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Biological Effects of Hawthorn Leaves Powder and its Extract on Biological and Biochemical Change of Induced Obese Rats

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Abstract: In present study aimed to clear effect of hawthorn leaves powder at (2.5, 5%) and its water extract at (250,500mg\kg) on obese rats. Thirty-six male rats (140±10g) were divided into six groups, Rats were treated with high fat diet (20% animal fat) to induce obese. The results showed that, (+ve) group had significant increase in weight gain, feed intake and feed efficiency ratio (FER), the best results showed in hawthorn powder 5% which decrease weight gain, feed intake and feed efficiency ratio compared to +ve group. The lower ALT, AST and ALP liver enzyme of treated group recorded for group fed on 500 mg/kg hawthorn extract with significant difference followed by hawthorn extract 250mg/kg in ALT, AST. Control (+ve) had increased significantly in Tc., TrG, LDLc and VLDLcc and decreased HDL compared to (–ve) control, The most decrease in Tc, TrG, LDLc, VLDLc and highest (HDL-c) levels recorded for rats fed on 500 mg/kg hawthorn extract. Hawthorn powder and extract showed a significant increase in GST, catalase, SOD and significant decrease NO compared to (+ve) group.
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1. Introduction

There is a true need to look for new natural materials curing dangerous disease. One of promising substances is Hawthorn (*Crataegus sp.*), It has been used as a medicinal drug and food in Europe and China. Extracts of hawthorn fruits and leaves have many health benefits including hypolipidaemic, anti-atherosclerotic, hypotensive, cardioprotective and blood vessel relaxing activities. Also, fruit extracts have some antioxidant and radical scavenging activities **(Pengzhan and Baoru 2012).** The same authors reported that phenolic compounds, procyanidins, flavonols and Cglycosyl flavones are considered among the major bioactive compounds in hawthorn. Moreover, hawthorn fruit is rich in vitamin C, triterpenoids, acids and sugar. **Alaghawani and Naser (2013)** investigated the curative effect of hawthorn has clear hypoglycemic effect against diabetic rats with streptozotocine **Shih, *et al.* (2013)** examined the effect of hawthorn by hepatic gluconeogenesis and lipogenesis on diabetes and obesity in high-fat-fed rats.

Obesity is the most grave health problem. It is a risk factor for development metabolic disorders such as type 2 diabetes, systemic hypertension, cardiovascular disease, dyslipidemia, and atherosclerosis. **(Cheng *et al.*, 2010)**. Obesity (WHO) defines as an increased of adipose tissue mass **(Roh *et al.*, 2012)**.

Hassan and El-Gharib, (2015) concluded that obesity is related to coronary heart diseases, diabetes type 2, metabolic syndrome, stroke, and cancers. Obesity is generally defined as the abnormal accumulation of fat in adipose tissue to the extent that health may be impaired **(Aronne and Segal, 2002)**.

This study aimed to investigate the effect of hawthorn leaves powder and extract consumption on obesity rats.

2. Materials and Methods

Materials:

Hawthorn (*Crataegus SP.*) fruits were obtained from Agricultural Research Center. Egypt.

Experimental animals:

A total of 36 adult normal male albino rats Sprague Dawley strain weighing 140±10 g were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan farm, Cairo, Egypt, animals were fed on standard diet according to **NRC (1995)**.

Methods:

The induction of experimental obesity:

Obesity was induces in normal healthy male albino rats by fed on high fat diet (20% animal lipid) supplemented in the basal diet and used as a positive control group.

Experimental design:

Thirty six adult male white albino rats, Sprague Dawley Strain, 10 weeks age, weighing (140±10g) were used in this experiment. All rats were fed on basal diet for 7 consecutive days. After this adaptation period, rats were divided into 6 groups, each group which consists of six rats as follows: group (I): rats fed on basal diet as (–ve). Group (2): Obese rats induced by fed on high fat diet (10% animal lipid) as (+ve). Group (3): A group obese rats fed on hawthorn powder by 2.5% of the weight of basal diet. Group (4): A group infected obese rats fed on hawthorn powder 5%. Group (5): A group infected obese rats fed on hawthorn water extract by 250 mg/kg of rat weight. Group (6): A group infected obese rats fed on hawthorn extract by 500 mg/kg. During the experimental period, the body weight and feed intake were estimated weekly and the general behavior of rats was observed. The experiment period was take 28 days, at the end of the experimental period each rat weight separately then, rats are slaughtered and collect blood samples. Blood samples were centrifuged at 4000 rpm for ten minute to separate blood serum, and then kept in deep freezer till using.

Total cholesterol, triglyceride and HDL-c were determined according to **Thomas (1992)** and **Young (1975), Fossati (1982)** and **Friedewaid (1972) and Grodon and Amer (1977)**. LDL-c and VLDL-c were calculated according to **Lee and Nieman (1996)**. Serum alanine and aspartae aminotransferase (ALT, AST), alkaline phosphates (AP) enzymes, creatinine and uric acid were estimated according to **Reitman and Frankel (1957), Kind and King (1954), Hare (1950) and Fossati, *et al.* (1980),** respectively. Plasma glutathione transferase (GST), catalase, superoxide dismutase enzymes (SOD) and nitric oxide (NO) according to **Habig (1974), Claiborne (1985), Beuchamp and Fridovich (1971) and Green et al. (1981)**, respectively.

