et al., 2012); milk thistle treated group (group III) was fed HFD alongside milk thistle (milk thistle in a dose of 200 mg/kg/day orally dissolved in distilled water using a gavage via intubation) (Haddad et al., 2011); dandelion/milk thistle combination treated group (group IV) was fed HFD alongside both dandelion and milk

thistle (Nnamdi Chinaka et al., 2012; Haddad et al., 2011). Obesity was induced in 8 weeks (Prieto-Hontoria et al., 2009) in which all groups received HFD or HFD alongside treatment. Composition of the HFD (g/kg diet) was according to the formula of Dourmashkin et al. (2006).

### **Experimental procedure**

The body weight gain was measured every one week. By the end of the experimental period, fasting rats (12 hours) were anaesthetized by ether, and while the heart was still beating, blood was collected and serum was separated. The abdomen was opened liver was taken, cleared of the adhering fat, weighed, washed by ice cold saline, dried by filter paper, and weighed then divided into specimens for preservation in 10% phosphate buffered formalin solution for histopathological examination using hematoxylin and eosin (H.&E.) and the other remaining liver tissues were divided into small pieces and underwent homogenization in phosphate buffer saline (PBS) 50 mM, pH 7.4. Supernatant of liver tissue homogenate, sera and remaining liver tissues were frozen at -80 °C for further evaluations.

### **Biochemical analysis of serum**

Serum aspartate transaminase (AST) and alanine transaminase (ALT) activities were determined using commercial diagnostics kits supplied by (Elitech, France). Fasting Blood Sugar (FBS) was measured by the oxidase method by supplied commercial kit (Biodiagnostic., Egypt), total Lipid profile including total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) levels were measured by enzymatic-colorimetric methods by supplied commercial kits (Biodiagnostic., Egypt). Enzyme linked immunosorbent assay (ELISA) was used to detect serum levels of insulin (USCN Life Science Inc, Wuhan, China) and oxidized-LDL (ox-LDL) (Cell Biolab, Inc., USA) using ELISA Reader (Star fax 2001). Insulin resistance was assessed by the homeostatic model assessment of insulin

resistance (HOMA-IR) (Duseja et al., 2007) calculated as: Fasting blood glucose level (mg/dl) \* fasting insulin level (μIU/mL)/405.

### **Biochemical analysis of liver tissue homogenate**

#### ***Assay hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) level***

By commercial kits supplied by Biodiagnostic, Egypt. In the presence of horse radish peroxidase (HRP), H<sub>2</sub>O<sub>2</sub> reacts with 3, 5-dichloro-2-hydroxybenzenesulfonic (DHBS) acid and 4-aminophenazone (AAP) to form a chromophore absorbance of the sample and absorbance of standard were read against blank at 510 nm using Biosystem spectrophotometer (BTS 350 semiautomatic analyzer) (Fossati et al., 1980; Aebi, 1984)

#### ***Assay of reduced glutathione (GSH) level***

By commercial kits supplied by Biodiagnostic, Egypt. The reduction of 2-nitrobenzoic acid (DTNB) with reduced glutathione (GSH) produced a yellow compound, the reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 (Ellman, 1959).

#### ***Assay of fatty acid synthase (FAS) activity***

Using a protocol as previously described (Nepokroeff et al., 1975), briefly, an aliquot of supernatant (50 μl) was pre-incubated with a buffer containing 200 mM potassium phosphate, pH 6.6, 1 mM DTT, 1 mM EDTA, 0.24 mM NADPH and 30 μM acetyl-CoA in a final volume of 0.2 ml and the reaction was monitored at 340 nm for 3 min to measure background NADPH oxidation. After the addition of 50 μM of malonyl-CoA, the reaction was assayed for an additional 15 min to determine FAS-dependent oxidation of NADPH. The rate of optical density (OD) at 340nm change was corrected for the background rate of NADPH oxidation.

