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Anti-anemic effect of *Thymus vulgaris* L. leaves powder on iron deficient rats

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Abstract

Herbal plants and their active ingredients are becoming more important for natural and safe use as compared to synthetic medications. For this purpose, the pharmacological impact of *Thymus vulgaris* (thyme) leaves powder to mitigate iron deficiency anemia was observed. Thyme leaves powder was subjected to nutritional analysis. To explore the anti-anemic effect, thyme leaves powder (G_1:10 g, G_2: 20 g and G_3: 30g) was fed to animal subjects. Forty female Sprague Dawley rats were allocated into four groups and each group fed for 6 weeks. The blood samples for serum biochemical parameters, hematological indices as well as liver and kidney function tests were analyzed at intervals of 0, 21 and 42 days. Results revealed that dried thyme leaves powder had high contents of proteins (20.43%), fiber (13.68%), nitrogen free extract (44.05%) as well as mineral contents. Feed intake and body weight gain were increased significantly ($p<0.05$) in experimental groups. The highest feed intake and body weight gain was observed in G_3 i.e. (22.35 ± 0.86 g per rat per day diet) and (112.97 ± 1.28 to 169.91 ± 1.12 g per rat), respectively. Similarly, G_3 showed more increase in serum biochemical profile and hematological indices during efficacy study as compared to G_1 and G_2. Liver and kidney functioning values showed decreasing trend throughout the study period of 42 days. The results of present study conclude that the strong nutritional composition of thyme leaves powder has a safe and therapeutic role against the treatment of iron deficiency anemia.

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Introduction

Dietary iron deficiency is one of the most prevalent nutritional problems worldwide that ultimately results in anemia. Iron deficiency affects around 2 billion people globally and most of the cases are children and women of reproductive age. Iron deficiency anemia has deteriorated effect on brain development as well as working capability in children, adults, fertile and pregnant women (Pasricha, 2012). Iron is an essential micronutrient and its unavailability along with marginal sufficiency and deficiency is very common in many countries (Bailey *et al*., 2015). The significance of this metal increases due to it pivotal roles in neuronal processes such as myelination of axons, neurotransmitter synthesis as well as provision of copious element in the brain. It is also an important metal for oxygen transportation to all the cells of the human body and accounts for 30% in ferritin, 65% in hemoglobin level, 5% in myoglobin and 0.3% in enzymes and cytochrome (Abbaspour *et al*., 2014; McCarthy and Kosman, 2015). Most of the dietary iron is taken in ferric form (Fe^{+3}) but it is readily absorbed in ferrous state (Fe^{+2}). Dietary sources of iron as classified as heme and non-heme iron and heme iron is highly bioavailable in meat and absorption of heme iron is not affected by other factors in foods. Non heme iron is present in dairy products, cereals, vegetables and herbal plants. Its absorption is inhibited by and tannins, phytates, calcium, phosphate and gastrointestinal acidity. Non heme iron absorption is influenced by various factors like vitamin C concentration, body needs, carbohydrates and protein intake (Sotelo *et al*., 2010; Weinborn *et al*., 2015; Wallace, 2016). Anti-anemic drugs due to limited efficacy and unfavorable interaction with nutrients are being replaced by alternate efficient and favorable natural products. Thus; there are definite preferences for natural herbal plants as alternative medicine which not only improve the nutritional status but also increase serum iron, serum ferritin level and overall hematologic parameters like Hb, RBC, MCV and hematocrit in nutritionally marginalized population. Currently, 25% of medicines available in market contain one or more active ingredients from natural herbs considering natural and safe where majority of contemporary drugs are still deprived of that perception (Orhan, 2014). Mint family *Lamiaceae* comprises aromatic plants like thyme of great economic and scientific importance. Thyme also known as common thyme is an aromatic herb. Bioactive compounds of thyme namely thymol, carvacrol and *p*-cymene are *p*-menthane type of aromatic monoterpenes, found in essential oil. These two phenolic monoterpenes are acknowledged for their antioxidant and antimicrobial potential. Leafy parts and essential oils of thyme have been used in foods for aroma, flavor and preservation and therefore added to meat, food products and herbal therapeutic products (Naghdi Badi *et al*., 2017). The mineral composition of thyme makes it an excellent source of calcium, potassium, magnesium, iron, zinc, manganese, copper as well as a good source of vitamin A, C and K. The phytochemicals, bioactive ingredients, iron and vitamin C enriched thyme can potentially be utilized in combating vitamin C and iron deficiency diseases such as scurvy, bleeding of gums, impaired wound healing and anemia (Dauqan and Abdullah, 2017). Keeping in view the therapeutic potential and mineral profile of thyme, the current project was designed to use thyme leaves powder to combat iron deficiency anemia using animal subjects and to explore the effect of leave powder on related biological parameters.

