Hepatorenal Protective Effect of Dandelion (Taraxacum officinale L.) and Its Role in Improving Exercise Capacity in Type 2 Diabetic Albino Rats: Possible Underlying Mechanisms

Walid Mostafa Said Ahmed [1], Amir Soliman [2], SafyEldin Mahmoud AboAli[3], Elsayed A. Eid [4], Rehab Mohamed El shahat[5], Amer M.Abdel hamid[6], Dania Abdelhady Mohammed[7], Omnia Saeed Mahmoud Ahmed[8], Asmaa M. Al-Emrany[9]

1. Lecturer, Medical Physiology Department, Faculty of Medicine, Al–Azhar University, Damietta, Egypt.
2. Assistant professor, Dpartment of Public Health and Community Medicine, Faculty of Medicine, Delta University for Science and Technology, Al Mansora Al Gadida, Egypt
3. Lecturer in Department of Physical Therapy for Pediatric Disorders, Faculty of Physical Therapy, Delta University for Science andTechnology, Gamasa, Egypt.
4. Associate professor, Dpartment of Internal Medicine and Endocrinology, Faculty of Medicine, Delta University for Science and Technology, Al Mansora Al Gadida, Egypt
5. Lecturer,Department of medical pharmacology, Al–Azhar University, Cairo, Egypt.
6. Lecturer, Department of medical biochemistry, Al–Azhar University, Damietta, Egypt.
7. Department of Physiology, Faculty of Medicine, Benha University.
8. Lecturer in Department of Physical Therapy for Internal Medicine and Geriatrics, Faculty of Physical Therapy, October University for Modern Sciences and Arts, (MSA).
9. Lecturer in Department of Physical Therapy for Internal Medicine and Geriatrics, Faculty of Physical Therapy, October University for Modern Sciences and Arts, (MSA).

Abstract

Background: The tremendous rise in the economic burden of type 2 diabetes (T2D) has prompted a search for alternative and less expensive medicines. Dandelion offers a compelling profile of bioactive components with potential hepato-renal and anti-diabetic properties. In many countries, it is used as food and in some countries as therapeutics for the control and treatment of T2D. **Objective:** To investigate hepatorenal protective effect of dandelion and its role in improving exercise capacity in type 2 diabetic albino rats and its possible underlying mechanisms. Materials and methods: Induction of partial diabetes in 30 rats by STZ followed by biochemical investigation of C- peptide and blood glucose level with several kidney and liver parameters. In addition to non-enzymatic and enzymatic anti-oxidants in vitro were evaluated. Also, the pro-inflammatory mediators and the expression levels of pro-oxidants/pro-inflammatory and anti-inflammatory mediators using qPCR. **Results:** Dandelion demonstrated improvement of liver, kidney functions and exercise capacity including improvement of the enzyme activity of ALT, AST, ALP, albumin, urea and creatinine. Treatment with Taraxacum officinale extract ameliorated the depletion of GSH level, GPx specific activity and SOD specific activity; it also significantly suppressed the increase in the level of TBARS and the expression level of both COX-2 and iNOS. The effect of dandelion on improving the exercise capacity of mice with liver dysfunction was observed and its mechanism was expounded. **Conclusion:** Dandelion serves as promising hepatorenal protective and anti-diabetic natural therapy in type 2 diabetes with improving exercise capacity due to the presence of chicoric acid, taraxasterol (TS), chlorogenic acid, and sesquiterpene lactones.

KeyWords: Dandelion, Exercise capacity, Liver function, kidney function and diabetes mellitus.

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Introduction:

Diabetes mellitus is a widespread and serious health issue leading to numerous global health problems. Although insulin and oral medications have shown significant therapeutic results in treating diabetes, patients who receive insulin injections or synthetic drugs still experience complications related to macrovascular, retinal, and neuropathic function **(Amanat et al., 2020).** However, by making lifestyle changes and taking

oral medications, the progression of type 2 diabetes mellitus (T2D) can be slowed, and the quality of life for those with T2D can be improved. The use of alternative medicine, particularly natural products.

with anti-diabetic properties, has increased. Research showed that several dietary patterns, including a high intake of plant foods, could help prevent obesity and T2D **(Eguilaz et al., 2016)**.

Diabetes in liver disease appears to be caused by insulin resistance in muscle and fat tissues, along with hyperinsulinemia. The impaired response of the pancreas' beta cells and resistance to insulin in the liver also plays a role. Non-alcoholic fatty liver disease, alcoholic cirrhosis, chronic hepatitis C (CHC), and hemochromatosis are commonly linked to DM. The treatment outcome for patients with CHC is worsened by insulin resistance, leading to an acceleration of fibrosis. Hepatogenous diabetes, unlike type 2 diabetes, is not commonly linked to microangiopathy, and cirrhotic patients are more likely to experience complications from their condition. The presence of diabetes increases the mortality risk for cirrhotic patients. Managing diabetes is challenging because of the liver damage and potential toxicity of oral hypoglycemic medications **(Elkrief et al., 2016)** .

Aging, metabolic imbalance, and fatigue can decrease liver function, but maintaining exercise can improve liver function, reduce fatigue, and sustain overall body health. Exercise capacity and aging have a reciprocal impact on overall body health, with good exercise capacity promoting sustained physical activity, thereby slowing aging **(Zhang et al., 2020).** The liver is vulnerable to oxidative stress since it depends on oxidative metabolism to produce energy. Inadequate synthesis of antioxidant enzymes can result in malfunction of the liver's mitochondrial respiratory complex, which ultimately leads to aging of liver cells, heightened autophagy, and damage from fibrinogen nitrification **(Luo et al., 2021).**

As the liver ages, free radicals accumulate, which hinders regular metabolism, causes fatigue, reduces exercise capacity, and reduces the quality of life of the elderly. Maintaining normal and healthy organ function, particularly the liver, improves the body's ability to withstand oxidative stress and slows down aging caused by reactive oxygen species **(Zhang et al., 2022).**