#### ***Assay Protein content***

According to Lowry et al. (1951) in which a standard curve of bovine serum albumin (from 0 to 1000 μg /ml of PBS) concentration was plotted, and the protein content of the unknown samples was measured at wave length 750 nm.

### **Liver TAG content**

Excessive accumulation of TAG in the liver is the hallmark of NAFLD. For hepatic TAG level, 100 mg of liver tissue was homogenized for extraction of lipid according to the method of Folch et al. (1957). Level of TAG was then quantified according to the manufacturer's procedures with commercial assay kit (Biodiagnostic., Egypt).

### **Estimation of SREBP-1c mRNA level in liver tissue**

The frozen liver samples were processed for total RNA extraction (MagNA Pure compact Nucleic Acid isolation kit I, Roche Diagnostics, GmbH, Mannheim, Germany) according to the manufacturer's recommendations. Total RNA was treated with DNase I to eliminate genomic DNA contamination, followed by synthesis of the first strand using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. SREBP-1c mRNA transcripts were quantified, relative to the house-keeping gene; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the Roche LightCycler® FastStart DNA MasterPLUSYBR Green I kits (Roche Diagnostics, Mannheim, Germany) following manufacturer's instructions. Sequence specific primers were designed by Primer3 software: (<http://bioinfo.ut.ee/primer3/>) as follows: SREBP-1c (No: NM\_001276707.1) (sense 5'-GGA GCC ATG GAT TGC ACA TT -3'), SREBP-1c (antisense 5'- CCT GTC TCA CCC CCA GCA TA -3'), GAPDH (No: NM\_017008.4) (sense 5'- GGT GAA GTT CGG AGT CAA CGG A- -3'), GAPDH (antisense 5'- GAG GGA TCT CGC TCC TGG AAG A -3'). PCR reaction was performed following the cycling protocol of 95 °C for 5 min, followed by 45 PCR cycles with 95 °C for 5 s, 58 °C for 15 s and 72 °C for 20 s. The values of the target gene under investigation and the value of the house-keeping gene (GAPDH) were calculated for each sample using standard curve. The final results were automatically calculated from the (Cp = crossing point) values of the target and

the reference genes by LightCycler® 4.0 Relative Quantification Software.

### **Histopathological study**

The liver tissue was visualized by hematoxylin and eosin (H.&E.). staining. The sections of liver were fixed in 10% formalin, dehydrated, embedded in paraffin and stained with H.&E. then photographed.

### **Statistical analysis**

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation by SPSS version.16. Analysis of variance (ANOVA) and Tukey's test were used to determine the significance between more than 2 groups: according to the computer program SPSS for Windows. P value < 0.05 was considered significant.

## **RESULTS**

### **Effect of dandelion, milk thistle and dandelion/milk thistle combination on body weight gain, total liver weight**

The body weight of the HFD control group (group I) was significantly higher than that of treated groups, after 8 weeks of feeding and remained significantly higher during the whole experiment (P<0.05), group II, group III and group IV had significantly lower body weight than group I and remained significantly lower during the experiment with significant lower results in group IV (P<0.05), there was no statistically significant difference between groups II and III (Table 1). The liver weight in group I rats was significantly increased when compared to those of treated groups (II, III and IV) (P<0.05), there was no statistically significant difference between groups II and III (Table 2).

### **Dandelion, milk thistle and dandelion/milk thistle combination improved the serum and hepatic lipid profiles as well as hepatic enzymes**

Rats of group I had significantly higher TAG, TC, LDL-C and ox-LDL levels than treated groups while HDL-C showed the opposite (P<0.05) (Table 2), however, dandelion/milk thistle combination group

(group IV) showed best results. The level TAG content in liver tissues was significantly lowered by dandelion, milk thistle and dandelion/milk thistle combination in groups II, III and IV respectively when compared to group I (P<0.05) (Table 2). In addition, ALT and AST activities were increased in the group I, but this was significantly lowered in treated groups with best results in group IV (P<0.05) (Table 2).