Materials and methods

Preparation of raw material

Dry thyme leaves were purchased from a local supermarket and ground into powder to pass through 1mm mesh size sieve using commercial grinder and stored in plastic bags at 5±1°C until use.

Nutritional composition of raw material

Chemical analysis

Moisture content of thyme leaves was analyzed using air forced draft oven. Samples were dried at 105 ± 5°C up to constant weight and values were calculated by using AACC (2000), method No. 44-15A. Crude proteins were measured by determining nitrogen contents through Kjeltech Apparatus and then multiplying percent nitrogen with conversion factor (AOAC, 2006). Crude fat was estimated using Soxtec System (Model: H-2 1045 Extraction Unit, Hoganas, Sweden).

Sample of 5g was taken out for extraction of crude fat using petroleum ether. Left over residue was dried until constant weight (AACC, 2000). After fat extraction, samples were referred to determine crude fiber through Labconco Fibertech (Labconco Corporation Kansas, USA). Digestion of 2g fat free sample was carried out with 1.25% NaOH and 1.25% H_2SO_4. After that the residue was dried at 130°C for 2h and ignited at 550±15°C and cooled ash was save for further analysis (AOAC, 2006). The sample was burned (charred) on burner till no smoke before ignition in the Muffle Furnace (MF–1/02, PCSIR, Lahore, Pakistan) to get residue of white-grayish color (AACC, 2000). Nitrogen free extract (NFE)% was calculated by difference of 100 minus moisture%, crude protein%, crude fat%, fiber% and total ash%).

Mineral profile

The mineral contents were determined by following the methods of AOAC (2006) through atomic absorption spectrophotometer (Model: Varian AA–240, Victoria, Australia).

Experimental animals and diet

Forty female rats of Sprague-Dawley strain, weighing 100-120g were purchased from National Institute of Health, Islamabad, Pakistan. The rats were fed on basal diet composed of corn oil (10%), casein proteins (10%), corn starch (66%) cellulose (10%), mineral (3%) and vitamin mixture (1%) for one week before the starting the experiment. The rats were randomly assigned to four groups (10 rats for each) and housed in stainless steel wire cages under controlled room conditions of 24±2°C and 55±5% relative humidity with 12 h of light - dark cycle. All rats had free access to drinking water and diets for 6 weeks duration. All groups of rats were induced anemic through intraperitoneal injection of 3.5mg phenylhydrazine/kg body weight (Uni-Chem Chemical Reagents).

Study design

The first group was fed on basal diet without the supplementation of thyme leaves powder throughout the study period denoted as control group. Second group was fed on diet containing 10g of thyme leaves powder and replacing with same percentage of starch. The third group was fed on diet containing 20g thyme leaves powder while the fourth group was fed on diet containing 30g thyme leaves powder. Gain in body weight of each rat in all groups was measured on 0, 21 and 42 day. The feed intake by each rat was determined by deducting the spilled and left-over diet from total feed provided each day. Blood collected at 0, 21 and 42 day after 12 h of food deprivation. The blood samples were collected from the portal vein in tube containing ethylene diamine tetra acetic acid (EDTA) for the determination of Hb, hematocrit, RBC, MCV and without EDTA for the determination of serum iron, serum ferritin, TIBC and transferrin saturation fraction.

Serum biochemical profile

Serum ferritin was determined by Radio-immuno assay with some modifications (Sera *et al.*, 2011). Serum iron was determined using atomic absorption spectrophotometer as described previously (AOAC, 2006). Transferrin was calculated in order to measure the total iron binding capacity (TIBC) and then transferrin saturation fraction by following the methods of Al-Buhairan and Oluboyede (2001). For the determination of TIBC, following equation was used:

TIBC = Transferrin × 24

Transferrin saturation fraction (%) was calculated using the following equation:

Transferrin saturation fraction =

$$\frac{\text{Serum Iron Concentration}}{\text{TIBC}} \times 100$$

Hematological indices

Blood Hb, hematocrit, RBC and MCV were measured using automatic blood CP analyzer (Medonic M-Series, Germany) as described previously (Al-Haj *et al.*, 2011).