Diabetic kidney disease (DKD) is the leading cause of chronic kidney disease affecting 30-40% of individuals with diabetes **(Bonner et al., 2020).** Bonner et al. presented evidence-based strategies for managing and preventing the DKD

and diabetic liver, combining traditional treatments for high blood sugar, high blood pressure, and protein in the urine with new natural products as phyto-chemical compounds **(Bonner et al., 2020).** Phyto-chemical compounds, such as phenolic acids, flavonoids, alkaloids, and sulfated polysaccharides, have been shown to have anti-oxidant properties **(Zengin et al., 2011).**

Flavonoids, which are believed to have beneficial effects on human health and are effective in scavenging free radicals, inhibiting hydrolytic and oxidative enzymes, and reducing inflammation **(Jucá et al., 2020).** Evidence suggests that their biological activities are related to their anti-oxidant properties. Dandelion, a perennial herb used in traditional Chinese medicine, is rich in flavonoids and has been used to treat many conditions, including acute mastitis, lymphadenitis, and urinary tract infections. Plant-based functional foods, such as dandelion, have positively affected liver health and function, particularly through their anti-oxidant properties **(Zhu et al., 2016).**

Our study aims to observe the effect of dandelion and its active ingredients on liver and kidney functions and, consequently, its role in exercise capacity in diabetic mice with impaired liver and kidney functions.

Materials and Methods:

Our study was conducted following the Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines **(Percie du Sert et al., 2020).**

Reagents and chemicals

The used Streptozotocin was purchased from Sigma-Aldrich (St Louis, MO, USA). Kits, reagents and chemicals used were of analytical grade; other chemicals were of the highest purity commercially available

Dandelion extract

The dandelion (Taraxacum officinale) was separately collected from the local market and identified at the Microbiology Department, Faculty of Science, Al-Azhar University. The dried leaves of dandelion (0.25-0.5 kg) were minced with (1-1.5 L) ethanol, stirred for three days, and then filtered off. Each ethanolic filtrate was collected, evaporated under reduced pressure using a rotary evaporator separately, then lyophilized and weighted. Each

1mg of lyophilized plant extract was dissolved in 50% (v/v) distilled water and ethanol.

Animals

Thirty male Wistar Albino rats (180–200gm) were

obtained from Nile pharmaceutical company.

The animals were kept in polypropylene cages (10 rats/ cage). The experimental cages, which housed rats during the study, were enclosed with metal grids and placed in a room that maintained optimal environmental conditions, including a 12-hour light-dark cycle and a regulated temperature (23±2ºC) and humidity. Each cage was supplied with standard chow diet and drinking water throughout the experimental period, and the minimum number of rats required for obtaining accurate scientific data was employed. The local committee approved the design of the experimental and protocol conforms to the guidelines of the National Institutes of Health (NIH).

Induction of diabetes

Streptozotocin causes a type of diabetes that resembles non-ketosis hyperglycemia in diabetes mellitus in certain animals. To experimentally induce diabetes in male adult rats weighing 180-200 grams and 75-90 days old, a Streptozotocin dose of 30 mg/kg was administered through intraperitoneal injection **(Akbarzadeh et al., 2007).**

Experimental design

The experimental animals were divided into 3 main groups, with 10 rats/group, a healthy control group and 2 diabetic groups injected with the drug streptozotocin and fed on the main meal for three days at adulthood to obtain severe DM:

Group I (healthy control group): fed on the basic diet.

Group II (diabetic control group): a group with diabetes, fed on the basic diet.

Group III (diabetic intervention group) (Taraxacum officinale treated group): a group with diabetes, which was injected with Taraxacum officinale for 2 months with a dose of (40 mg/kg) every 24 h IP

Running experiment

The rats underwent running and swimming tests until exhaustion. The rats were made to run on the exercise wheel at a rate of 20 revolutions per minute and subjected to five consecutive electric shocks until they reached exhaustion. The duration of their running time was measured and documented. **(YHCS, Wuhan Yihong Technology Co., Ltd, Wuhan, Hubei, China).**

Exhaustive swimming experiment

After the procedure involving gastric samples, the rats were immersed in a water tank with a temperature of 28 ± 2°C and a water depth of 20 cm, which was used to regulate the temperature. The rats were forced to swim until they became exhausted, which was defined as the inability to stay afloat for more than 10 seconds, and the duration of their swimming was measured and noted.

Blood samples and tissue preparation

At the end of the experimental period, the rats were fasted for 12 h and were sacrificed under anesthesia. Using a sterile syringe, the blood samples were obtained from the heart and centrifuged at 3000 rpm for 10 minutes. The serum samples were separated and stored at -80°C for the biochemical analysis.

The liver and kidney tissues were quickly removed and cleaned in normal saline ice. Small pieces from the tissues were homogenized (9 times; w/v) with ice phosphate-buffered solution (0.1 M, pH 7.4) and centrifuged at 1000 mg for 10 min at 4 °C using a centrifuge (PLC-05, Taiwan). The supernatants were separated and stored at -80 °C for the biochemical assays.