**Dandelion, milk thistle and dandelion/milk thistle combination lowered insulin resistance-related biomarkers**

HFD fed rats exhibited increased fasting blood glucose, serum insulin level and HOMA-IR index (P<0.05) (Table 3). However, dandelion, milk thistle and dandelion/milk thistle combination lowered the aforementioned parameters which are more obvious in group IV.

**Dandelion, milk thistle and dandelion/milk thistle combination ameliorated biomarkers of oxidative stress in liver tissue**

Supplementation with dandelion, milk thistle and dandelion/milk thistle combination in groups II, III and IV resulted in significant reduction in elevated H<sub>2</sub>O<sub>2</sub> level and

significant elevation in the lowered GSH level in group I (P<0.05) (Table 4).

**Dandelion, milk thistle and dandelion/milk thistle combination reduced the expression of SREBP-1c genes and FAS activity in the liver**

HFD fed rats had higher mRNA expression of SREBP-1c as well as higher FAS activity in their liver tissues than animals in the groups II, III and IV with best lower significant results in group IV (P<0.05) (Table 4).

**Histopathological findings**

There were histopathological changes with structural damage of hepatic lobules in the liver of group I (Figure 1A), the prominent lesions were many lipid-droplets vacuoles, fatty cysts and cytoplasmic shrinkage with dark and small nuclei as well as many perivascular inflammatory infiltration. In groups II and III, hepatocytes had displayed less fatty and inflammatory infiltration (Figures 1B&C). The fatty and inflammatory changes of hepatocytes decreased obviously in group IV (Figure1D).

**Table 1:** Comparison of body weight gain (gm) throughout the experimental study between all studied groups.

Week/Group	Group I <sup>a</sup> n=15	Group II <sup>b</sup> n=15	Group III <sup>c</sup> n=15	Group IV <sup>d</sup> n=15
1 week	112.6±3.2 <sup>bcd</sup>	106.2±5.1 <sup>ad</sup>	109.1±1.2 <sup>ad</sup>	100.2±2.9 <sup>abc</sup>
2 weeks	122± 5.2 <sup>bcd</sup>	119±4.3 <sup>ad</sup>	120.3±2.9 <sup>ad</sup>	109.2±2.1 <sup>abc</sup>
3 weeks	134±3.3 <sup>bcd</sup>	129.1±2.1 <sup>ad</sup>	130.2±1.9 <sup>ad</sup>	120.9±3.1 <sup>abc</sup>
4 weeks	146±2.4 <sup>bcd</sup>	139.1±2.2 <sup>ad</sup>	141.4±1.2 <sup>ad</sup>	131.4±4.1 <sup>abc</sup>
5 weeks	159±2.5 <sup>bcd</sup>	150.9±1.3 <sup>ad</sup>	152.4±1.1 <sup>ad</sup>	145.2±2.2 <sup>abc</sup>
6 weeks	173.2±2.2 <sup>bcd</sup>	161.2±3.9 <sup>ad</sup>	162.3±2.1 <sup>ad</sup>	152.3±1.9 <sup>abc</sup>
7 weeks	186.2±3.1 <sup>bcd</sup>	172.1±3.1 <sup>ad</sup>	174.3±2.3 <sup>ad</sup>	159.1±2.7 <sup>abc</sup>
8 weeks	193.1±2.1 <sup>bcd</sup>	180.4±3.4 <sup>ad</sup>	179.1±4.1 <sup>ad</sup>	164.4±2.6 <sup>abc</sup>

<sup>a-d</sup>: significant difference between groups at p<0.05\* <sup>a</sup>: significant difference with group I, <sup>b</sup>: significant difference with group II. <sup>c</sup>: significant difference with group III. <sup>d</sup>: significant difference with group VI. Data are mean ± standard deviation of 15 rats of each. Statistical analysis was carried out using one way analysis of variance (ANOVA) with Tukey's post-hoc test, SPSS computer program.

