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Biochemical Studies on Carob

Nadia Y.A. Attia.[1]; Ibrahim M. Abd El - Aleem[1]; Kareem M.K. El Tobgy[2] and Hala F. Mohamed[2]

1: Department of Biochemistry, Faculty of Agriculture, Moshtohor, Banha University.
2: Department of Medicinal and Aromatic Plants Research, Horticulture Research Institute, Agriculture Research Center.

Abstract

Water, methanol and ethanol were used to extract total phenols, total tannins, and flavonoids from carob (Ceratonia siliqua L) by using different methods. The obtained extracts were used to study their antioxidant activity and their antimicrobial effect against some Gram positive bacteria (*Staphylococcus aureus*, *Listeria monocytogenese, Bacillus megaterium* and *Bacillus cereus*), some Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumonia* and *Salmonella typi*) and some fungi (*Aspergillus niger* and *Candida albicans*). Also, carob fiber and water extract were used to study their biological effects against diabetic and hypercholesterolemia in Wister rats. Water extract at 50°C for 20 min showed the highest capacity for extracting total phenols, total tannins and total flavonoids compounds from carob. Methanol extract at 50°C for 20 min and at 25°C for 24 h recorded the highest antioxidant activity. Some carob extracts recorded inhibition for the tested microorganisms except *salmonella typhi* which did not affected. Administration of carob fiber and water extract showed significant decrement in blood serum glucose, triglycerides, total cholesterol, low density lipoprotein (LDL), urea and creatinine in diabetic and hypercholesterolemia rats while, high density lipoprotein (HDL) elevated. Administration of carob fiber and water extract induced significant increment in white blood cells (WBC) in diabetic groups. While, hypercholesterolemia group administrated with carob fiber showed significant increment in Red blood cells (RBC) count and carob water extract showed increment in RBC count and decrement in WBC count.

Key words: Carob – antioxidant – antimicrobial – lipid profile – liver functions.

Introduction

Several years scientists are interested in studying the effect reactive oxygen species (ROS) that are implicated in many human diseases (**Lobo *et al*., 2010**). Increased ROS led to oxidative stress and a degenerative signaling cascade triggered by oxidation of vital cellular components, which induced cellular damage and cell death (**Farrugia and Balzan, 2012**). The carob tree has been widely cultivated for years in Mediterranean countries. Various chemical and physiological aspects of carob plants have been investigated**. Hussein *et al.* (2011)** found that the total protein, fiber, total carbohydrate, fat, ash and moisture contents in dried carob were 8.95%, 8.91%, 73.14%, 5.48%, 3.52% and 10.1% respectively. Carob extracts have several beneficial effects on health such as cholesterol lowering activities in humans suffering from hypercholesterolemia (**Zunft *et al*., 2001, 2003**) and antioxidant properties *in vitro* test systems (**Custodio *et al*., 2005**). Recent studies discovered that Tunisian leaf carob extract presented some ameliorative effects against CCl4-induced oxidative damage in rats tissues (**Hsouna *et al*., 2012**).

Benchikh and Louailèche (2014) showed that solvents (acetone, ethanol, methanol and water) at concentration (40–100%) with solid-to-solvent ratio (15/10 to 75/10 mg/ml) at extraction time (60–120 min) and extraction temperature (25–90°C) had statistically significant effects on phenolic compounds extracted from carob pulp and antioxidant activities. The best extraction conditions were 70% acetone, 25 mg/10 ml, 90 min and 90°C. Also, phenolic compounds content was positively correlated with antioxidant activities. **Sebai *et al* (2013)** showed that the carob polar extracts were richer in total polyphenols, total flavonoids and condensed tannins than the nonpolar extracts with quantitative variation of phenolic compounds between seeds and pulp.