Statistical analysis:

The obtained results of biological evaluations were statistically analyzed according (SAS, 1999). LSD at 5% level of significance was used to compare between means according to **Snedecor and Cochran (1967)**.

3. Results and Discussion

Data recorded in Table (1) illustrated that (+ve) group showed increased significantly in weight gain, feed intake and feed efficiency ratio (FER) compared to (-ve) group. The hawthorn powder, (2.5 and 5%) groups showed significant decrease in weight gain, feed intake and FER compared to (+ve) group.

These results are in harmony with those obtained by (**Pengzhan and Baoru 2012).** suggest that extracts of hawthorn leaves have many health benefits including hypolipidaemic, anti-atherosclerotic, hypotensive, cardioprotective and blood vessel relaxing activities. Also, fruit extracts have some antioxidant and radical scavenging activities.

As shown in Table (2), the positive control group showed a significant increase in serum (ALT, AST & ALP) enzymes at (96.24, 85.44 and 67.03) compared to (–ve) which were (34.11, 30.65 and 29.40) respectively, the best results showed in hawthorn extract 500mg\kg were (47.50, 28.16 and 37.52) followed by extract 250mg\kg (63.65, 45.34 in ALT and AST) while rats fed on hawthorn powder 5% decreased significantly in ALP at 42.25. **Shih, *et al.* (2013)** examined the effect and molecular mechanism of hawthorn by quantifying the expression of hepatic gluconeogenesis and lipogenesis.

Feeding on high fat diet increase Tc, TG, LDL-c and VLDL-c at (154.23, 137.03, 85.86 and 27.40) respectively, but decrease HDL-c to 36.15(g\dl). On the other hand, treatment with hawthorn decrease lipid profile as shown in Table (3 & 4). Hawthorn extract 500mg\kg which was the best results decreased Tc, TG, LDLc and VLDL-c at (75.40, 65.25, 39.89 and 13.05) and increased significantly in HDL-c at 47.50g\dl. The prefer concentration of extract at 500 followed by 250mg\kg. On the other hand the prefer concentration of powder hawthorn at 5%. This is in accordance to **Onody *et al.* (2003) who** demonstrated that, consumption of hawthorn reduce blood lipid and cholesterol levels. It is related to catechins, triterpene, saponins, and quercetin in hawthorn (**Zhu 1998).**

These results are in agreement with **Krentz (2003)**, they reported that could reverse the hyperlipidemia in experimental diabetic rats, and thus may lead to a decrease in the risk of micro- and macrovascular disease and related complications.

As shown in Table (5), (+ve) group showed decreased significantly in plasma glutathione transferase (GST), catalase and superoxide dismutase (SOD) and a significant increase in nitric oxide (NO) compared to (-ve) group. Hawthorn powder and extract showed a significant increase in GST, catalase, SOD and significant decrease NO compared to (+ve) group. Increase of GST activity is an indication of a cellular failure in compensating the induced oxidative stress **(Virgil and George 1996)**. These data are in same direction with those obtained by **Nsimba *et al.*, (2008)** who revealed that natural anti-oxidants have an important action in scale down free radicals and oxidative reactions in tissue and membrane levels. **Jan *et al.* (2009)** found that HPLC analysis of hawthorn, epicatechin, chlorogenic acid, hyperoside, isoquercitrin, protocatechuic acid at (178, 65, 25, 13 and 3 mg/100 g dry fruit) respectively, Previous studies show that hawthorn has high polyphenolic and flavonoids compunds (**Rabiei *et al.*, 2012**).

From the present study, it is concluded that hawthorn powder and water extract have an antioxidant effect and it can be used as antiobesity.

Table 1: Antioxidant activity of hawthorn extract

Antioxidant assay	FRAP (µmol Fe++/g)	TRAP (µmol TE/g)
Hawthorn	1354.05	732.20

FRAP: Ferric reducing antioxidant TRAP: total radical-trapping antioxidant

Table (1): Effect of hawthorn leaves powder and its extract on body weight gain, feed intake and feed efficiency ratio of obese rats

Parameters	**BWG (g)**	**FI (g/day)**	**FER (%)**
Control group (-)	31.73 ±0.32^{c}	25.15 ±1.23^{a}	0.045^{b} ± 0.004
Control group (+)	58.49 ±0.61^{a}	23.10 ±1.12^{b}	0.090^{a} ±0.003
Obese rats with hawthorn powder (2.5%)	18.68 ±0.50^{d}	17.67 ±1.25^{c}	0.037^{c} ±0.002
Obese rats with hawthorn powder (5%)	15.03 ±0.11^{e}	18.88 ±1.10^{c}	0.028^{c} ± 0.001
Obese rats with hawthorn leaves extract (250 mg/kg)	21.77 ±0.20^{d}	18.45 ±1.24^{c}	0.042^{b} ± 0.002
Obese rats with hawthorn leaves extract (500 mg/kg)	26.11 ±0.12^{d}	22.52±1.31^{b}	0.041^{b} ±0.005

Values with the same letters indicate insignificant difference and vice versa.