Assessment of pro-oxidants

Determination of lipid peroxidation end product

Lipid peroxidation as a major indicator of oxidative stress was evaluated by measuring the thiobarbituric reactive substances (TBARS) in the liver and kidney **(Tappel and Zalkin, 1959).**

Glutathione peroxidase specific activity assay (GPx)

The Glutathione peroxidase family of enzymes plays an important role in protecting organisms from oxidative damage. GPx converts reduced glutathione (GSH) to oxidized glutathione (GSSG) while reducing lipid hydroperoxides or hydrogen peroxide to their corresponding alcohols or water, respectively. Low levels of GPx have been correlated with free radical-related disorders. Glutathione peroxidase activity was determined by subtracting the excess GSH after the enzymatic reaction from the total GSH in the absence of the enzyme. Reduced glutathione reacts with DTNB to form a yellow color. This assay was performed

according to **(Rotruck et al., 1973).**

Cu/Zn Superoxide dismutase (SOD) specific activity assay

Cu/Zn Superoxide dismutase (SOD) is an anti-oxidant enzyme involved in the defence system against reactive oxygen species (ROS) by catalyzing the dismutation of superoxide anion radical ($O2\bullet^-$) to hydrogen peroxide, which is then detoxified by glutathione peroxidase and catalase. Cu/Zn SOD activity was determined using a simple and rapid method based on the ability of the enzyme to inhibit the auto-oxidation of pyrogallol at pH 8.2. The principle of this method is based on the competition between the pyrogallol auto-oxidation by $O2\bullet^-$ and the dismutation of this radical by SOD. This assay was performed according to **(Marklund and Marklund, 1974).**

Assessment of the anti-oxidant parameters

Determination of reduced glutathione

The glutathione (GSH) was allowed to react with Ellman's reagent, DTNB (5,5'-dithiol-bis-2-nitrobenzoic acid). This reaction generates a yellow-colored 2-nitro-5-thiobenzoic acid product **(Ellman, 1959).**

Biochemical evaluation

The collected blood was examined for liver function tests (ALT **(Gella et al., 1985),** AST **(Gella et al., 1985).,** ALP **(Tietz et al., 1983)** and albumin **(Doumas et al., 1971).** Also, their blood will be examined for kidney function tests (urea **(Fawcett & Scott, 1960),** creatinine **(Fabiny and Ertingshausen, 1971)**).

Estimation of the diabetic profile

Determination of serum glucose level

One ml of the working reagent was added to 0.01 ml of serum sample or standard. The mixture was mixed well and incubated at 37 °C for 10 min. The absorbance values of the sample (A Sample) and standard (A Standard) were measured at 510 nm against a reagent blank.

ELISA detection of tumor necrosis factor-alpha (TNF-α)

The tumor necrosis factor (TNF)-α kit is a solid phase sandwich enzyme linked-immuno-sorbent assay (ELISA). The intensity of this colored product is directly proportional to the concentration of TNF-α present in the original specimen. This assay was performed using TNF alpha ELISA kit

forward primer: CCCCAAAGGGATGAGAAGTTC

reverse primer:GGCTTGTCACTCGAATTTTGAGA

Molecular analysis

The total RNA was extracted from the liver and kidney (100 mg) using an easy-RED TM total RNA extraction kit **(Gauthier et al., 1997).** Quantitative polymerase chain reaction (qPCR) was used to evaluate the Taraxacum officinale effect on the relative change in expression of several parameters such as COX-2 and iNOS expression level by real-time polymerase chain reaction (PCR). The CT values of each target gene were normalized to that of β-actin according to the manufacturer's instructions, and the change in expression (2−ΔΔCT) was calculated **(Thermo Scientific, USA).** The amount of PCR product (amplicon) using the inexpensive fluorescence synergy brands (SYBR) green kit. Fold change in gene expression = log (2-ΔΔCT).

Statistical analysis of the data

The data are expressed as mean ± SEM, and the significant values were considered at $p < 0.05$. One-way analysis of variance (ANOVA) by Duncan's test was used to evaluate the difference between the mean values of the results obtained using plant extracts. The analysis was done for three measurements using SPSS software version 25 **(IBM, 2019).**

Results:

Effect of Taraxacum officinale on cellular non-enzymatic and enzymatic anti-oxidants in diabetic-induced rats (GSH, GPx and SOD)

As observed from Table (2) and Figure (1A), There was a significant depletion of the endogenous anti-oxidant GSH level in diabetic-induced rats (0.339 ± 0.01 mmol/ml, p≤ 0.05) in comparison with the normal group (0.71± 0.03 mmol/ml), Treatment with Taraxacum officinale (0.6± 0.021 and 0.5 ± 0.029 b mmol/ml, respectively) significantly restored the GSH level in liver and kidney of diabetic induced rats compared to induced group (p≤0.05).

As observed from Table (2) and Figure (1C), there was a significant depletion of the endogenous anti-oxidantGPx-specific activity in diabetic-induced mice (0.4±0.05 mU/µg, p≤0.05) in comparison with the normal group (7.9±0.3 mU/µg). Treatment with Taraxacum officinale (7.1±0. 29 and 5.5±0.15 mU/µg, respectively) significantly restored the specific activity of GPx in the liver and kidney of diabetic induced rats compared to the induced group.

Data in Table (2) and Figure (1B) demonstrated a significant depletion of the endogenous anti-oxidant SOD-specific activity in diabetic-induced rats (0.055±0.003 U/µg, p≤0.05) in comparison with the normal group (0.16±0.003 U/µg). The administration of Taraxacum officinale ethanolic plant extract (0.1±0.002 and 0.8±0.002 U/µg, respectively) significantly restored the depletion of SOD in the liver and kidneys of diabetic-induced rats.

Effect of plant extracts on cellular pro-oxidants or pro-inflammatory mediators in diabetic-induced rats (MDA and TNF-α)

As shown in Figure (1D) and Table (2), STZ administration to rats resulting in diabetes significantly (p≤ 0.05) elevated the level of MDA in diabetic-induced rats (3.55±0.52 nmoles/ml) when compared to normal cells (0.73±0.1 nmoles/ml). However, Taraxacum officinale administration to diabetic-induced rats significantly reduced the MDA levels in the liver and kidney (0.65±0.15 and 0.6±0.13 nmoles/ml, respectively).

To identify the anti-inflammatory mechanism, the inflammatory reaction was induced by STZ-induced diabetes, which significantly increased TNF-□ production (861.6±0.01 pg/ml) compared to the normal group (175.6±0.01 pg/ml). As shown in Figure (3B) and Table (2), treatment with Taraxacum officinale significantly p≤0.05 reduced the TNF-□ production in liver and kidney of diabetic-induced rats (148.8±1.98 and 150.1±1.3 pg/ml, respectively) compared to the diabetic induced group.

