Potential Effects of Thyme (*Thymus vulgaris L.*) and Nettle (*Uritca dioica L.*) on Oxidative Stress and Inflammation in Rats

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Abstract

This research examined nutrition value of thyme and nettle in diet and how were protected rats from the damaging effects of oxidative stress and inflammation. Forty-eight albino Sprague-Dawley strain rats weighing 180±5 g has been randomly assigned into eight groups, six of each, following an adaptation period for seven days. Group 1 has been fed on basal diet (negative control), whereas groups from(2-8) were induction with Lipopolysaccharide (LPS) (10 mg/ kg.bw. intraperitoneally). Group 2 positive control (+ve). Group 3 fed on basal diet supplemented with 5% thyme. Group 4 fed on basal diet addition to 5% nettle. Group 5 fed on basal diet supplemented with thyme 10%. Group 6 fed on basal diet supplemented with nettle 10%. Group 7 fed on basal diet supplemented with thyme 5% +5% nettle. Group 8 fed on basal diet supplemented with thyme 10% +10% nettle for 8 weeks. chemicals analysis for thyme and nettle were elevated. Hispathological examination for liver was determined. Outcomes demonstrated that, the groups of rats fed on thyme and nettle experienced greater gains in body weight than the (+ve) group. Compared with (+ve) control, concentration of enzymes of liver which include AST, ALP, ALT and total protein reduced significantly in groups fed on diet supplemented with thyme & nettle. When rats were given a diet consisting of thyme and nettle powder, both their levels of urea nitrogen as well as creatinine show considerable improvements. Lipid profile elevated in (+ve) group in comparison with negative control group. However, MDA have been reduced significantly in the Groups (3-8), in comparison with the (+ve) control group. Also, inflammatory markers reduced in groups of rats fed on thyme and nettle leaves compared to (+ve) control group. As a result, the thyme and nettle leaves had a positive influence on the oxidative stress and inflammation in rats.

Key Words: Lipid profile - kidney function – Antioxidant –Liver enzymes.

الملخص العربى

تم اجراء هذا البحث لمعرفه القيمه الغذائيه للزعتر والقراص وكيفيه تاثير ذلك على الحمايه من الاجهاد التاكسدى والالتهاب فى الفئران. تم استخدام ثمانيه واربعون فار من سلاله الالبينو تزن تقريبا (١٨٠± ٥ جم) .تم تقسيمهم عشوائيا الى ثمانيه مجاميع بعد اسبوع من التاقلم.المجموعه الاولى تغذت على الغذاء الاساسى كمجموعه ضابطه سالبه، بينما المجاميع من(٢-٨) تم حقنهم بماده الليبوبولى سكاريد (10 ملجم /كجم/من وزن الجسم فى الغشاء البريتونى) .وكانت المجموعه الثانيه مجموعه ضابطه موجبه .بينما المجموعه الثالثه تغذت على الغذاء المدعم ب٥% من الزعتر.تغذت المجموعه الرابعه على ٥% من القراص. بينما المجموعه الخامسه تغذت على ١٠% زعتر .والمجموعه السادسه تغذت على ١٠% من القراص .بينما تغذت المجموعه السابعه على خليط من ٥% زعتر +٥%قراص. والمجموعه الثامنه تغذت على خليط من ١٠%زعتر +١٠% قراص لمده ٨ اسابيع. تم عمل تحليل كيميائى لكلا من الزعتر والقراص وتم عمل هستوباثولوجى للكبد. اظهرت النتائج حدوث زياده فى معدل الوزن فى المجاميع التى تغذت على الزعتر والقراص مقارنه بالمجموعه الضابطه الموجبه. وايضا كان هناك هناك انخفاض ملحوظ فى انزيمات الكبد والبروتين الكلى مقارنه بالمجموعه الضابطه الموجبه فى المجاميع التى تغذت على الغذاء المدعم بالزعتر والقراص.ادى الغذاء المدعم بالزعتر والقراص الى تحسن فى مستويات اليوريا والكرياتينين فى السيرم. كان هناك زياده ملحوظه فى مستويات الدهون فى المجموعه الضابطه الموجبه مقارنه بالمجموعه الضابطه السالبه.على الرغم من ذلك كان هناك انخفاض فى مستويات المالونديهيد فى المجاميع (٣-٨) مقارنه بالمجموعه الضابطه الموجبه. بالنسبه لمؤشرات الالتهاب فى السيرم انخفضت فى المجاميع التى تغذت على الغذاء المدعم بالزعتر والقراص. ونتيجه لهذه النتائج يعتبر الزعتر واوراق القراص ذات تاثير ايجابى ويقلل الاجهاد التاكسدى والالتهاب فى الفئران.

الكلمات المفتاحيه: مستويات الدهون – وظائف الكلى –مضادات الاكسده –انزيمات الكبد.

Introduction

The production of reactive oxygen species (ROS) has a crucial role in the pathogenesis of multiple illnesses. Its potential association with inflammation has led to the suggestion that there may be a connection among oxidative stress & the activation/production of cytokines in precancerous states **(Adelani *et al*.,2020).** Oxidative stress a significant role in the activation of a variety of signaling pathways, which result in damage to tissues and inflammatory illnesses. **(Chatterjee, 2016).** Continuous damage to tissues and inflammation were the causes of the pathogenesis of chronic illnesses, such as tumors **(Khansari *et al*.,2009).** Inflammation that is induced by damage to tissue is a consequence of an interaction among both adaptive and innate immunity is defined by the simultaneous production of biomarkers of inflammation, such as cytokines and chemical mediators like ROS, persistent inflammatory stimuli **(Cardin *et al*., 2014).** ROS are produced by the response of cells to xenobiotics, including the production of cytokines, pathogenic attack, and oxidative metabolism of mitochondria. Nevertheless, such products are scavenged by a variety of antioxidant defense mechanisms, like superoxide & hydroxyl radical **(Mittler ,2017).** There are several defense mechanisms, like glutathione-related enzymes and superoxide dismutase (SOD). The pathogenesis of chronic and degenerative illnesses, such as tumors, is fundamentally influenced by an disturbance in the production of ROS in comparison to antioxidant activities, which results in oxidative stress **(Chang *et al*., 2014 ; Frijhoff *et al*., 2015and Liu *et al*., 2018).**