Hussein *et al.* (2011) reported that the water extract of carob had antioxidative, antibacterial and antifungal activities against some pathogenic bacteria (*Escherichia coli Staphylococcus auraus, Enterococcus feacalis, Bacillus subtilis* and *B. megaterium)* and some yeast *(Debaryomyces hansenii, Zygosaccharo mycesrouxii, Rhodotorula rubra, Candida shehatae* and *Candida tropicalis*). **Aissani *et al.* (2012**) found that methanolic extract of carob leaves inhibited the growth of *Listeria monocytogenes* at 28.12 µg/mL. The effect of this bacteriostatic concentration on the growth of this bacterium revealed a pattern of inhibition characterized by (a) resumed growth phase, which showed a lower rate of growth if compared with controls; and (b) first a lag and then a stationary phase at a lower bacterium concentration. **Abd Razik *et al.* (2012)** reported that the methanol extract of *Ceratonia Siliqua* had antibacterial activity on Gram positive bacteria (Lactobacillus *sp*. and *Staphylococcus aureus)* and Gram negative bacteria

(*Proteus sp., Pseudomonas aeruginosa ,Escherichia coli* and *Enterococcus sp.*). The extract produced inhibition zones against Gram positive bacteria sensitive to concentration ranged from 500 - 1000 (mg/ml) and Gram negative bacteria sensitive to concentration ranged from 125-1000 (mg/ml), *Escherichia coli* and *Enterococcus sp.* sensitive to concentration ranged from 1000-500 (mg/ml). Solvent (negative control) used for preparation different concentrations showed no activity against any tested bacteria.

Zunft *et al.* (2003) reported that carob fiber consumption lowered triglycerides in females by 11.3 %. Lipid lowering effects were more pronounced in females than in males. The consumption of carob fiber reduced LDL cholesterol by 10.5 %. The LDL: HDL cholesterol ratio was marginally decreased by 7.9 % in the carob fiber group compared to the placebo group. **Roso *et al.* (2010)** found that the carob fiber rich in polyphenols reduced the total cholesterol by 17.8%, LDL cholesterol by 22.5%, LDL: HDL cholesterol ratio by 26.2% and triglycerides by 16.3% at the end of the study. No significant differences were found in the glucose, creatinine, uric acid, bilirubin, alkaline phosphatase, AST, and ALT) between the beginning and end of treatment. **Mokhtari *et al.*(2011)** stated that in diabetic adult male wistar rats received 150, 300 and 600 mg/kg hydro-alcoholic seed extract of *Ceratonia siliqua*, the concentration of glucose, total cholesterol and LDL-C decreased significantly in respect to diabetic control group while, triglyceride level was only declined in group received 200 mg/kg extract. In addition, the serum level of HDL-C showed a considerable elevation. **Ali *et al.* (2012)** reported that rats feeding on high fructose diet led to significant increment in cholesterol, triglycerides, LDL-c, vLDL-c, uric acid, urea nitrogen and creatinine and decrement in HDL-c. Feeding rats on high fructose diet with the different levels of carob(2.5%, 5% and 7.5%) improved all parameters and kidney weight, especially when used the high level from carob.

The aim of this investigation is to study the best method for extracting the antioxidants of carob, evaluate carob extracts as antimicrobial and evaluate carob water extract and fiber biological effects on diabetic and hypercholesterolemia rats.

Materials and methods:

Plant Material

Carob (Ceratonia *siliqua* L) was obtained from Horticulture Research Institute, Agriculture Research Center, Giza, Egypt (August 2013).

Preparation of the samples:

Carob pods were cleaned, dried and ground to fine powder.

Preparation of carob fiber:

Sugars and soluble tannins were removed from carob powder by two sequentially water extractions (15 min at 60°C followed by 30 min at 110°C). The residue was washed and dried at 45°C under vacuum and reground (**Wursch, 1979**).

Proximate analyses

Moisture, ash, crude protein, crude fiber and total lipids contents were determined in carob according to **A.O.A.C. (2005**).Total hydrolysable carbohydrate was determined according to **Dubois *et al.* (1956).**

Extraction methods

Water, methanol and ethanol were used at 25C for 24h, at 50°C for 20min and by using Soxhelt apparatus also ,boiling water for 5, 10, 20 min were used to identify the most suitable solvent for the extraction of polyphenol, tannins, flavonoids and antioxidant. All extracts were then passed through filter paper and dried in oven at 50˚C.

Chemical composition

Total polyphenols content was estimated by the Folin-Ciocalteu method reported by **Elfalleh *et al.* (2009).** Hydrolysable tannins content was determined by the method of **Çam and Hişil (2010).** The amount of total flavonoids in the extracts was measured spectrophotometrically by the method of **Djeridane *et al.* (2006).** The scavenging activity on DPPH radical of different extracts was determined according to the method reported by **Okonogi *et al.* (2007**).