Liver and kidney functioning tests

Liver function tests including AST and ALT were measured as previously by (DNPH-method) using Sigma Kits and ALP was performed by adopting the alkaline phosphates (DGKC- method) (Basuny *et al.*, 2009). Renal response for the tested fortified diets was estimated using Commercial Kits. Serum urea (GLDH-method) and creatinine (Jaffe-method) levels were recorded to assess the renal functionality of different groups (Kumari *et al.*, 2017).

Statistical analysis

A randomized, controlled, double-blind study for diet was conducted for the period of 6 weeks. The descriptive data presentation is shown as means with standard deviations. Differences between groups were assessed by two-way ANOVA for ordinal data with group using statistical analysis system, version 8.1. The significance level was set at $p < 0.05$ for all the tests.

Results and discussion

Nutritional composition of raw material

The proximate and mineral analysis of dry thyme leaves powder is presented in Table 1. The results showed that thyme is rich in macronutrients. The moisture, crude protein, crude fat, ash, NFE and fiber were 7.42 ± 0.17, 20.43 ± 0.24, 4.59 ± 0.23, 12.33 ± 0.29, 44.05 ± 1.79 and 13.68 ± 1.51% respectively. Tomescu *et al.* (2015) also conducted proximate analysis of six different medicinal plants including thyme and observed some variations in moisture, proteins and fat content.

The macronutrients composition of wild thyme cultivated in hot season is different from that one cultivated in winter. The nutritional composition of thyme like other plants varied according to the plant's age, harvesting time and season, difference in variety, changes in weather pattern and type of fertilizers as well as minerals provided in the soil (Rehman and Adnan, 2018). Data regarding mineral profile shows that thyme is also a mineral rich herbal source. Its leaves had highest concentration of calcium among macro minerals followed by potassium and sodium. Calcium, sodium and potassium were determined as 1892.01 ± 2.69, 51.37 ± 1.76 and 817.77 ± 3.77mg/100g respectively, whereas, iron and zinc content in dry leaves were detected as 122.80 and 5.88mg/100g respectively.

Similar trend as in current study was reported regarding mineral composition of *Thymus capitatus* which detected highest concentration of calcium among macro minerals followed by potassium and sodium and among micro minerals concentration, iron level was higher than zinc (Khalil *et al.*, 2012).

Most frequent effect was observed on mineral composition due to climate changes, soil condition, soil type and harvesting time besides the genetic type.

Table 1. Nutritional composition of thyme leaves powder (*Thymus vulgaris*).

	Nutrients	Composition value
Proximate composition	Moisture (%)	7.42±0.17
	Crude protein (%)	20.43±0.24
	Crude fat (%)	4.59±0.23
	Crude fiber (%)	13.68±1.51
	Ash (%)	12.33±0.29
	Nitrogen free extract (%)	44.05±1.79
Mineral composition	Calcium (mg/100g)	1892.01±2.69
	Sodium (mg/100g)	51.37±1.76
	Potassium (mg/100g)	817.77±3.77
	Iron (mg/100g)	122.80±1.56
	Zinc (mg/100g)	5.88±0.24

Feed intake and body weight gain

It was observed that thyme leaves powder supplementation acts as appetizer and led to increment the feed intake. The results of feed intake and body weight gain are shown in Fig. 1 and 2, respectively. The feed intake showed significant differences as function of dose of thyme leaves powder and study period. The highest feed intake was observed in G_3 followed by G_2 and G_1 that consumed 22.35 ± 0.86, 21.13 ± 0.94 and 19.27 ± 0.79g per rat per day diet, respectively. Whereas, the least preferred diet was observed in G_0 anemic control group (17.65 ± 0.72g per rat per day). The results of present study showed that the feed intake of the groups that received thyme diet increased significantly as the level of thyme powder increases. It was observed that consumption of thyme acted as appetizer and led to increase the feed intake and body weight gain due to higher intake of feed compared to rats that took less quantity of thyme or to rats that were not fed on thyme diet (El-Sheikh, 2008). Previous studies demonstrated that concentration of thyme less than 5g/kg had no significant effect on body weight gain however; significant difference in body weight gain was observed when thyme leaves powder fed on above 5g/kg of diet (Saleh *et al.*, 2014).