Thymus is an herbaceous subshrub or perennial belonging to the *Lamiaceae* family that is extensively scattered across the globe. **(Dong *et al*.,2023).** The Mediterranean diet has utilized thyme species as a traditional medicine for thousands of years, as they are crucial medicinal & aromatic plants **(Salehi *et al*., 2019).** Essential oils of thyme possess potent antimicrobial, antioxidant, antifungal, and anti-inflammatory characteristics **(Hong *et al*.,2020; Kim *et al*.,2012 and Ren *et al*.,2019).** Through assessment of antioxidant capacities of eight thymes, discovered that linalool and thymol were primarily responsible for their activity **Cutillas *et al*., (2018)**. The essential oils of thyme contain a variety of complex components, and the combination of multiple plant antimicrobials may have effects that are either additive or synergistic **(Pinto *et al*.,2023).** The Chinese native thyme species *Thymus quinqueco* status is also extensively utilized in folk medicine for management of a variety of conditions, including toothache, stroke dyspepsia, hypertension, chronic eczema, and acute gastroenteritis. The majority of essential oils are usually considered safe at a specific concentration are frequently used as agricultural products, agents for preservation of food, and medications due to their high biodegradability, ephemeral nature & volatility **(Falleh *et al*.,2020).**

Thyme was widely utilized in the culinary and pharmaceutical industries throughout Asia as a result of its condimental, nutritional, & therapeutic properties **(Salehi *et al*., 2018).** Investigations have demonstrated that extracts of thyme, principally polyphenols, exhibit anti-inflammatory, antioxidant, anti-tumor and anti-diabetic activities **(Kim *et al*.,2022).**

Nettle (*Urtica dioica L*.,) is a plant that is frequently detected in Europe, Asia, and Africa. This plant is currently obtaining attention as a highly nourishing diet. The fresh leaves are dried in powder or other forms. Numerous bioactive compounds are present in leaves. Phenolic acids, fatty acids, carotenoids, and flavonoids are among the constituents that are bioactive chemicals that were isolated from stinging nettle to date. Extracts of stinging that it contains, such as rutin, kaempferol, and vit. A, have been exploited for their nutritional benefits as well as their antioxidant & anti-inflammatory characteristics **(Devkota *et al*., 2022).**

The perinea herbaceous plant *Urtica dioica L*., which is frequently referred to as stinging nettle **(Figure 1)**, is a member of the *Urticaceae* family. In numerous regions of the globe, like Europe, North Africa, North America, and Asia, it is found in temperate regions at elevations of up to 1800 meters **(Jan *et al*., 2017).** It is frequently utilized as a vegetable and, following drying, as a stock food through shortages of food **(Grauso *et al*., 2020).** *Urtica dioica* is utilized in the treatment of pain in the joints through a process known as urtication (external stinging), which involves the local application of fresh stems as well as leaves **(Randall *et al*., 2000).** It has a lengthy history of being utilized as a traditional medicine and vegetable. *Urtica dioica* has been utilized as a diuretic, in the management of colds, wounds, cuts, and coughs in a variety of ethno medicinal investigations **(Bhusal *et al*., 2022).** This plant has recently garnered attention as a diet that is extremely nutritious. The fresh leaves are dried and utilized in powder or other forms. The leaves are abundant in a variety of compounds that are bioactive, including phenolic acids, flavonoids , and amino acids **(Grauso *et al*., 2020).**

The objective of this research is to point out natural antioxidants and nutrition value from thyme and nettle & their impact on oxidative stress & inflammation in experimental rats.

Figure 1. Photographs of *Urtica dioica*

Materials and Methods

Materials

1- Plant materials

Thyme has been attained from the Agricultural Research Center, Giza, Egypt. Nettle leaves have been obtained from Imtenan Health Shop, Obour City, Egypt.

2-Preparation of plant

Thyme and nettle have been cleaned, then milled with a blender to get the powder. The powder was subsequently stored in a polyethylene container at 5 ºC until it was needed for analysis.

3-Chemicals and kits for biochemical analysis

Lipopolysaccharide (LPS) and all other compounds have been purchased from Sigma Chemical Company for Trading Drug, Chemicals, and Medical Instruments, Cairo, Egypt. Kits for biochemical analysis of lipid profile (total cholesterol, HDL and total glycerides), liver functions (ALT, total protein, ALP, and aspartate aminotransferase), kidney functions (creatinine and urea), malondialdehyde (MDA), (GSH) , catalase, and inflammatory indicators (TNF-α and IL-6) have been acquired from Gama Trade Company in Dokki, Egypt.

4-Rats and diet

The Laboratory Food Technology Research Institute, Agricultural Research Center Ministry of Agriculture , Egypt, provided adult albino rats of the Sprague Dawley strain, weighing 180 ± 5grams. Al-Gomhoria Company for Trading Drugs and Chemicals, Cairo, Egypt, was the source of all constituents of the basal diet, including cellulose, D-L methionine, choline chloride, casein, minerals and vitamins. Starch, sucrose and soybean oil have been purchased from the local market in Cairo, Egypt.