Expression levels of cellular pro-oxidants or pro/anti-inflammatory mediators in diabetic-induced rats (COX-2 and iNOS expression level)

Figure (3A) and Table (3) induction of diabetes by STZ has been demonstrated to upregulate COX-2 expression five times (5.2± 0.12) when compared to the normal group (1.3± 0.04). Taraxacum officinale significantly downregulated COX-2 expression in the liver and kidney of diabetic-induced rats (0.91± 0.08 and 0.9±0.1, respectively) compared to the induced group at p≤ 0.05.

Figure (3C) and Table (3) showed that induction of diabetes by STZ has been demonstrated to upregulate iNOS expression five times (4.8±0.14) when compared to the normal group (1.079±0.15). Taraxacum officinale significantly downregulated iNOS expression in the liver and kidney of diabetic-induced rats (0.81±0.03 and 0.85±0.04, respectively) compared to the induced group at p ≤ 0.05.

Biochemical investigation of liver function test of diabetic induced rats

A high AST level often means there is some liver damage, but it is not necessarily caused by hepatitis C. A high AST with a normal ALT may mean that the AST comes from a different body part. As observed from Figure (3) and Table (4) for AST, the mean values of AST of rats groups that were divided into negative control group (that fed on the basic diet) and positive control group were (23.2±2.5 and 58.4±3.6 U/L, respectively). Statistically, the AST increased significantly (p<0.05) in the positive control group compared to the negative control group. On the other hand, all diabetic rats groups treated with Taraxacum officinale significantly had improvement in their AST (35.6±1.4 U/L) when compared to the induced group

Elevated levels of ALT, AST, GGT and ALP are related to higher odds of diabetes. Also, increased levels of ALT, GGT and ALP, even within the normal range, were independently related to the increased odds of diabetes. These results indicated the potential of elevated liver enzymes as biomarkers for the possible presence of diabetes. As observed from Figure (3) and Table (4) for ALT, the mean values of ALT of rats groups that were divided into negative control group (that fed on the basic diet), positive control group were (82.4±7.2 and 33.8±6.5 U/L, respectively). Statistically, all diabetic rats treated with Taraxacum officinale significantly improved their ALT (51.1± 1.3 U/L) compared to the induced group.

An alkaline phosphatase (ALP) test measures the amount of ALP in the blood. It is commonly used to diagnose liver damage or bone disorders. As observed from Figure (3) and Table (4), the mean values of ALP of rats groups that were divided into negative control group (that fed on the basic diet) and positive control group were (48.6±1.2 and 137.4±6.9 U/L, respectively). Statistically, the ALP increased significantly at (p<0.05) in the positive control group compared to the negative control group. All diabetic rats groups treated with Taraxacum officinale significantly improved their ALP (82.4±2.4 U/L) compared to the induced group.

Albumin is a protein made by your liver. Albumin enters your bloodstream and helps keep fluid from leaking out of your blood vessels into other tissues. It also carries hormones, vitamins, and enzymes

throughout your body. As observed from Figure (3) and Table (4), the mean values of albumin of rats

groups that were divided into negative control group (that fed on the basic diet) and positive control group were (3.2±0.8 and 7.4±1.6 g/dL, respectively). Statistically, the albumin increased significantly at ($p<0.05$) in the positive control group compared to the negative control group. All diabetic rats treated with Taraxacum officinale significantly improved their albumin (4.6±1.5 g/dL) compared to the induced group.

Biochemical investigation of kidney function test of diabetic induced rats

Creatinine and Urea impairment was relatively common among type-2 diabetic patients. As observed from Figure (3) and Table (4), the mean values of urea (mmol/L) of rats groups that were divided into negative control group (that fed on the basic diet), positive control group were (28.8±1.2 and 46.7±5.41 mmol/L, respectively). Statistically, urea increased significantly at ($p<0.05$) in the positive control group compared to the negative control group. All diabetic rats groups treated with Taraxacum officinale significantly improved their urea level (35.2±2.32 g/dL) compared to the induced group.

Diabetic nephropathy is the kidney disease that occurs as a result of diabetes. As observed from Figure (3) and Table (4), the mean values of creatinine of rats groups that were divided into negative control group (that fed on the basic diet), positive control group were (0.77±0.12 and 1.34±0.21 mg/dL, respectively). Statistically, creatinine increased significantly at ($p<0.05$) in the positive control group compared to the negative control group. On the other hand, all diabetic rats groups treated with Taraxacum officinale significantly improved their creatinine level (1.13±0.13 g/dL) compared to the induced group.

The Effect of Taraxacum officinale treatment on diabetic rat's blood glucose level

As observed from Figure (3) and Table (4), the results showed the highest significant increase at ($p<0.05$) in serum blood glucose in the positive control group compared with the negative control group (99.2±3.4 and 301.4±10.9 mg/Dl, respectively), that increase might be due to STZ injection. On the other hand, all diabetic rats groups treated with Taraxacum officinale significantly improved their blood glucose level (140.2±12.9 mg/dL) compared to the induced group.

The running and exhaustive swimming times in rats

The administration of dandelion significantly increased the duration of running and swimming until exhaustion ($P < 0.05$). The difference among comparable groups can be seen in Figure 2D and Table 5

Discussion:

Our study assessed the effect of Taraxacum officinale in diabetic albino rats. It revealed the following: Taraxacum treated group showed significant improvement in the anti-oxidant levels (GSH, GPx, and SOD) and blood glucose levels. Additionally, Taraxacum improved ALT.AST, ALP, urea, creatinine, and albumin levels. Regarding time to exhaustion, Taraxacum significantly increased the time needed to exhaustion. Concerning pro-inflammatory and pro-oxidant levels, Taraxacum significantly decreased their levels in the kidney and liver.