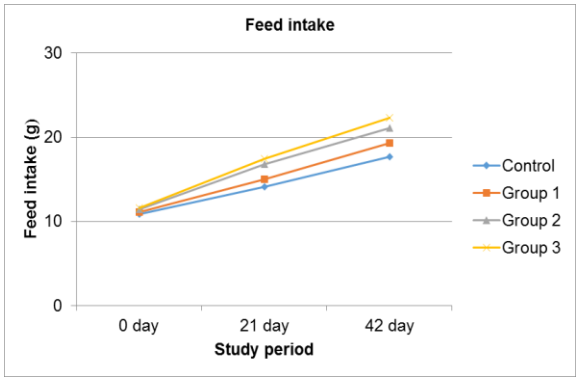

Fig. 1. Shows the effect of thyme leaves powder on feed intake in rats during 6 weeks. Four groups of rats were induced anemia through intraperitoneal saline injection of 3.5mg phenylhydrazine/kg body weight.
Control: Anemic group feeding on diet without thyme leaves powder
Group 1: Anemic group feeding on diet containing 10 g thyme leaves powder
Group 2: Anemic group feeding on diet containing 20 g thyme leave powder
Group 3: Anemic group feeding on diet containing 30 g thyme leave powder

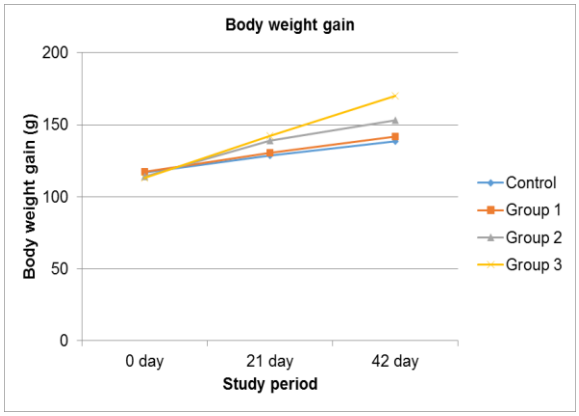

Fig. 2. Shows the effect of thyme leaves powder on body weight gain in rats during 6 weeks. Four groups of rats were induced anemia through intraperitoneal saline injection of 3.5mg phenylhydrazine/kg body weight.
Control: Anemic group feeding on diet without thyme leaves powder
Group 1: Anemic group feeding on diet containing 10 g thyme leaves powder
Group 2: Anemic group feeding on diet containing 20 g thyme leaves powder
Group 3: Anemic group feeding on diet containing 30 g thyme leaves powder

The body weight of rats in different groups varied significantly as function of thyme dose and study duration. The highest gain in body weight was observed in G_3 (112.97 ± 1.28-169.91 ± 1.12g per rat) followed by G_2 and G_1 as (114.23 ± 0.83-153.12 ± 1.87g per rat) and (117.34 ± 1.52-141.71 ± 1.62g per rat) during study period from 0 day to 42 days, respectively. While, the lowest body weight gain (116.67 ± 1.98-138.53 ± 1.69g per rat) was observed in anemic control group G_0 from day 0 to 42 days of study. Higher body weight can be positively correlated with increased feed intake. Thymol, an essential oil present in thyme leaves is supposed to increase nutrient absorption in small intestine that stimulates the appetite resulting in increased rate of feed intake that further improves the body weight gain (Gumus *et al.*, 2017).