Methods

Preparation of basal diet and diets with thyme and nettle

The nutritional needs of rats through the duration of the experiment were satisfied by the standard AIN-93 M basal diet, which has been prepared in accordance with the approved quantities of **Reeves *et al.*, (1993).** A complementary diet has been created by incorporating thyme and nettle into the basal diet at a rate of 5 & 10 %, respectively, separately or in combination.

Induction of oxidative stress and inflammation

A physiological saline solution was used to dissolve lipopolysaccharide (LPS). LPS (10 mg/kg b.w.) was administered intraperitoneally to the rats for five days in order to induce subclinical inflammation and oxidative stress. In alternative to LPS, the control group was given 2 ml/kg/week of saline. Five days later, optical blood samples were collected at random to test the levels of tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6).

Chemical analysis

Determination of (ash, fat, fiber, protein, moisture and carbohydrates calculated by difference), was carried out following the technique defined by **A.O.A.C. (2010).**

Experimental design

Following an adaptation period for one week, 48 Sprague-Dawley albino rats, have been randomly assigned to one of two primary groups that consisted of 6 rats. The 1st group has been maintained on basal diet as the (-ve) control, whereas the 2nd group consisted of forty-two rats that were fed on basal diet and injected with lipopolysaccharide (LPS) at a concentration of (10 mg/ kg .bw. intraperitoneally) for a period of five days according to **Asgharzadeh *et al*.,(2018).** The rats have then been divided into seven groups. Group 2 positive control (+ve). Group 3 fed on a basal diet supplemented with 5% thyme. Group 4 has been fed with a basal diet in addition to 5% nettle. Group 5 was provided with a basal diet that was supplemented with 10% thyme. Group 6 fed on a basal diet that was supplemented with 10% nettle. Group 7 fed on with a basal diet that was supplemented with 5% thyme and 5% nettle. Group 8 was administered a basal diet that has been supplemented with 10% thyme and 10% nettle ,for 8 weeks.

The animals have been housed in the Animal House of the Agricultural Research Center in Giza, Egypt, where they were confined in hygienic conditions at a temperature of 25±2 ºC , a moderate humidity of 56 %. Basic diet and water were accessible on an *ad libitum* basis. Blood samples were taken from the central canthus of the rats eyes using fine capillary glass tubes and placed in a centrifuge tube without any anticoagulant. The centrifuge was run for 15 minutes at 3000 r.p.m. The serum kept at -20°C until it was needed for further analysis. Liver was removed from each rat for histopathological examination.

Biological evaluation

Body weight has been monitored on a weekly basis throughout the 8 weeks experiment. The biological assessment of various diets has been conducted by estimating gain to body weight percentage (BWG %) in accordance with **Chapman *et al*., (1959).**

Biochemical analysis

The lipid profile, total cholesterol (TC), triglycerides (TG), and HDL-C of the serum have been assessed in accordance with **Allain *et al*., (1974); Trinder and Ann (1969),** and **Lopes-Virella *et al*., (1977),** respectively. Also, the LDL-C has been estimated in accordance with **Friedwald *et al*., (1972).** Alanine aminotransferase, alkaline phosphatase, total protein, and aspartate aminotransferase have been assessed in accordance with the reports of **Bergmeyer *et al*., (1985), Roy (1970), and Zaia *et al*., (2000),** respectively. **Fossati *et al*., (1980)** technique has been utilized to quantify the concentration of serum urea nitrogen. The measurement of creatinine was carried out utilizing a technique published by **Henry (1974).** malondialdehyde (MDA) , antioxidant enzyme activity glutathione (GSH) and catalase (CAT) with regard to the instructions of the manufacturer by **Draper and Hadly (1990); Hamad *et al*.,(2020) & Sinha, (1972).** Interleukin-6(IL-6) and tumor necrosis factor-alpha (TNF-α) have been evaluated according to **Hirano, (1998) and George *et al*.,(1999)**.

Histopathology investigation

The liver has been promptly fixed in 10% buffered neutral formalin after the animals were excised. The fixed tissue has been subsequently processed for histopathology examinations in accordance with the method established by **Carleton (1979).**

Statistical analysis

The SPSS application was used to examine the data. To determine whether there were statistically noteworthy variations among the groups, an ANOVA was performed (**SPSS, 1986).**

Results and Discussion

Chemical composition of thyme and nettle

Table (1): Chemical composition of thyme and nettle

Nutrients		Thyme	Nettle
Nutrients (g)	**Protein**	11.12	18.60
	Fat	3.60	1.15
	Carbohydrate	47.50	29.24
	Fiber	29.13	17.16
	Ash	4.90	24.05
	Moisture	3.75	9.8

Table (1) demonstrated averages of the chemical composition of thyme and nettle leaves powder which include (protein, carbohydrate, fat, fiber, ash and moisture). Carbohydrate had greatest average, followed by fiber, protein, ash, moisture, and fat, correspondingly, according to the chemical composition of thyme powder. According to the chemical composition of nettle, ash had the greatest average, followed by carbohydrate, protein, fiber, moisture, and fat, correspondingly.

These findings are disagreement with **Maria *et al*., (2019),** who illustrate that the powder of nettle leaves contained a high concentration of carbohydrates, protein, and minerals. Moisture and crude fat contents were relatively low. The nutritional characteristics of powder of dried leaves of *Urtica dioica* were compared to those of barley and wheat flours by **Adhikari *et al*., (2016).** In comparison to wheat flours and barley, the leaf powder of *Urtica dioica* contained a greater concentration of crude protein (33.8 %), crude fiber (9.1 %), crude fat (3.6 %), and carbohydrates (37.3 %), according to their report. The energy value has been determined to be 307 kcal/100 g . In terms of gallic acid equivalents, total carotenoid, tannin & phenolic contents were 129 mg, 3497 µg / g, and 0.93 mg/100 g, correspondingly. **El-Refai, (2020)** observed that the contents of ash (7.86 %), crude protein (9.67 %), and total carbohydrates (61.40 %) in dried thyme .