The way Taraxacum helps improve the functioning of the kidneys and liver could be due to its various bioactive components and their pharmacological effects **(González-Castejón et al., 2012; Schütz et al., 2006).** Abdul Kadir et al. investigated that the alcoholic extract of Taraxacum officinale contained glycosides, alkaloids, phenolic compounds, tannins, flavonoids and proteins. Also, Leaves have (K, Ca, Na, Fe) and low concentrations of (Zn, Cd, Cu) **(Abdul Kadir et al., 2012).** A phenolic compound in Taraxacum officinale may be responsible for its anti-oxidant activity that helped explain many Taraxacum officinale favorable outcomes **(Amin et al., 2013).** Taraxacum is not the only plant-based treatment turned into a pharmaceutical drug. Metformin, the most widely used anti-diabetic drug, was originally derived from galegine found in the plant Galega officinalis **(Bailey and Day, 2004).** Similarly, Acarbose used to treat diabetes by blocking the enzyme alpha-glucosidase, was discovered from a bacteria**(Brunkhorst and Schneider, 2005).**

Our study showed that using Taraxacum officinale extract in ethanol form improved blood sugar levels in rats with induced diabetes. This finding aligns with the study conducted by Wirngo et al., who concluded that the benefits of polyphenolic components in plants on Type 2 Diabetes Mellitus

are due to various mechanisms such as increasing cAMP levels, which leads to the release of insulin, reducing degradation of insulin, preventing oxidative stress, promoting the regeneration of beta cells, repairing and increasing cell size, and increasing cell proliferation in the islets of Langerhans **(Wirngo et al., 2016).**

Regarding hepatocellular injury, preliminary studies linked pro-inflammatory cytokines as TNF-α and non-alcoholic steatohepatitis patients, suggesting a possible genetic link or predisposition to fatty liver and impaired liver function **(Tian et al., 2021).** As we found in our study, Taraxacum officinale affects the inflammatory process by decreasing pro-inflammatory and pro-oxidants, thus improving liver functions (ALT, AST, ALP, and Albumin). **(Seo et al., 2005)**

As to Tian et al., the ethanolic extract of Taraxacum officinale (dandelion) improved kidney function by reducing urea and creatinine levels. In vivo and in vitro experiments also found that dandelion sterol can inhibit the TLR4/NF-κB (p65) signaling pathway by regulating miR-140-5p, thereby reducing damage to renal tissue cells induced by diabetes mellitus **(Tian et al.,2021).** These findings are consistent with the results of the current study.

According to **Zhang et al., 2022** physical activity can decrease cell membrane damage from free radicals, help maintain normal cellular functions, and improve liver health In the current study, STZ harmed the pancreas and caused oxidative stress, which led to aging and reduced liver function in rats. However, dandelion extract improved exercise tolerance and improved liver function, as demonstrated by rats' prolonged capacitance to swim and run till exhaustion. This finding is consistent with previous literature

Regarding the role of Taraxacum officinale in the regulation of inflammatory process and anti-oxidant properties, previous literature enhanced our finding as they declared that Taraxacum officinale could control and inhibit pro-inflammatory cytokines for example, IL-1β, IL-6, IL-8, and TNF-α, and cyclooxygenase-2 (COX-2) enzyme **(Tian et al., 2021; Owczarek and Lewandowska, 2017).** Our study confirmed the findings of previous studies that reported the protective effects of dandelion sterol on lipopolysaccharide-damaged macrophages. The sterol inhibited the expression of inducible nitric oxide synthase and cyclooxygenase-2 by regulating the ERK1/2 and p38 signaling pathways. **(Xiong et al., 2014; Tang et al., 2011;**

Piotr et al.,2014; Yoon et al., 2010; Hu and Kitts, 2005).

Limitations we faced in our study were as follows: We need a more comprehensive analysis of various dandelion components to know the exact role of each and each of which would be responsible for definite intracellular pathways. Also, to know the efficiency of Taraxacum officinale as a liver protector, further studies should add a separate arm or stratify studied animals according to their liver status to avoid confounders. Additionally, there is a need for more research on the effects of dandelion components in human clinical trials. Studies using human diabetic patients would easily determine the potency and viability of dandelion components against T2D.

Conclusion:

Our study demonstrated that dandelion showed anti-hyperglycemic, anti-oxidative, hepato-renal protective and anti-inflammatory properties. Additionally, it significantly increased the time needed to exhaustion. Concerning pro-inflammatory and pro-oxidant levels, dandelion significantly decreased their levels in the kidney and liver. More research is also necessary on the bioavailability and metabolism of these components in humans. Research in this area would pave the way for the further development of dandelion-derived compounds as drugs and provide more comprehensive information to those in need of treatment that is not currently available.

Figure ligands:

Figure (1): Effect of Taraxacum officinale on cellular non-enzymatic and enzymatic anti-oxidants in STZ diabetic induced rats (A): GSH mmol/ml (B): specific activity of SOD (U/µg) (C): Specific activity of GPx (mU/µg) (D): Lipid peroxidation level (nmoles/ml).

Figure (2): Fold change in gene expression of TNF, COX-2 and iNOS in diabetic induced rats (A): TNF-α levels (pg/ml) (B): Fold change in gene expression of COX-2 (C): Fold change in gene expression of iNOS. (D): Exercise capacity

Figure (3): Biochemical investigation of diabetic induced rat

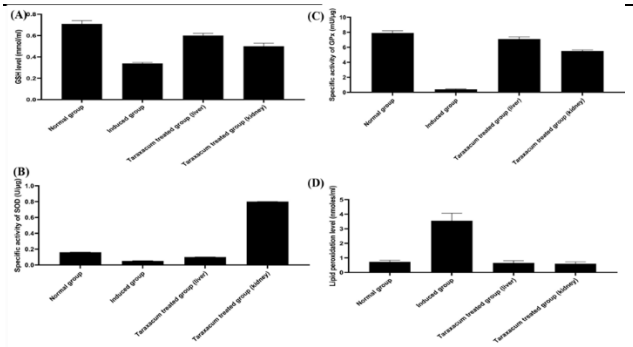

Figure (1)