Serum biochemical profile

The effect of thyme leaves powder supplementation on serum iron, serum ferritin, TIBC and transferrin saturation fraction is shown in Table 2. The results showed that a substantial increase was observed in serum iron concentration in all the groups. A maximum increase was observed in G_3 followed by G_2 and G_1 as compared to anemic control group (G_0) at the end of study period. There was non-significant increase in serum iron level in G_0 group that did not fed on thyme leaves powder. There was observed a significant ($p<0.05$) increase in serum iron, serum ferritin and transferrin saturation in rats fed on different concentrations of thyme leaves powder however, the highest effect was observed with 30g of thyme leaves powder supplementation. Serum ferritin level varied in all experimental groups and means for serum ferritin showed significant differences ($p<0.05$) in all the groups compared to anemic control group (G_0). Maximum increase was observed in G_3 and values ranged between 18.63 ± 1.73 and 32.54 ± 1.18ng/mL at initiation and termination of study, respectively. However, there was non-significant increase of serum ferritin concentration in G_0 (20.55 ± 0.03 to 20.91 ± 0.62ng/mL) from 0 to 42 days. It is worth mentioning that overall means of serum ferritin in experimental rats were significantly different and indicated anti- anemic effect through the iron status of respective subject.

Table 2. Effect of thyme leaves powder on serum biochemical parameters.

Biochemical parameters	Treatment	Study period		
		0 Day	21 Day	42 Day
Serum iron (µg/dL)	G_0	40.45 ± 1.16^{Bb}	40.75 ± 0.67^{Dab}	41.45 ± 0.71^{Da}
	G_1	38.78 ± 1.55^{Cc}	47.94 ± 1.05^{Cb}	58.07 ± 0.71^{Ca}
	G_2	41.06 ± 2.10^{Bc}	50.74 ± 1.70^{Bb}	61.10 ± 1.48^{Ba}
	G_3	43.80 ± 0.97^{Ac}	54.01 ± 0.54^{Ab}	66.14 ± 1.19^{Aa}
Serum ferritin (ng/mL)	G_0	20.55 ± 0.03^{Aa}	20.65 ± 0.60^{Ca}	20.91 ± 0.62^{Da}
	G_1	19.95 ± 0.02^{Ac}	22.54 ± 0.15^{Bb}	29.04 ± 1.41^{Ba}
	G_2	17.81 ± 1.22^{Bc}	20.61 ± 1.51^{Cb}	27.49 ± 0.22^{Ca}
	G_3	18.63 ± 1.73^{Bc}	24.30 ± 1.22^{Ab}	32.54 ± 1.18^{Aa}
TIBC (µg/dL)	G_0	514.83 ± 1.79^{Aa}	514.81 ± 1.80^{Aa}	514.75 ± 1.78^{Aa}
	G_1	509.11 ± 1.62^{Ca}	497.60 ± 1.01^{Bb}	483.28 ± 2.35^{Bc}
	G_2	511.40 ± 1.04^{Ba}	496.96 ± 1.03^{Bb}	478.71 ± 0.80^{Cc}
	G_3	507.67 ± 1.44^{Da}	489.12 ± 1.37^{Cb}	466.00 ± 1.18^{Dc}
Transferrin saturation fraction (%)	G_0	7.85 ± 0.16^{BCb}	7.91 ± 0.12^{Db}	8.05 ± 0.14^{Da}
	G_1	7.61 ± 0.31^{Cc}	9.63 ± 0.21^{Cb}	12.01 ± 0.15^{Ca}
	G_2	8.03 ± 0.42^{Bc}	10.20 ± 0.34^{Bb}	12.76 ± 0.30^{Ba}
	G_3	8.62 ± 0.19^{Ac}	11.04 ± 0.12^{Ab}	14.19 ± 0.25^{Aa}

Means with different lower case letters (a, b, c) are statistically different ($p < 0.05$) from 0 – 42 days. Means with different upper case letters (A, B, C) are statistically different ($p < 0.05$) within control and all other anemic groups.

G_0: Control (anemic group feeding on diet without thyme leaves powder)