Table (2):The effect of thyme and nettle on gain of body weight & liver organ weight on rats with oxidative stress and inflammation.

Parameters / Groups	Body weight gain (BWG) (%)	Liver relative weight (%)
Group1:(-Ve)	263.40± 1.07[a]	3.27±0.13[a]
Group 2 :(+Ve)	115.20± 1.02[f]	2.16±0.27[b]
Group 3: (5% thyme)	194.60±1.36[e]	3.23±0.15[a]
Group 4: (5% nettle)	227.00 ± 1.67[c]	3.07±0.06[a]
Group 5: (10% thyme)	172.60 ±0.92[f]	2.84±0.13[b]
Group 6: (10% nettle)	214.60 ±1.43[d]	2.45±0.20[b]
Group 7: (5% thyme +5% nettle)	234.20±1.56[b]	2.78±0.15[b]
Group 8: (10% thyme +10% nettle)	273.20±1.46[a]	2.83±0.19[b]

*Values are represented as means ±SE.

* Values at the same column with various superscript letters are significantly different at $p< 0.05$.

In Table (2), the positive control group showed a reduction in BWG in comparison to the control (-ve). A significant rise has been detected in the mean values of all managed rats that were fed thyme & nettle leaves in comparison to the control (+ve) group. The group that achieved the highest BWG (%) findings was in group 8, which was fed 10% thyme and 10% nettle leaves. This group was comparable to the control group.

Table (2) illustrates the results of modifications to the relative weight of the liver. In comparison to the (-ve) group, the average value of the relative weights of the liver in the rats in the (+ve) control group reduced. In contrast to the group of positive controls, the liver relative weight was elevated in all groups except for the rats that were fed a diet supplemented with 5% thyme and 5% nettle.

No variance in the relative weight of the liver has been discovered in group of rats supplemented with essential oil and powder of thyme. Nevertheless, the relative weight of liver of the positive control group increased **(Sobhy *et al*., 2020).** Our findings are in disagreement with **Saeidi *et al*., (2019),** who found that groups of rat supplemented with thyme powder, essential oil exhibited reductions in feed intake and gain in weight. These decrements might be ascribed to reduced serum leptin concentration, which subsequently inhibits appetite. The composition of thyme contains salicylic acid, which resulted in a decrease in feed intake & an elevation in thermogenesis. The thermogenic effect subsequently induced a reduction in body weight **(Vizzari *et al*., 2019).**

Table (3): The effect of thyme and nettle on kidney function on rats with oxidative stress & inflammation.

Groups \ Parameters	Urea	Creatinine
	(mg/dl)	
Group1:(-Ve)	41.60±1.33^{b}	٠.78±0.02^{b}
Group 2 :(+Ve)	49.22±2.03^{a}	١.99±0.04^{a}
Group 3: (5% thyme)	34.64±1.15^{d}	١.71±0.01^{b}
Group 4: (5% nettle)	35.94±2.14^{d}	٠.76±0.02^{b}
Group 5: (10% thyme)	37.34±0.76^{c}	٠.78±0.03^{b}
Group 6: (10% nettle)	37.72±4.76 ^{c}	٠.76±0.06^{b}
Group 7: (5% thyme +5% nettle)	40.56±0.69^{b}	٠.75±0.01^{b}
Group 8: (10% thyme +10% nettle)	41.28±0.71^{b}	٠.77±0.05^{b}

*Values are represented as means ±SE.

* Values at the same column with various superscript letters are significantly different at $p < 0.05$.

The information in Table (3) demonstrates the impacts of thyme & nettle leaves on the serum levels of urea and creatinine in oxidative stress and inflammation rat models. The results exhibited a substantial variance between the lower value for serum urea levels obtained using the control negative group and the higher value obtained using the positive control group, which were 41.60±1.33 & 49.22±2.03 mg/dl, respectively.

Serum creatinine ranges varied extensively between the (+ve) control group & the(-ve) control group, with greater values being documented in the +ve control group. The averages for the relative means have been 1.99±0.04 and 0. 78±0.02 mg/dl. Contrarily group 7(5%thyme+5%nettle) created the best creatinine ranges amongst treated groups with variants that had been statistically significant.

Furthermore, every group of rats that were kidney-damaging given various diets contain nettle leaves powder indicates a significant decrease in mean values of urea, creatinine and uric acid concentrations in comparison the control positive group. The lowest values were documented for the group fed on 5% nettle powder with insignificant variance in comparison with the negative group **(Hassan *et al*.,2020).**

Table (4):The effect of thyme and nettle on enzymes of liver on rats with oxidative stress & inflammation.

Groups \ Parameters	AST	ALT	ALP	Total protein
	(U/L)			(g/dl)
Group1:(-Ve)	35.40±1.20[c]	23.60±0.92[d]	341.40±0.92[e]	3.68±0.13[b]
Group 2 :(+Ve)	54.60± 1.56[a]	39.20±1.31[a]	694.40±1.69[a]	4.81±0.06[a]
Group 3: (5% thyme)	41.80± 0.97[b]	31.80±1.82[b]	571.80±1.78[b]	3.80±0.24[b]
Group 4: (5% nettle)	37.20±0.97[c]	26.20±0.86[c]	404.00±2.91[c]	3.61±0.26[b]
Group 5: (10% thyme)	42.40±0.92[b]	30.40±2.65[b]	422.60±3.28[d]	3.73±0.26[b]
Group 6: (10% nettle)	40.00±0.70[b]	23.80±2.59[d]	305.20±1.71[f]	3.77±0.29[b]
Group 7: (5% thyme +5% nettle)	32.20±0.86[c,d]	25.80±1.15[c,d]	411.80±1.39[d]	3.53±0.17[c]
Group 8: (10% thyme +10% nettle)	31.80±0.91[d]	29.80±1.77[c]	333.60±2.66[e]	3.67±0.20[b]

*Values are represented as means ±SE.