Table (2): Effect of hawthorn leaves powder and its extract on liver functions of obese rats

Treatment Parameter	**ALT (U/L)**	**AST (U/L)**	**ALP (U/L)**
Control group (-)	34.11 ± 2.90^{f}	30.65 ± 1.6^{f}	29.40 ± 5.60^{f}
Control group (+)	96.24 ± 2.13^{a}	85.44 ± 6.12^{a}	67.03 ± 6.11^{a}
Obese rats with hawthorn powder (2.5%)	80.45 ± 1.63^{b}	53.40 ± 7.10^{b}	56.20 ±4.30^{b}
Obese rats with hawthorn powder (5%)	65.17 ± 5.21^{c}	46.20 ± 4.25^{c}	42.25 ±3.16^{d}
Obese rats with hawthorn extract (250 mg/kg)	63.65 ± 5.10^{d}	45.34 ± 6.31^{d}	48.20± 6.23^{c}
Obese rats with hawthorn extract (500 mg/kg)	47.50 ± 4.23^{e}	28.16 ± 4.20^{e}	37.52 ± 7.18^{e}

Values with the same letters indicate insignificant difference and vice versa.

Table (3): Effect of hawthorn leaves powder and its extract on serum total cholesterol and triglycerides of obese rats

Treatment/Parameter	**Total cholesterol (mg /dl)**	**Triglycerides (mg /dl)**
Control group (-)	70.25±6.74^{e}	66.33±9.24^{d}
Control group (+)	154.23±15.18^{a}	137.03± 13.61^{a}
Obese rats with hawthorn powder (2.5%)	98.40±4.23^{b}	85.43±4.52^{b}
Obese rats with hawthorn powder (5%)	95.20±5.66^{c}	81.11±11.61^{c}
Obese rats with hawthorn extract (250 mg/kg)	75.40± 4.26^{d}	77.10± 9.51^{d}
Obese rats with hawthorn extract (250 mg/kg)	80.35± 8.77^{e}	65.25± 7.72^{e}

Values with the same letters indicate insignificant difference and vice versa.

Table (4): Effect of hawthorn leaves powder and its extract on lipid profile of obese rats

Treatment/Parameter	(HDL_{-C}) (g/dl)	(LDL_{-C}) (g/dl)	($VLDL_{-C}$) (g/dl)
Control group (-)	49.22±2.60[d]	30.42± 4.74[f]	13.26±0.21[c]
Control group (+)	36.15±4.50[a]	85.86±1.77[a]	27.40±0.13[a]
Obese rats with hawthorn powder (2.5%)	43.00±10.28[b]	37.40±1.75[e]	17.08±0.20[b]
Obese rats with hawthorn powder (5%)	42.09±8.26[b]	34.66±6.87[e]	16.22±0.42[b]
Obese rats with hawthorn extract (250 mg/kg)	44.44±9.11[c]	34.68±4.35[e]	15.42±0.10[c]
Obese rats with hawthorn extract (500 mg/kg)	47.50±10.47[c]	39.89±7.80[e]	13.05±0.70[c]

Values with the same letters indicate insignificant difference and vice versa.

Table (5): Effect of Hawthorn leaves powder and its Extract on plasma glutathione transferase (GST), catalase, superoxide dismutase (SOD) enzymes and nitric oxide (NO).

Treatment/Parameter	GST (µ /l)	Catalase (µ /l)	SOD (µ /l)	NO (µmol /l)
Control group (-)	**271.31±33.27[a]**	**385.21±55.14[a]**	**70.13±5.22[a]**	**2.17±0.33[b]**
Control group (+)	**77.85±8.40[c]**	**115.55±10.14[c]**	**21.25±3.47[b]**	**13.99±1.4[a]**
Obese rats with hawthorn powder (2.5%)	**278.15±31.71[a]**	**384.11±39.11[a]**	**73.14±7.81[a]**	**2.01±1.21[b]**
Obese rats with hawthorn powder (5%)	**188.35± 22.17[b]**	**230.77± 32.11[ab]**	**63.14±7.16[a]**	**4.33±1.11[b]**
Obese rats with hawthorn extract (250 mg/kg)	**211.31±23.81[b]**	**291.61±31.61[a]**	**68.33±6.35[a]**	**3.22±1.03[b]**
Obese rats with hawthorn extract (500 mg/kg)	**240.21±23.71[a]**	**277.11±30.91[a]**	**71.31±9.23[a]**	**3.11±1.05[b]**

Values with the same letters indicate insignificant difference and vice versa.

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