**Table 2:** Effects of dandelion, milk thistle and dandelion/milk thistle combination on liver weight, transaminases activity and serum/lipid profiles among all studied groups.

Parameter/Group	Group I <sup>a</sup> n=15	Group II <sup>b</sup> n=15	Group III <sup>c</sup> n=15	Group IV <sup>d</sup> n=15
liver weight (grams)	6.51±0.25 <sup>bcd</sup>	3.9±0.65 <sup>ad</sup>	4.01±0.09 <sup>ad</sup>	3.08±0.69 <sup>abc</sup>
Serum ALT activity (U/L)	75.1 ±1.5 <sup>bcd</sup>	65.5± 3.51 <sup>ad</sup>	64.1± 3.6 <sup>ad</sup>	52.3±2.7 <sup>abc</sup>
Serum AST activity (U/L)	80.5 ±0.95 <sup>bcd</sup>	64.2± 1.9 <sup>ad</sup>	64.13± 2.3 <sup>ad</sup>	54.5±4.4 <sup>abc</sup>
Hepatic TAG level (mg/gm tissue)	13.4±3.9 <sup>bcd</sup>	8.2±0.89 <sup>acd</sup>	8.2±0.6 <sup>abd</sup>	6.3±0.94 <sup>abc</sup>
Serum TAG level (mg/dl)	169.1±10.7 <sup>bcd</sup>	96.2±5.8 <sup>ad</sup>	96.6±4.9 <sup>ad</sup>	83.7±5.1 <sup>abc</sup>
Serum TC level (mg/dl)	222.2 ±46 <sup>bcd</sup>	192.5± 8.7 <sup>ad</sup>	191.1± 8.7 <sup>ad</sup>	106.1 ±20.8 <sup>abc</sup>
Serum LDL-C level (mg/dl)	162.1 ±4.2 <sup>bcd</sup>	135.4±23.1 <sup>ad</sup>	132.2±23.9 <sup>ad</sup>	60.1 ±24.8 <sup>abc</sup>
Serum HDL-C level (mg/dl)	26.8 ±1.4 <sup>bcd</sup>	30.9± 1.6 <sup>ad</sup>	30.6± 2.0 <sup>ad</sup>	33.8 ±2.3 <sup>abc</sup>
Serum ox-LDL level (ng/ml)	96.6 ±4.6 <sup>bcd</sup>	63.4± 5.7 <sup>ad</sup>	65.8± 1.8 <sup>ad</sup>	28.9±9.9 <sup>abc</sup>

<sup>a-d</sup>: significant difference between groups at p<0.05\* <sup>a</sup>: significant difference with group I, <sup>b</sup>: significant difference with group II, <sup>c</sup>: significant difference with group III, <sup>d</sup>: significant difference with group VI. Data are mean ± standard deviation of 15 rats of each. Statistical analysis was carried out using one way analysis of variance (ANOVA) with Tukey's post-hoc test, SPSS computer program. ALT: alanine transaminase. AST: aspartate transaminase. TAG: triacylglycerol. TC: total cholesterol. LDL-C: low density lipoprotein cholesterol. HDL-C: high density lipoprotein cholesterol. ox-LDL: oxidized low density lipoprotein.

**Table 3:** Effects of dandelion, milk thistle and dandelion/milk thistle combination on insulin resistance related biomarkers among all studied groups.