* Values at the same column with various superscript letters are significantly different at $p < 0.05$.

Data in Table (4) confirmed the impacts of thyme & nettle leaves as powders on liver enzymes ranges (ALT, ALP, AST, and total protein) of rats. In the instance of the liver enzyme AST, the control (+ve) group had greater levels than the control(–ve) group, who had a significantly lower number. The average values were, respectively, 54.60± 1.56 and 35.40±1.20 U/L. On the other side group 7 recorded the best AST liver enzyme value of treated groups, whereas group 8 recorded the lowest value, with significant variations. The corresponding mean values were 32.20±0.86 and 31.80±0.91 U/L.

It is clear to point out that the greater ALT liver enzyme ranges recorded for the control (+ve) group, while control negative group recorded the decrease value with a substantial difference. The average numbers were 39.20±1.31 and 23.60±0.92 U/L, respectively.

Regarding the ALP liver enzyme, control positive group levels were greater, while control negative group levels were lower, with a significant difference. The average values were, respectively, 694.40±1.69 and 341.40±0.92 U/L. On the other hand, there was a substantial difference between the ALP liver enzyme levels of the managed groups, with the highest value being documented for group 3 and the lowest value being reported in group 6, the corresponding mean values were 571.80±1.78 & 305.20±1.71 U/L.

Data in Table (4) illustrated that total protein in the positive control group increased compared with the negative control group .All groups fed on a diet supplemented with thyme and nettle leaves had improvement in total protein compared to the positive group.

Normal functioning of the liver is indicated by the existence of normal concentrations of ALT and AST. The serum ALP concentration is elevated in obese rats due to the intracellular enzyme’s leakage from the liver cytosol into the bloodstream, which suggests hepatotoxicity

(Dauqan *et al*., 2012). Additionally, the administration of toxins, liver cirrhosis, hepatitis, and liver cancer all result in an increase in serum AST, ALP & ALT concatenation. Consequently, these enzymes might be regarded as indicators of the extent of liver injury **(Cui *et al*., 2011).**

Diabetic rats that were subjected to *Urtica dioica* leaves in their basal diet exhibited a decrease in AST and ALT levels when in comparison with control rats that were positive **Eldamaty, (2018).** Our findings corroborated those of **Joshi *et al*., (2015)** who claimed that the nettle has a powerful antioxidant fraction which supports the highest level of hepatoprotective potential because of its capacity to operate as a free radical scavenger, as illustrated by *in-vitro and in-vivo* antioxidant potential.

Table (5): The effect of thyme and nettle on malondialdehyde, catalase and glutathione on rats with oxidative stress & inflammation.

Parameters / Groups	Malondialdehyde	Catalase	Glutathione
	(nmol/g tissue)	**(U/g tissue)**	**(U/mg protein)**
Group1:(-Ve)	4.54 ± 0.52^{d}	30.58 ± 1.87^{c}	3.57 ± 0.13^{b}
Group 2 :(+Ve)	33.70 ± 1.54^{a}	22.04 ± 1.66^{e}	2.41 ± 0.20^{c}
Group 3: (5% thyme)	21.62 ± 0.70^{b}	41.20 ± 1.88^{a}	3.61 ± 0.15^{b}
Group 4: (5% nettle)	14.42 ± 0.84^{c}	$32.64 \pm 1.97^{b,c}$	3.56 ± 0.04
Group 5: (10% thyme)	$10.96 \pm 0.89^{c,d}$	29.59 ± 1.20^{d}	3.94 ± 0.05^{b}
Group 6: (10% nettle)	$10.64 \pm 0.25^{c,d}$	36.50 ± 1.95^{b}	3.62 ± 0.10^{b}
Group 7: (5% thyme +5% nettle)	7.34 ± 0.77^{d}	34.17 ± 1.50^{b}	4.09 ± 0.10^{a}
Group 8: (10% thyme +10% nettle)	7.24 ± 0.38^{d}	28.40 ± 1.40^{d}	3.13 ± 0.11^{b}

*Values are represented as means ±SE.

* Values at the same column with various superscript letters are significantly different at p< 0.05..

Compared to the normal control group, the serum MDA concentration in rats in (+ve) control group has significantly been elevated ($p < 0.05$), whereas the concentrations of catalase and GSH have been significantly reduced ($p<0.05$) (Table 5). Conversely, the positive control group exhibits significant ($p<0.05$) increases in MDA concentrations in comparison with the groups that are fed thyme, nettle leaves, or a combination of both. Compared to the positive control group, the same examined materials exhibit statistically significant ($p<0.05$) elevated concentrations of catalase and GSH. Group 8 exhibited the greatest enhancement in MDA and GSH, while groups 3was the best result in catalase.