G_1: Anemic group feeding on diet containing 10 g thyme leaves powder

G_2: Anemic group feeding on diet containing 20 g thyme leaves powder

G_3: Anemic group feeding on diet containing 30 g thyme leaves powder

Mean values regarding TIBC indicated that it varied among different groups and maximum value was observed in G_0 (anemic control) as compared to all other groups from 0 to 42 days. Thyme significantly declined TIBC and maximum decline was observed in G_3 (507.67 ± 1.44 to 466.00 ± 1.18µg/dL) from initiation to termination of study, respectively. More iron in blood, less will be total iron binding capacity because there will be less empty spaces on transferrin (carrier protein) to remain unbound and it becomes saturated with already present blood iron. The ratio of serum iron to total iron binding capacity is transferrin saturation which depicts saturation of transferrin protein with elemental iron. Thyme supplementation showed significant ($p<0.05$) increase in transferrin saturation fraction in all the experimental groups except G_0 (anemic control) and maximum increase was observed in G_3 followed by G_2 and G_1 from day-0 to 42 days. Thyme leaves powder supplementation in diet also resulted in improvement of iron level in blood serum of rat. The high concentration of iron present in leaves led to absorb more iron but several studies also showed that there are various phytochemicals present in thyme which help in iron absorption into blood circulation. For example, ascorbic acid in thyme can make iron more available; inhibit the activity of saponins, tannins and phytates that improves iron status in iron deficiency anemic patients. It was observed previously by some researchers that 0.5 g of ascorbic acid given to vegetarians for 2 months improves hemoglobin in anemic subjects (Singh *et al.*, 2016; Sultana *et al.*, 2016). Moreover, with increase in iron intake, its storage protein (ferritin) as marker of iron was also increased. El-Din Yossef (2010) reported a substantial increase in serum ferritin concentration in rat experimental model and a considerable increment in iron remarkably decreased TIBC and increased transferrin saturation fraction.

Hematological indices

Mean values of Hb, hematocrit, RBC and MCV are shown in Table 3. The mean values of Hb showed that it varied significantly ($p<0.05$) with the study intervals in different groups. Non-significant ($p>0.05$) increase was observed in G_0 (anemic control). Maximum increase was observed in G_3 (10.00 ± 0.36 to 13.91 ± 0.25g/dL) followed by G_2

(9.29 ± 0.23 to 13.16 ± 0.31g/dL) and G_1 (8.98 ± 0.39 to 12.82 ± 0.33g/dL). Hematocrit level exhibited significant ($p<0.05$) difference between all experimental groups. The maximum mean value was observed in G_3 followed by G_2 and G_1 ranging from 24.36 ± 0.26 to 34.74 ± 0.54, 24.37 ± 0.49 to 33.12 ± 0.52 and 22.77 ± 0.26 to 29.82 ± 0.56% respectively from 0 to 42 days. The minimum increase was observed in G_0 (27.27 ± 0.78 to 28.39 ± 0.49%). Mean values of RBC increased significantly ($p<0.05$) from G_1 to G_3 and maximum increase was noted in G_3 followed by G_2 and G_1, respectively, while non-significant increase was observed in G_0 (anemic control). Similar effect of thyme leaves powder supplementation was observed on MCV. The mean values showed significant difference among all the experimental rat groups except control group. Maximum increase in MCV was observed in G_3 (73.97 ± 0.22-79.11 ± 0.77fL), whereas, non-significant increase was observed in anemic control group (71.52 ± 1.80-72.43 ± 0.90fL). The significant increment in iron concentration also increased Hb, MCV, hematocrit and RBC (El-Sheikh, 2008). The improvements in RBC, hematocrit, Hb and MCV of current study may be attributed to the unique composition of thyme minerals as well as other phytochemicals which may become helpful in the treatment of iron deficiency anemia (Chaturvedi *et al*., 2014).

Table 3. Effect of thyme leaves powder on hematological indices.

Hematological indices	Treatment	Study period		
		0 Day	21 Day	42 Day
Hb (g/dL)	G_0	11.18 ± 0.75^{Ab}	11.32 ± 0.58^{Bab}	11.80 ± 0.38^{Da}
	G_1	8.98 ± 0.39^{Cc}	10.88 ± 0.29^{Cb}	12.82 ± 0.33^{Ca}
	G_2	9.29 ± 0.23^{Cc}	11.21 ± 0.10^{Bb}	13.16 ± 0.31^{Ba}
	G_3	10.00 ± 0.36^{Bc}	11.94 ± 0.21^{Ab}	13.91 ± 0.25^{Aa}
Hematocrit (%)	G_0	27.27 ± 0.78^{Ab}	27.75 ± 0.36^{Bb}	28.39 ± 0.49^{Da}
	G_1	22.77 ± 0.26^{Cc}	25.70 ± 0.94^{Cb}	29.82 ± 0.56^{Ca}
	G_2	24.37 ± 0.49^{Bc}	28.40 ± 0.37^{ABb}	33.12 ± 0.52^{Ba}
	G_3	24.36 ± 0.26^{Bc}	29.02 ± 1.26^{Ab}	34.74 ± 0.54^{Aa}
RBC (million/µL)	G_0	3.81 ± 0.03^{Ab}	3.86 ± 0.06^{Ab}	3.92 ± 0.07^{Ca}
	G_1	3.18 ± 0.01^{Dc}	3.58 ± 0.13^{Bb}	4.03 ± 0.08^{Ba}
	G_2	3.44 ± 0.07^{Bc}	3.87 ± 0.04^{Ab}	4.36 ± 0.07^{Aa}
	G_3	3.29 ± 0.03^{Cc}	3.79 ± 0.16^{Ab}	4.40 ± 0.06^{Aa}
MCV (fL)	G_0	71.52 ± 1.80^{Ba}	71.92 ± 0.89^{Ca}	72.43 ± 0.90^{Da}
	G_1	69.89 ± 0.50^{Cc}	71.85 ± 0.25^{Cb}	73.98 ± 0.03^{Ca}
	G_2	70.96 ± 0.54^{Bc}	73.46 ± 0.53^{Bb}	75.98 ± 0.24^{Ba}
	G_3	73.97 ± 0.22^{Ac}	76.51 ± 0.36^{Ab}	79.11 ± 0.77^{Aa}