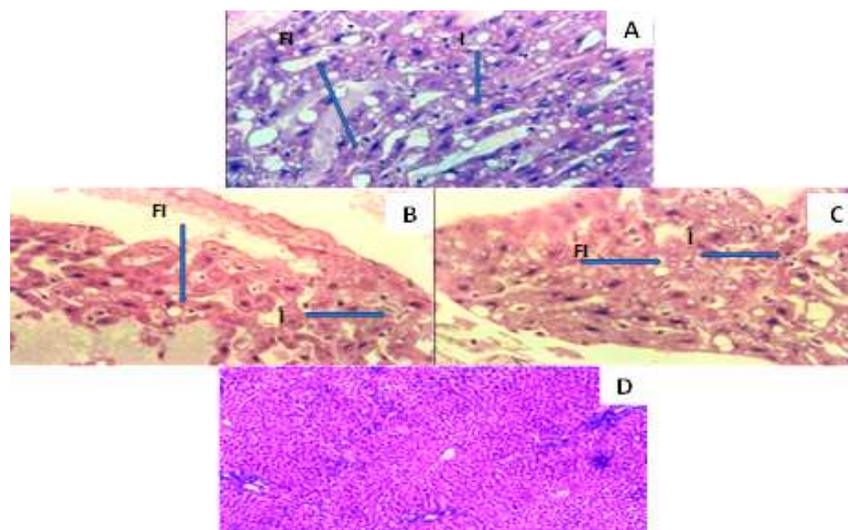
Parameter/Group	Group I <sup>a</sup> n=15	Group II <sup>b</sup> n=15	Group III <sup>c</sup> n=15	Group IV <sup>d</sup> n=15
FBS level (mg/dl)	137.9 ±4.9 <sup>bcd</sup>	121.7± 4.5 <sup>ad</sup>	120.2± 4.7 <sup>ad</sup>	92.9 ±7.5 <sup>abc</sup>
Fasting insulin level (µIU/ml)	29.8 ±2.6 <sup>bcd</sup>	14.3± 1.7 <sup>ad</sup>	14.6± 1.6 <sup>ad</sup>	5.99 ±2.2 <sup>abc</sup>
HOMA <sub>1</sub> IR index	10.1 ±1.0 <sup>bcd</sup>	4.31± 0.53 <sup>ad</sup>	4.33± 0.47 <sup>ad</sup>	1.46±1.06 <sup>abc</sup>

<sup>a-d</sup>: significant difference between groups at p<0.05\* <sup>a</sup>: significant difference with group I, <sup>b</sup>: significant difference with group II, <sup>c</sup>: significant difference with group III, <sup>d</sup>: significant difference with group VI. Data are mean ± standard deviation of 15 rats of each. Statistical analysis was carried out using one way analysis of variance (ANOVA) with Tukey's post-hoc test, SPSS computer program. FBS: fasting blood glucose. HOMA<sub>1</sub>IR: homeostatic model assessment of insulin resistance.

**Table 4:** Effects of dandelion, milk thistle and dandelion/milk thistle combination on biomarkers of oxidative stress and lipogenesis in hepatic tissue among all studied groups.

Parameter/Group	Group I <sup>a</sup> n=15	Group II <sup>b</sup> n=15	Group III <sup>c</sup> n=15	Group IV <sup>d</sup> n=15
Hepatic H <sub>2</sub> O <sub>2</sub> (nmol/min/gm tissue)	0.88 ±0.12 <sup>bcd</sup>	0.54± 0.22 <sup>ad</sup>	0.55± 0.15 <sup>ad</sup>	0.21±0.25 <sup>abc</sup>
Hepatic GSH (mg/gm tissue)	1.34 ±0.14 <sup>bcd</sup>	2.36± 0.13 <sup>ad</sup>	2.38± 0.08 <sup>ad</sup>	3.06±0.24 <sup>abc</sup>
Hepatic FAS activity (nmol /mg protein / min)	5.8±0.29 <sup>bcd</sup>	4.43±0.19 <sup>acd</sup>	4.15±0.1 <sup>abd</sup>	3.98±0.20 <sup>abc</sup>
Hepatic relative SREBP-1C mRNA expression	1.13±0.19 <sup>bcd</sup>	0.59±0.09 <sup>acd</sup>	0.59±0.07 <sup>abd</sup>	0.45±0.11 <sup>abc</sup>

<sup>a-d</sup>: significant difference between groups at p<0.05\* <sup>a</sup>: significant difference with group I, <sup>b</sup>: significant difference with group II, <sup>c</sup>: significant difference with group III, <sup>d</sup>: significant difference with group VI. Data are mean ± standard deviation of 15 rats of each. Statistical analysis was carried out using one way analysis of variance (ANOVA) with Tukey's post-hoc test, SPSS computer program. H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide. GSH: reduced glutathione. FAS: fatty acid synthase. SREBP-1C: sterol regulatory element-binding protein-1c.