Lipopolysaccharide (LPS) has the potential to induce the accumulation of reactive oxygen species, which in turn reduces lipid peroxidation and antioxidant defenses of biological membranes. This is followed by a rise in the production of MDA, which ultimately aggravates kidney , liver injury and leads to numerous pathological alterations **(Gao *et al* ., 2021).**

Any abnormal condition leads to free radicals being extremely generated, resulting in oxidative stress a disturbance in the oxidant per antioxidant system. In contrast to their overproduction and inadequate elimination, which leads to irreversible and destructive damage to cells, the production of free radicals is an essential characteristic of cells that are normal. In the body natural state, the system of natural defense that has a significant role in detoxifying free radicals is composed of multiple enzymes, including CAT, and nonenzymatic antioxidants, like GSH. The correlation of many illnesses with oxidative stress has led to the widespread utilization of food supplements or foods that are rich in antioxidants (**Saravanan and Pari, 2015; Yu *et al*., 2016& Vetvicka and Vetvickova, 2016).** Antioxidant effect of thyme powder is attributed to presence of a variety of compounds, including phenolic components and flavonoids according to **(Thamer *et al*., 2018).**

It has been established through a variation of in *vitro and in vivo* approaches that thyme possesses antioxidant properties. For instance, **Wisam *et al*., (2017)** demonstrated that *T. vulgaris* had both antioxidant activity and reducing capacity. This may be attributed to the increased concentration of total phenols and flavonoids that has been detected in the plant. The redox properties of the phenolic compounds were principally responsible for the antioxidant effects that they exhibited. These qualities could have a significant role in the neutralization and adsorption of free radicals. An investigation into the *in vitro* antioxidant activity of thymol, an important polyphenolic compound in thyme. The findings indicated that thymol exhibited antioxidant activity also, could prevent development of hyperlipidemia associated with a diet high in fat and atherosclerosis through minimizing oxidative stress , serum lipids, as well as by reducing aortic intimal lipid lesions **(Yu *et al*., 2016)** .

Table (6):The effect of thyme and nettle on lipid profile on rats with oxidative stress & inflammation.

Groups \ Parameters	TC	TG	HDL	LDL
	(mg/dl)			
Group1:(-Ve)	81.76±0.99[c]	86.16±2.08[c]	61.88±1.24[b]	64.18±1.22[b,c]
Group 2 :(+Ve)	110.34±3.77[a]	121.34±1.53[a]	44.40±1.87[c]	76.48±3.31[a]
Group 3: (5% thyme)	85.08±1.81[c]	98.82±1.20[b]	59.40±1.61[b]	65.78±1.58[b]
Group 4: (5% nettle)	91.16±3.64[b]	83.50±1.18[c]	62.52±1.95[a,b]	68.42±3.68[b]
Group 5: (10% thyme)	74.88±1.45[d]	73.60±1.34[d]	58.46±2.55[b]	67.66±2.32[b]
Group 6: (10% nettle)	82.02±0.99[c]	75.80±1.90[d]	64.42±3.83[a]	63.66±2.31[c]
Group7:(5%thyme+5% nettle)	78.70±2.31[d]	73.46±2.34[d]	64.68±2.42[a]	63.96±1.44[c]
Group8:(10%thyme+10% nettle)	75.46±1.59[d]	71.98±0.67[d]	63.54±1.96[a]	62.80±2.18[c]

*Values are represented as means ±SE.

* Values at the same column with various superscript letters are significantly different at $p < 0.05$.

The outcomes in table (6) indicate that there was a significant rise ($p < 0.05$) in (cholesterol, triglyceride, and LDL) in the (+ve) control group in comparison to (-ve) control group. While these parameters reduced in all managed groups, particularly group 5 and group 8, correspondingly. Conversely, the HDL variable in (+ve) control group decreased in comparison to (-ve) control group. Nevertheless, HDL levels elevated in rats that have been fed a diet that contained either thyme or nettle leaves, either individually or in combination.

These findings are consistent with **Eldamaty, (2018)** observation that powder of leaves of *Urtica dioica* has reduced the concentrations of lipoproteins and lipids in the blood. Total cholesterol, cholesterol fractions, and ratios of LDL/HDL were all found to have significantly decreased due to lower LDL levels. Our results corroborate the claims of **Nassiri-Asl *et al*., (2009)**, who asserted that the pulverized nettle leaves had a reduction in blood lipoprotein and lipid concentration. On the lipid profile, which includes total cholesterol, cholesterol fractions, and ratios of LDL/HDL, reduced concentrations of low-density lipoprotein cholesterol & plasma total apo-protein B have been demonstrated to be highly influencing.

The composition of essential oil produced by *Urtica dioica* stem and leaves comprises numerous compounds that are bioactive, like 2-pentyl furan, linalyl acetate, α-terpineol, cadina, nonanal, copaene, eugenol, butylidene phthalide, limonene, calamenenemethyl chavicol, β-caryophyllene, carvone, (E)-geranyl acetone, linalool, β-selinene, carvacol, furanone, cumin aldehyde, pentyl benzene, caryophyllene oxide, cadinenen, hexagydrofarnesyl acetone, bisabolene, phytol, anethol, naphthalene, and kessane **(Ahmadipour and Khajali, 2019).** A significant elevation in the expression of antioxidant genes like glutathione peroxidase, glutathione, SOD, and CAT in the hippocampal tissue of streptozotocin-induced diabetes Wistar rats that were nourished with daily oral gavage of leaves of *Urtica dioica* hydro alcoholic extract for a period of 6 weeks. Additionally, the extract significantly reduced the amount of lipid peroxidation in hippocampus tissue as compared to diabetic rats **(Rahmati *et al*., 2021).** Additionally, **Eldamaty ,(2018)** described that the nettle leaves very excessive polyphenol content material can also extensively contribute to fitness benefits such as lowering TC, and TG levels.

Table (7):The effect of thyme and nettle on serum concentrations of interleukin -6and tumor necrosis factor-alpha on rats with oxidative stress & inflammation.