Means with different lower case letters (a, b, c) are statistically different ($p < 0.05$) from 0 – 42 days. Means with different upper case letters (A, B, C) are statistically different ($p < 0.05$) within control and all other anemic groups.

G_0: Control (anemic group feeding on diet without thyme leaves powder)

G_1: Anemic group feeding on diet containing 10 g thyme leaves powder

G_2: Anemic group feeding on diet containing 20 g thyme leaves powder

G_3: Anemic group feeding on diet containing 30 g thyme leaves powder

Liver and kidney functioning tests

The effect of thyme leaves powder supplementation on liver function tests i.e. ALT, AST and ALP is depicted in Table 4. The mean values of ALT showed that it varied significantly ($p<0.05$) with the study period in all experimental rat groups. Non-significant effect was observed in G_0 (anemic control). Maximum decrease in ALT was reported in G_3 (53.68 ± 0.42-46.88 ± 0.94IU/L) followed by G_2 (54.89 ± 0.44-47.54 ± 0.51IU/L) and G_1 (54.46 ± 0.67-49.03 ± 0.59IU/L).

Table 4. Effect of thyme leaves powder on liver and kidney function tests.

Parameters	Treatment	Study period		
		0 Day	21 Day	42 Day
ALT (IU/L)	G_0	55.14 ± 1.12^{Aa}	55.12 ± 1.14^{Aa}	55.09 ± 1.13^{Aa}
	G_1	54.46 ± 0.67^{Ba}	52.52 ± 0.58^{Bb}	49.03 ± 0.59^{Bc}
	G_2	54.89 ± 0.44^{ABa}	52.28 ± 0.64^{Bb}	47.54 ± 0.51^{Cc}
	G_3	53.68 ± 0.42^{Ca}	51.41 ± 0.94^{Cb}	46.88 ± 0.94^{Cc}
AST (IU/L)	G_0	108.70 ± 1.78^{Ca}	108.67 ± 1.77^{Aa}	108.65 ± 1.75^{Aa}
	G_1	112.59 ± 1.02^{Aa}	106.59 ± 1.27^{Bb}	102.87 ± 2.33^{Bc}
	G_2	111.40 ± 1.03^{Ba}	105.40 ± 1.05^{BCb}	100.69 ± 1.09^{Cc}
	G_3	111.89 ± 1.06^{ABa}	104.88 ± 1.25^{Cb}	99.95 ± 1.69^{Cc}
ALP (IU/L)	G_0	187.79 ± 2.83^{Aa}	187.44 ± 2.95^{Aa}	187.01 ± 2.64^{Aa}
	G_1	189.85 ± 2.99^{Aa}	177.85 ± 2.44^{Bb}	155.83 ± 2.40^{Bc}
	G_2	183.88 ± 2.25^{Ba}	176.97 ± 2.09^{Bb}	149.08 ± 2.27^{Cc}
	G_3	185.06 ± 2.29^{Ba}	176.49 ± 2.50^{Bb}	148.85 ± 2.49^{Cc}
Urea (mg/dL)	G_0	35.19 ± 1.54^{Ba}	34.98 ± 1.37^{Aa}	34.59 ± 1.57^{Aa}
	G_1	37.22 ± 1.30^{Aa}	35.79 ± 1.03^{Ab}	26.80 ± 0.91^{Bc}
	G_2	36.04 ± 1.32^{ABa}	34.74 ± 1.21^{Ab}	25.68 ± 1.5^{BCc}
	G_3	36.17 ± 1.37^{ABa}	33.63 ± 1.02^{Bb}	25.04 ± 1.53^{Cc}
Creatinine (mg/dL)	G_0	1.03 ± 0.02^{Ca}	1.02 ± 0.01^{Ba}	0.98 ± 0.02^{Ab}
	G_1	1.18 ± 0.02^{Ba}	1.08 ± 0.03^{Ab}	0.87 ± 0.02^{Bc}
	G_2	1.22 ± 0.03^{Aa}	1.07 ± 0.05^{Ab}	0.86 ± 0.04^{Bc}
	G_3	1.21 ± 0.03^{Aa}	1.06 ± 0.04^{Ab}	0.85 ± 0.04^{Bc}