**Figure 1:** The liver tissue stained with hematoxylin and eosin (H.&E.) (200 folds of amplification) in rats at the end of 8 week. A: group I with severe steatosis; B: group II with improvement of steatosis; C: group III with improvement of steatosis; D: group IV with restoration of nearly normal architecture. FI: fatty infiltration; I: inflammatory cell infiltration

## DISCUSSION

NAFLD is gaining increasing recognition as a component of the epidemic of obesity worldwide and is the most common cause of liver damage (Cheung and Sanyal, 2010). At present, no pharmacological treatment has been convincingly efficient against steatohepatitis.

In fact, slight but consistent weight loss, healthy eating regimen and exercise together with a number of therapeutic avenues remain the center of all strategies to improve or reverse steatohepatitis (Cheung and Sanyal, 2010). With the rising interest in complementary and alternative medicine, several natural products became under focusing for their potential beneficial effects in liver diseases. Among such attractive novel therapeutic possibilities are dandelion and milk thistle, which are widely consumed around the world (Xu et al., 2010).

Results herein showed that dandelion and milk thistle alone or combined reduced body weight gain and lowered the weights of liver tissues when compared to rats on HFD as previously reported (Choi et al., 2010; Jahan et al., 2015). Other studies have revealed that

HFD promote hyperlipidemia and hyperglycemia so it can be used to generate a valid rodent model for the analysis of the pathophysiology of dyslipidemia (Puccinelli et al., 2015).

Results herein showed that dandelion and milk thistle could ameliorate the abnormal blood lipid alteration in obese rats (group I) when compared to treated groups with best results obtained in the dandelion/milk thistle combination group as previously reported (Choi et al., 2010; Jahan et al., 2015). Additionally, dandelion, milk thistle and dandelion/milk thistle combination significantly reduced the elevated liver transaminases in group I when compared to treated groups that may be due to their antioxidant or free radical-antagonizing actions that increase stability of cellular membranes of hepatocytes and thereby promoting hepatic tissue regeneration that is more obvious in group IV (Shaker et al., 2010; You et al., 2010).

Hepatic steatosis arises from imbalance in TAG acquisition and removal (Puccinelli et al., 2015). dandelion, milk thistle and dandelion/milk thistle combination

significantly reduced the elevated liver TAG content in group I when compared to treated groups with best results were obtained in dandelion/milk thistle combination group as herbal supplementation can reduce intestinal absorption level of dietary lipid, thereby decreasing serum and hepatic TAG (Yao et al., 2011; Mingarro et al., 2015).

Insulin resistance may play an important role in the development of NAFLD (Bugianesi et al., 2005). In this current study, the FBG, fasting insulin as well as HOMA-IR were significantly increased in group I when compared to other treated groups, however, dandelion and milk thistle alone or combined improved insulin sensitivity, as demonstrated by a significant reduction in serum insulin and glucose levels as well as HOMA-IR specially in group IV. This can be explained that both herbs together can activate adenosine monophosphate (AMP)-activated protein kinase (AMPK) in liver which is resulted in significantly suppression of lipid accumulation in the liver, improve insulin resistance as previously reported (Hardie, 2011; Yao et al., 2011; Mingarro et al., 2015).

In obesity fat accumulation correlated with systemic oxidative stress in humans and rodent as the production of reactive oxygen species (ROS) increased selectively in adipose tissue (Holvoet et al., 2008; Iantorno et al., 2014). In this study, the ox-LDL levels were significantly increased in group I when compared to treated groups, however, dandelion and milk thistle supplementation improved this which is more obvious in combination group as reported previously by Wallace et al. (2008) and Colle et al. (2012) who reported the protective effects of both herbs most likely through antioxidant and free radical scavenging mechanisms.