Parameters / Groups	IL-6	TNF-α
	pg/mL	
Group1:(-Ve)	129.06±1.59^{d}	154.56±1.41^{c}
Group 2 :(+Ve)	147.08±1.80^{a}	200.94±1.68^{a}
Group 3: (5% thyme)	130.02±1.66^{b,c}	153.26±1.05^{c}
Group 4: (5% nettle)	133.74±1.49^{b}	163.80±1.52^{b}
Group 5: (10% thyme)	125.78±5.03^{e}	114.12±1.95^{d}
Group 6: (10% nettle)	131.16±2.02^{c}	166.00±1.99^{b}
Group 7: (5% thyme +5% nettle)	135.22±1.38^{b}	164.82±1.57^{b}
Group 8: (10% thyme +10% nettle)	122.96±2.01^{e}	165.34±1.75^{b}

*Values are represented as means ±SE.
* Values at the same column with various superscript letters are significantly different at $p < 0.05$.

Table (7) indicates that interleukin -6 is elevated in the (+ve) control group in comparison with the (-ve) control group with a mean value of 147.08±1.59pg/mL and 129.06±1.59 pg/mL, respectively. when inflammation rats were nourished on diet supplemented with thyme and nettle leaves at any levels of intake interleukin -6 concentration in serum was decreased significantly compared to (+ve) group. The best findings were in group 8 which fed on 10%thyme and 10% nettle leaves.

For serum tumor necrosis factor-alpha concentration elevated significantly in the positive control group compared to negative control group with a mean value of 200.94±1.68 pg/mL and 154.56±1.41 pg/mL, respectively. when inflammation rats were fed on a diet supplemented with thyme and nettle leaves at any levels of intake tumor necrosis factor-alpha concentration in serum has been reduced significantly than positive group. The best outcomes were in group 5 which nourished on 10%thyme.

According to **Li *et al*., (2014),** inflammation is thought to be an important contributor to a number of illnesses, including osteoarthritis, cardiovascular illness, neurodegenerative illnesses, and obesity. Inflammation is induced by oxidative stress, which is mediated by the activation of NF-κB. This is followed by the expression of pro-inflammatory genes, including IL-8, tumor necrotic factor alpha, IL-1β, & inducible nitric oxide synthase (iNOS) **(Han *et al*., 2007).** Thyme and its extracts have been traditionally utilized worldwide for the management of inflammatory illnesses, & many investigations have demonstrated their anti-inflammatory properties **(Lorenzo *et al*., 2019).**

An investigation on fifty rats with rheumatoid arthritis to assess the anti-inflammatory impacts of thymol (100 mg/kg orally) or nicotine (2.5 mg/ kg orally) either individually or in combination. The results obtained indicated that thymol and nicotine each reduced IL-1β, IL-6, tumor necrotic factor alpha, IL-17, & IFN levels. However, the combination of thymol and nicotine (15 and 1.25 mg /kg, correspondingly) resulted in a more significant reduction in IL-17, IL-1β, C-reactive protein, & myeloperoxidase (**Golbahari and Abtahi Froushani ,2019).** In addition, **Habashy *et al*., (2018)** investigated the anti-inflammatory properties of Greek *T. vulgaris* oil , water extracts and their capacity to detoxicate lipopolysaccharide (LPS)-induced inflammation and oxidative stress. These extracts had the ability to decrease the LPS-induced rise in cyclooxygenase (COX)-2, nuclear factor-kappa B (NF-κB), tumor necrotic factor alpha, inducible nitric oxide (NO) synthase, NO, and they produced a stronger attenuating effect compared to dexamethasone for the majority of investigated inflammatory mediators, based on their findings.

Comparative analysis of *Urtica dioica* extract from a variety of plant portions (leaves, stems, flowers and roots) in aqueous hexane, dichloromethane & methanol solvents. The dichloromethane extract of stems, leaves and roots exhibited a stronger anti-inflammatory activity in the LPS-stimulated murine macrophage cell line than any other solvent extract in the NF-κB luciferase assay. This investigation implies that the lipophilic dichloromethane extract of leaves stems, and roots of nettle may be a more promising anti-inflammatory compared to the conventional tincture, which is prepared from methanol, ethanol, & an aqueous solvent (**Johnson *et al*.,2013).**

In streptozotocin-induced diabetes, the expression of protein of pro-inflammatory cytokines IL-1β and tumor necrotic factor alpha in hippocampal tissue was significantly reduced. In comparison to diabetic rats that weren't administered the extract, Wistar rats that were administered a daily dose of hydroalcoholic extract *Urtica dioica* leaves (15mg protein /kg body weight) for six weeks. This investigation highlights the possibility of extract o *Urtica dioica* to reduce neuroinflammation in hippocampus **(Rahmati *et al*.,2021).**

Histopathological examination of liver

Light microscopic examination of sections of liver of rats from group one indicates no histopathological alterations (Photo 1). Meanwhile, liver of rats from group two demonstrated hepatocellular vacuolization, sparsely necrosis of hepatocytes (Photo 2). On the other hand, liver of rats from group three exhibited slight hydropic degeneration of hepatocytes (Photo 3). Furthermore, liver of rats from group four revealed slight cytoplasmic vacuolization of some of hepatocytes (Photo 4). Otherwise, liver of rats from group 5 exhibited normal histological structure of hepatic tissue (Photo 5). In adverse, liver of rats from group 6 demonstrated hepatocellular vacuolar degeneration (Photo 6). Meanwhile, liver of rats from group seven demonstrated mild lesions characterized by slight hydropic degeneration of some hepatocytes, slight congestion of central vein (Photo 7). Furthermore, marked regression of lesions has been observed in liver of rats from group 8, examined sections demonstrated no histopathological alterations (Photo 8). These results are in accordance with the outcomes of (**El-Newary *et al*., 2017 and Elgaml and Hashish ,2014)** According to their findings, thymus vulgaris extract can protect rats from cadmium-induced hepatotoxicity, according to liver histopathology data. The acute hepatotoxicity brought on by cadmium poisoning was lessened by thyme extract. By reducing oxidative stress and scavenging the free radicals that cause tissue damage, the antioxidant protection system prevents the oxidation of lipids **(Lee *et al*., 2005).**