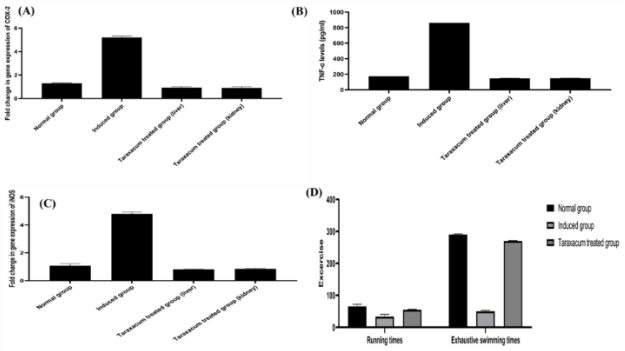

Figure (2)

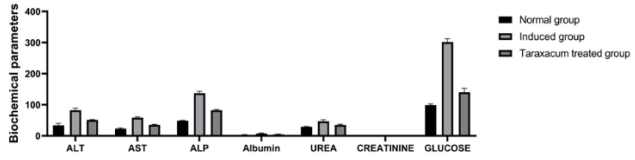

Figure (3)

Table (1): Real time-PCR forward and reverse primer sequences

Gene	Forward primer	Reverse primer
iNOS	5'-ACAAG CTGGC CTCGC TCTGG AAAGA-3'	5'-TCCAT GCAGA CAACC TTGGG GTTGA A
COX-2	5'-TTCAAATGAGATTGTGGAAAAAT 3'	5' AGATCATCTCTGCCTGAGTATCTT 3'
β-actin	5'-GGGAAATCGTGCGTGACATC-3'	5'-GCGGCAGTGGCCATCTC-3'

Table (2): Effect of Taraxacum officinale on cellular non-enzymatic and enzymatic anti-oxidants in STZ diabetic induced rats

imal group	GSH level (mmol/ml)	Specific activity of GPx (mU/µg)	Specific activity of SOD (U/µg)	Lipid peroxidation level (nmoles/ml)	TNF-α
rmal group	0.71± 0.03^{a}	7.9 ± 0.3^{a}	0.16± 0.003^{a}	0.73 ± 0.1^{a}	175.6±
luced group	0.339 ± 0.01^{c}	0.4 ± 0.05^{d}	0.05± 0.003^{d}	3.55 ± 0.52^{c}	861.6 ±
raxacum officinale er tissue	0.6± 0.021^{b}	7.1 ± 0. 29^{b}	0.1± 0.002^{b}	0.65 ± 0.15^{b}	148.8±
raxacum officinale lney tissue	0.5 ± 0.029^{b}	5.5 ± 0.15^{c}	0.8± 0.002^{c}	0.6 ± 0.13^{b}	150.1±

Table (3): Fold change in gene expression of COX-2 and iNOS in diabetic induced rat

Animal group	Fold change in gene expression of COX-2	Fold change in gene expression of iNOS
Normal group	1.3± 0.04^{a}	1.079±0.15^{a}
Induced group	5.2± 0.12^{c}	4.8±0.14^{c}
Taraxacum officinale Liver tissue	0.92± 0.08^{b}	0.81±0.03^{b}
Taraxacum officinale kidney tissue	0.9± 0.1^{b}	0.85±0.04^{b}

Results are expressed as Mean ± SEM (n=3).

-Means with different letters were significantly different at p≤0.05.

-If two means have the same letter, there were not significantly different, at p<0.05.

Table (4): Biochemical investigation of diabetic induced rat

Animal group	ALT (U/L)	AST (U/L)	ALP (U/L)	ALBUMIN g/dL	UREA mg/dL	CREATININE mg/dL	GLUCO mg/dL
Normal group	33.8^{a} ± 6.5	23.2^{a}±2.5	48.6^{a}±1.2	3.2^{a}±0.8	28.8^{a}± 1.20	0.77^{a}±0.12	99.2^{a}±3.
Induced group	82.4^{c}± 7.2	58.4^{c}± 3.6	137.4^{c}±6.9	7.4^{c}±1.6	46.7^{c}±5.41	1.34^{c}±0.21	301.4^{c}±1
Taraxacum officinale treated rats	51.1^{b}± 1.3	35.6^{b}±1.4	82.4^{b}±2.4	4.6^{b}±1.5	35.2^{b}±2.32	1.13^{b}±0.13	140.2^{b}±1

Results are expressed as Mean ± SEM (n=3).

-Means with different letters were significantly different at p≤0.05.

-If two means have the same letter, there were not significantly different, at p<0.05.

Table (5): The running and exhaustive swimming times in mice

Animal group	Running times	exhaustive swimming times
Normal group	65.5^{a} ± 6.5	290^{a}±2.5
Induced group	33.4^{c}± 7.2	50^{c}± 3.6
Taraxacum officinale treated rats	55.1^{b}± 1.3	270^{b}±1.4

Declarations

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Availability of data and materials

All data and materials are fully presented in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

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REFERENCES

1. **Akbarzadeh, A.; Norouzian, D.; Mehrabi, M.R.; Jamshidi, S.h.; Farhangi, A.; Verdi, A.A.; Mofidian, S.M. and Rad, B.L. (2007):** Induction of diabetes by Streptozotocin in rats. Indian, J. Clin. Biochem., 22(2): 60-4.
2. **Amanat, S.; Ghahri S.; Dianatinasab, A.; Fararouei, M. and Dianatinasab, M. (2020):** Exercise and type 2 Diabetes. Adv. Exp. Med. Biol., 1228: 91-105
3. **Amin, MM; Sawhney, S.S. and Manmohan, S.J. (2013):** Comparative Anti-oxidant Power Determination of Taraxacum officinale by FRAP and DTPH Method Pharmaceut Anal Acta, 4:221.
4. **Arct, J. and Pytkowska, K. (2008):** Flavonoids as components of biologically active cosmeceuticals. Clin. Dermatol, 26: 347-57.