Means with different lower case letters (a, b, c) are statistically different ($p < 0.05$) from 0 – 42 days. Means with different upper case letters (A, B, C) are statistically different ($p < 0.05$) within control and all other anemic groups.

G_0: Control (anemic group feeding on diet without thyme leaves powder)

G_1: Anemic group feeding on diet containing 10 g thyme leaves powder

G_2: Anemic group feeding on diet containing 20 g thyme leaves powder

G_3: Anemic group feeding on diet containing 30 g thyme leaves powder

Mean AST values for G_0 showed non-significant results as compared with all other experimental groups. Maximum decline in AST level was observed in G_3 (111.89 ± 1.06-99.95 ± 1.69IU/L) consuming 30 g thyme leaves powder and minimum decrease was reported in G_0 (108.70 ± 1.78-108.65 ± 1.75IU/L). Means pertaining to ALP values showed significant results for all the experimental rat groups as compared to anemic control group. Feeding of rat groups on thyme leaves powder supplemented diet significantly decreased the ALP level which was lifted earlier because of the phenylhydrazine induced anemia. Maximum decrease in ALP was measured in G_3 followed by G_2 and G_1. Mean values of serum urea decreased significantly ($p<0.05$) from G_1 to G_3 and maximum decline was noted in G_3 followed by G_2 and G_1, respectively, while non-significant decline was observed in G_0 (anemic control). In G_0 group, rats showed uplifted blood urea level i.e. 34.59 ± 1.57mg/dL, whereas its level reduced to 26.80 ± 0.91mg/dL, 25.68 ± 1.5mg/dL and 25.04 ± 1.53mg/dL in G_1, G_2 and G_3, respectively. Similar effect of thyme leaves powder supplementation was observed on serum creatinine. The mean values were significantly different among all the experimental groups except the control group. Maximum decreasing trend was observed in G_3 (1.21 ± 0.03-0.85 ± 0.04mg/dL), whereas, non-significant decline was reported in anemic control group (1.03 ± 0.02-0.98 ± 0.02mg/dL).

Phenylhydrazine has been investigated to cause hepatotoxicity and nephrotoxicity in animals. The activities of liver enzymes (ALT, AST and ALP) in anemic rats increased significantly because of the membrane damage of hepatocytes (Ebuehi and Mbara, 2011). Therefore, the protective effect of thyme leaves was observed on liver enzymes which restored them to the normal level because of the presence of phytochemicals and antioxidants as well as membrane stabilizing activity of thyme. These findings of liver enzymes were in accordance with the earlier studies by Arthur *et al.* (2012) as they

evaluated the anti-hepatotoxic effect of aqueous extract of *Annona muricata* leaf because of the good antioxidant profile against carbon tetrachloride induced liver injury. Moreover, administration of thyme leaves powder supplementation lessens the harmful effects on kidney function because of its free radical scavenging ability and hence reduces the oxidative stress (Hamzawy *et al.*, 2012).

List of abbreviation

Hb: Hemoglobin
MCV: Mean corpuscular volume
RBC: Red blood cells
TIBC: Total iron binding capacity
EDTA: Ethylene diamine tetra acetic acid
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
ALP: Alkaline phosphatase

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