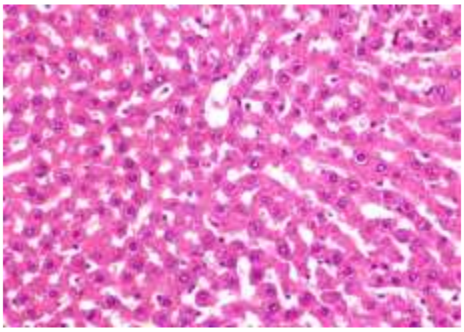

Photo (1): Photomicrograph of liver of rat from group 1 demonstrating no histopathological changes (H & E X 400).

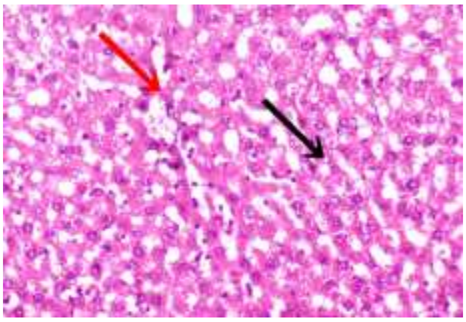

Photo (2): Photomicrograph of liver of rat from group 2 demonstrating hepatocellular vacuolization (black arrow) & sparsely necrosis of hepatocytes (red arrow) (H & E X 400).

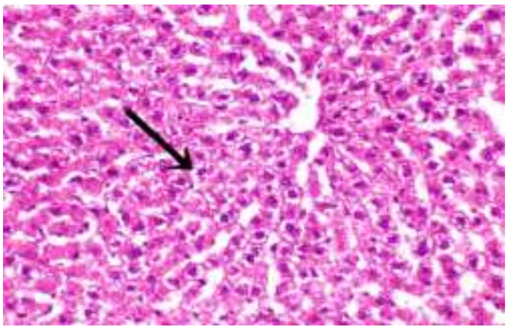

Photo (3): Photomicrograph of liver of rat from group three demonstrating slight hydropic degeneration of hepatocytes (black arrow) (H & E X 400).

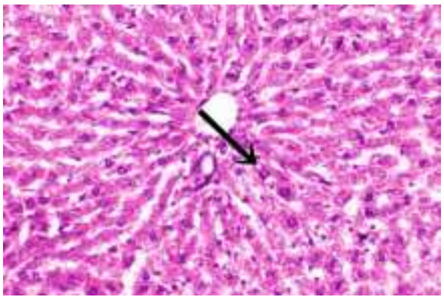

Photo (4): Photomicrograph of liver of rat from group four demonstrating slight cytoplasmic vacuolization of some of hepatocytes (black arrow) (H & E X 400).

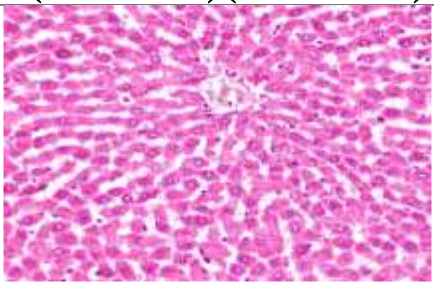

Photo (5): Photomicrograph of liver of rat from group 5 demonstrating normal histological structure of hepatic tissue (H & E X 400).

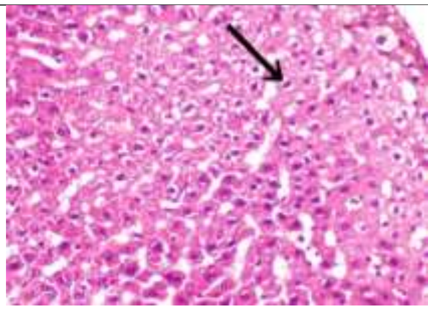

Photo (6): Photomicrograph of liver of rat from group 6 demonstrating hepatocellular vacuolar degeneration (black arrow) (H & E X 400).

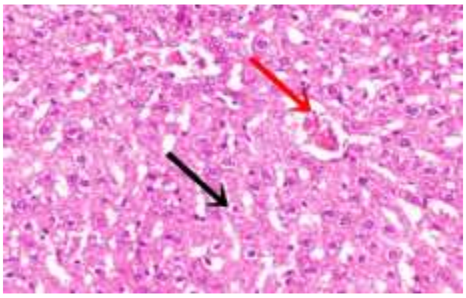

Photo (7): Photomicrograph of liver of rat from group 7 demonstrating slight hydropic degeneration of some hepatocytes (black arrow) & slight congestion of central vein (red arrow) (H & E X 400).

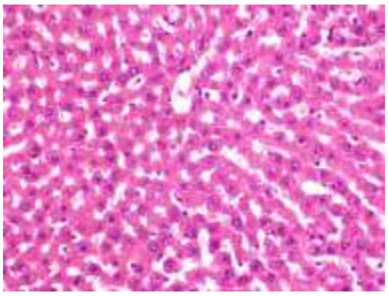

Photo (8): Photomicrograph of liver of rat from group 8 demonstrating no histopathological changes (H & E X 400).

In conclusion

Thyme and nettle leaves worked well to reduce inflammation and oxidative stress. Our findings demonstrated that the active ingredients in thyme and nettle improved chemical parameters and decreased free radicals. Because nettle leaves and thyme have strong nutritional and therapeutic benefits, particularly for patients with oxidative stress and inflammation, we recommended including them in our daily meals and drinks.

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