5. Bailey C, Day C. Metformin: its botanical background. Pract Diabetes Int 2004;21:115–7. https://doi.org/10.1002/pdi.606.
6. **Bancroft, J.D. and Gamble, M. (2008):** Theory and practice of histological techniques: Elsevier health sciences.
7. **Bonner, R.; Albajrami, O.; Hudspeth, J. and Upadhyay, A. (2020):** Diabetic Kidney Disease. Prim. Care. 47 (4) : 645-659.
8. **Borneo, R.; Leon, E.A.; Aguirre A.; Ribotta, P. and Cantero, j.j. (2008):** Anti-oxidant capacity of medicinal plants from the Province of Cordoba Argentina) and their in vitro testing in model food system. Food Chem., 112: 664 -70.
9. **Brunkhorst C, Schneider E. (2005):**Characterization of maltose and maltotriose transport in the acarbose-producing bacterium Actinoplanes sp. Res Microbiol; 156:851–7. https://doi.org/10.1016/j.resmic.2005.03.008.
10. **Di Carlo, G.; Mascolo, N.; Izzo, A.A. and Capasso F. (1999):** Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sci., 65:337-53.
11. **Doumas, B. T., Watson, W. A., & Biggs, H. G. (1971).** Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta, 31(1), 87-96.
12. **Duke, J.; Duke, P.K. and Cellier, JL (2002):** Handbook of Medicinal Herbs (second ed.), CRC Press, United States., 595.
13. **Eguilaz, M.; Batlle, MA; Morentin, B.; San-Cristóbal, S.; Pérez-Díez, S.; Navas-Carretero, S. and Martínez, G.A. (2016):** , Alimentary and lifestyle changes as a strategy in the prevention of metabolic syndrome and diabetes mellitus type 2: milestones and perspectives, An. Sist. Sanit. Navar., 39: 269 —289.
14. **Elkrief L, Rautou P-E, Sarin S, Valla D, Paradis V, Moreau R.**(2016):Diabetes mellitus in patients with cirrhosis: clinical implications and management. Liver Int ;36:936–48. https://doi.org/10.1111/liv.13115.
15. **Ellman, G.L. (1959):** Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82(1):70-7.
16. **Fabiny, D.L. and Ertingshausen, G. (1971):** Automated reaction-rate method for determination of serum creatinine with the CentrifiChem. Clin. Chem., 17(8): 696-700.
17. **Garcia-Compean, D.; Jaquez-Quintana J.O; Gonzalez-Gonzalez, J.A. and Maldonado-Garza, H.(2009):** Liver cirrhosis and diabetes: risk factors, pathophysiology, clinical implications and management. World J. Gastroenterol., 15(3):280-8.
18. **Gargouri, M.; Ghorbel-Koubaa, F.; Bonenfant-Magné, M.; Magné, C., Dauvergne, X., Ksouri, R., Krichen, Y.; Abdelly, C. and El Feki, A. (2012):** Spirulina or dandelion-enriched diet of mothers alleviates lead-induced damages in brain and cerebellum of newborn rats. Food Chem. Toxicol., 50:2303–2310
19. **Gauthier, E. R.; Madison, S. D. and Michel, R. (1997):** Rapid RNA isolation without the use of commercial kits: application to small tissue samples. PflügersArchiv., 433(5): 664-668.
20. **Gella, F. J.; Olivella, T.; Pastor, M. C.; Arenas, J.; Moreno, R.; Durban, R. and Gomez, J. A. (1985):** A simple procedure for the routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. Clinica. Chimica. Acta., 153(3): 241-247.
21. **Georgakouli, K.; Manthou, E.; Fatouros, I.G; Deli, CK; Spandidos, D.A; Tsatsakis, A.M; Kouretas, D.; Koutedakis, Y.; Theodorakis, Y. and Jamurtas, A.Z. (2015):** Effects of acute exercise on liver function and blood redox status in heavy drinkers. Exper. Ther. Med., 10:2015–2022
22. **González-Castejón M, Visioli F, Rodriguez-Casado A.(2012):** Diverse biological activities of dandelion. NutrRev.;70:534–47. https://doi.org/10.1111/j.1753-4887.2012.00509.x.
23. **Gornall, A. G.; Bardawill, C. J. and David, M. M. (1949):** Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177(2): 751-766.
24. **Hu, C. and Kitts, D.D. (2005):** Dandelion (Taraxacum officinale) flower extract suppresses both reactive oxygen species and nitric oxide and prevents lipid oxidation in vitro. Phytomedicine, 12(8):588-97.
25. **IBM. IBM SPSS Statistics for Windows**, Version 25.0. Armonk, NY: IBM Corp. 2019.
26. **Jucá MM, Cysne Filho FMS, de Almeida JC, Mesquita D da S, Barriga JR de M, Dias KCF, et al**.(2020): Flavonoids: biological activities and therapeutic potential. Nat Prod Res.;34:692–705. https://doi.org/10.1080/14786419.2018.1493588.
27. **Livak, K.J. and Schmittgen, T. D.(2001):** Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods, 25(4):402-408.
28. **Luo, X.L.; Liu, W.X.; Zhong, H.; Yan, Y.Q.; Feng, F.Q. and Zhao, M.J. (2021):** Synergistic effect of combined oyster peptide and ginseng extracts on anti-exercise-fatigue and promotion of sexual interest activity in male ICR mice. J. Funct. Foods, 86:104-700
29. **Marklund, S. and Marklund, G. (1974):** Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-74.
30. **Montgomery, H. and Dymock, J. (1961):** The determination of nitrite in water. Analyst, 86: 414-416.
31. **Owczarek, K. and Lewandowska, U. (2017):** The Impact of Dietary Polyphenols on COX-2 Expression in Colorectal Cancer. Nutr. Cancer., 69(8):1105-1118.
32. **Padmanabhan, P. and Jangle SN (2012):** Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. Int. J. Basic. Appl. Med. Sci.; 2 (1):109116.
33. **Pei, Z.; Deng, S.; Xie, D.; Lv, M.; Guo, W.; Liu, D. and Long, X. (2018):** Protective role of fenofibrate in sepsis-induced acute kidney injury in BALB/c mice. RSC advances, 8(50): 28510-28517.
34. **Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al.(2020):** The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLOS Biol.;18:e3000410. https://doi.org/10.1371/journal.pbio.3000410.
35. **Rock, R.; Walker, W. and Jennings, C. (1987):** Nitrogen metabolites and renal function. In 'Fundamentals of clinical chemistry'.(Ed NW Tietz) pp. 669–704: WB Saunders Company: Philadelphia.
36. **Rotruck, J.T.; Pope, AL; Ganther, H.E.; Swanson, A.B.; Hafeman, D.G. and Hoekstra W.G. (1973):** Selenium: biochemical role as a component of glutathione peroxidase. Science, 179(4073):588-90.
37. **Schütz K, Carle R, SchieberA.(2006):** Taraxacum—A review on its phytochemical and pharmacological profile. J Ethnopharmacol;107:313–23. https://doi.org/10.1016/j.jep.2006.07.021.
38. **Amanat, S.; Ghahri, S.; Dianatinasab, A.; Fararouei, M. and Dianatinasab, M. (2020):** Exercise and type 2 diabetes, Adv. Exp. Med. Biol., 1228 :91 —105
39. **Seo, S.; Koo, H.; An, H.; Kwon, K.; Lim, B.; Seo, E.; Ryu, D.; Moon, G.; Kim, H. and Hong S.H. (2005):** Taraxacum officinale protects against cholecystokinin-induced acute pancreatitis in rats. World J. Gastroenterol., 11(4):597–599.
40. **Shetty, S.; Kapoor, N.; Bondu, J. D.; Thomas, N. and Paul, T. V. (2016):** Bone turnover markers: Emerging tool in the management of osteoporosis. Indian Journal of Endocrinology and Metabolism, 20(6): 846.
41. **Singh, K.P.; Ahmad, A.H.; Hore, S.K.; Singh, V.; Lohani, M. and Rahal, A. (2006).:** Effect of Emblica officinalis on anti-oxidative and haematological parameters following mercury induced toxicity. in Proceedings of the 6th Annual conference of ISVPT, Patna, India.
42. **Ştefănescu, R.; Farczadi, L.; Huţanu, A.; Ősz, B.E.; Măruşteri, M.; Negroiu, A. and Vari, C.E.(2021):** Tribulus terrestris Efficacy and Safety Concerns in Diabetes and Erectile Dysfunction, Assessed in an Experimental Model. Plants (Basel).10(4):744.
43. **Tappel, A. L. and Zalkin, H. (1959):** Inhibition of lipid peroxidation in mitochondria by vitamin E. Arch. Biochem. Biophys., 80: 333–333.
44. **Tian, L.; Fu, P.; Zhou, M. and Qi, J. (2021):** Dandelion sterol improves diabetes mellitus-induced renal injury in in vitro and in vivo study. Food Sci. Nutr., 9(9):5183-5197
45. **Tietz, N.; Burtis, C.; Duncan, P.; Ervin, K.; Petitclerc, C.; Rinker, A. and Zygowicz, E. (1983):** A reference method for measurement of alkaline phosphatase activity in human serum. Clinical chemistry, 29(5): 751-761.
46. **Tilwari, N.P. and Shukla U.D. (2011):** Effect of five medicinal plants used in Indian system of medicines on immune function in Wistar rats Afr. J. Biotechnol., 10:16637–45.
47. **Usman, H.; Abdulrahman, F. and Ladan, A. (2007):** Phytochemical and anti-microbial evaluation of Tribulus terrestris L. growing in Nigeria Res. J. Biol. Sci., 2: 244–47.
48. **Wirngo, F.E.; Lambert, M.N. and Jeppesen, P.B. (2016):** The Physiological Effects of Dandelion (Taraxacum Officinale) in Type 2 Diabetes. Rev. Diabet. Stud.13(2-3):113-131.
49. **Zengin, G.; Guler, G.; Cakmar, Y. and Abdurrahman, A. (2011):**Anti-oxidant capacity and fatty acid profile of Centaurea kotschyi (Boiss. &Heldr.) Hayek var. Persica (Boiss.). Wagenitz from Turkey. Grasas. Y.