In the current study, there were marked increase in the levels of H<sub>2</sub>O<sub>2</sub> as well as a distinct diminution in GSH level in liver tissue in group I when compared with treated groups which is improved by dandelion and milk thistle supplementations with best improvement in group IV, a result in accordance with results herein Choi et al.

(2010) and Yao et al. (2011) who reported the antioxidant properties of silibinin because of its capacity to decrease lipid peroxidation, to reduce the release of O<sub>2</sub><sup>-</sup> and to restore hepatic GSH level while Colle et al. (2012) who reported that dandelion scavenger activities against ROS and reactive nitrogen species are attributed to the content of its phenolic compounds.

Sterol regulatory element binding proteins (SREBPs) are master transcription factors for de novo lipogenesis (Zhao et al., 2014). The three SREBP isoforms, SREBP-1a, SREBP-1c and SREBP-2, play different roles in lipid synthesis. Studies using transgenic and knockout mice suggest that SREBP-1c plays an essential role in the regulation of most lipogenic genes involved in fatty acid and TAG synthesis (Zhao et al., 2014). The present study showed significant increase in SREBP-1c mRNA level as well as FAS activity in the liver tissues of HFD fed rats when compared to treated groups, however dandelion and or milk thistle supplementations attenuated this increase in treated groups. A recent study by Xiao et al. (2013) showed that dandelion and milk thistle inhibited hepatic lipid accumulation through AMPK activation, the activated AMPK further phosphorylates acetyl-CoA carboxylase, which switches off fatty acid synthesis and accelerates the transport of long-chain fatty acyl groups into the mitochondria to undergo  $\beta$ -oxidation (Hardie, 2011).

Other possible mechanism of the antiobesity activity of both herbs is that oxidative stress associated obesity which is attenuated by herbal supplementation can increase SREBP-1c mRNA expression, leading to enhanced transcription of FAS with subsequent hepatic fat accumulation as reported by Abd Eldaim et al. (2010).

To our knowledge, no previous studies studied the hypolipidemic effect of dandelion or milk thistle on the lipogenic gene expression.

Abnormal lipid metabolism associated obesity in the liver leads to fatty liver

formation. Histopathologically, liver sections from rats fed HFD showed a clear difference from those of other treated groups in which fat accumulates in the liver hepatocytes with marked degenerative changes, necrosis, disrupted architecture were observed, these results are in agreement with previous study of Samuhasaneeto et al. (2007) who reported the effects of HFD associated inflammation and oxidative stress in inducing the early stage steatohepatitis.

Improvement of the histopathological results was observed with herbal supplementation in treated groups II and III with nearly restoration of normal architecture to some extent in combination group IV reflecting their anti-oxidative, anti-fatty infiltration, anti-inflammatory and liver regenerating effects as previously reported (Kim et al., 2012)

### Conclusion

HFD induced obesity associated with a disturbed lipid profile, defective antioxidant stability, and high values of insulin resistance biomarkers; this may have implications for the progress of obesity related steatohepatitis. Treatment with dandelion, milk thistle and dandelion/milk thistle combination improved obesity and its associated steatohepatitis so they may be of particular benefit to individuals who are unable or unwilling to reduce their intake of high-fat foods. Moreover we can conclude that when herbs are used in combination it helps the body for better managing. It is therefore preferable to use herbal combinations instead of depending on single herbs. Although further research is required to confirm that, similar effects occur in humans.

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### COMPETING INTERESTS

The authors have no conflict of interest to declare.

### AUTHORS' CONTRIBUTIONS

NAS participated in animal preparation, sample collection, biochemical and molecular assays, and together with SAE-D participated in the design of the study, performed statistical analysis, and drafted the manuscript. All authors read and approved the final manuscript.

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