aceites. 62(1): 90-95..
50. **Zhang, Y.; Oliveira, A.N and Hood, D.A. (2020):** The intersection of exercise and aging on mitochondrial protein quality control. Exper. Gerontol., 131:110824
51. **Zhang, Y.; Sheng, L.; Liu, X.W.; Wei, J.; Liu, X.J.; Zhang, N.Y. and Wang, Z.Y. (2022):** Effects of different exercise on liver lipid accumulation and FGF21 secretion in obese rats. Chinese J. Appl. Physiol., 38:47–52

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52. **Zhao, G.y.; Di, D.h.; Wang, B.; Huang, X. and Xu, Y.j. (2015):** Effects of Mouse Hepcidin 1 Treatment on Osteoclast Differentiation and Intracellular Iron Concentration. Inflammation, 38(2):718-727.
53. **Zhou, G.; Ye, L.; Zhang, L.; Zhang, L.; Zhang, Y.; Deng, L. and Yao, H. (2014):** Association of myeloid cells of triggering receptor-1 with left ventricular systolic dysfunction in BALB/c mice with sepsis. Mediators of inflammation, 2014, 391492
54. **Zhu, K.X, Nie, S.P.; Tan, L.H.; Li, C.; Gong, D.M and Xie, M.Y. (2016):** A polysaccharide from Ganoderma atrum improves liver function in type 2 diabetic rats via anti-oxidant action and short-chain fatty acids excretion. J Agri Food Chem 64:1938–1944