Bacterial and fungal isolates

Clinical isolates of *E .coli* NRRL B/210, *Staph. aeureus* NRRL B/3 B , *Bacillus cereus* NRRL B / G 43 , *Bacillus megtarin* NRRL B/1366 , *Listeria monocytogenase* serotype NRRL Y/477, *Klabsila pneumonia* ATTCC700603 , *Candida albicans* NRRL Y/477, and *Aspergillus niger* NRRL/3 and *Salmonella typhi* ATTC5647006 were obtained from chemical department of Natural and Microbial product, National Research Center, and were kept in the laboratory in the frozen state until used. The antimicrobial effect of the carob extracts was evaluated using disk inhibition zone by the method described by **Orak *et al.* (2011).**

Biological evaluation of carob water extracts and fiber.

a. Experimental animals.

A total of 45 of adult's male albino rats (Wister Strain) weighed 200g were obtained from Organization of Biological Products and Vaccines from Helwan breeding farm, Cairo, Egypt. The rats were housed in stain lasted cages with wire mesh bottoms in a room temperature maintained at 25 ºC ± 2ºC. Rats were kept under normal healthy conditions

for one week and fed on basal diet. The diet contained Casein 10%, Corn oil 10%, Salt mixture 4%, Vitamin mixture1%, Cellulose 5% and starch 70% (**Reeves *et al.* 1993).**

Dosage and administration of decoction: The decoction was administered at a dosage of carob fiber 15% (15% from the starch of basil diet was substituted by 15% carob fiber) (**Forestieri *et al.* 2006)** and carob water extract 600 mg/kg/day **(Mokhtari *et al.* 2011)**, using a Sondi needle by gastric gavage method **(Iddamaldeniya *et al.* 2006).**

After that the rats were divided into three groups,15 rats each. The first main group (control) was divided into three subgroups (5rats each) the first (control) was fed with basil diet for another 6 weeks. The second was fed on basil diet and administered of carob fiber The third was fed on basil diet and administered of carob water extract.

The second main group was the diabetic group. The rats were injected with a single dose of alloxan solution 150 mg\kg body weight (**Buko *et al* 1996**). After 24hours of alloxan injection, the presence of diabetes was confirmed (glucose blood was higher than 180 mg/dl). Rats were left for one week without any treatment to stabilize diabetes, and then were divided into three subgroups. The first (control diabetic) fed with basil diet for another 6 weeks. The second was fed on basil diet and administered of carob fiber. The third was fed on basil diet and administered of carob water extract.

The third main group was hypercholesterolemia group. The rats were fed on high fat diet similar to the control diet but differed in more fat content which was 10% sheep fat, 2% cholesterol and 0.25% bile salts and starch 57.75%. for 2 weeks **(Abdel-Rahim *et al* 2013)**, and then were divided into three subgroups. The first (control hypercholesterolemia) fed with basil diet for another 6 weeks. The second was fed on basil diet and administered of carob fiber. The third was fed on basil diet and administered of carob water extract.

Blood sample

At the end of experiment blood was collected in tubes from retro-orbital vein in two separated tubes , one tube with EDTA (ethylene diamin tetra acetic acid) for determination of haematological parameters , and the other tube was centrifuged at 3000 rpm for 20 min , for serum preparation.

Serum analysis

Serum parameters were determined by enzymatic colorimeteric methods, glucose was determined according to the procedure of **Trinder (1969)**. Serum triglyceride and total cholesterol were determined according to the methods of **Fossati and Prencipe (1982)** and **Allain *et al.* (1974)**. Low density lipoprotein (LDL-cholesterol) and high density lipoprotein (HDL - cholesterol) were determined according to the method of **Tietz *(*1976 a).** Total bilirubin, total protein and albumin in serum were determined according to the methods of **Walters and Gerarde (1970); Vassault *et al.* (1986)** and **Young *et al.* (1975)**. Alkaline phosphatases (ALP) was determined according to the method of **Young *et al.* (1972)**. Serum aspartate transferease (AST) and alanine transferease (ALT) activities were measured colorimetrically according to the method of **Tietz (1976 b).** Serum urea, Uric acid and Creatinine were determined according to **Tietz (1990), Vassault *et al.* (1986) and Tietz (1986).**

Haematology

The red blood cells (RBC), white blood cells (WBC), and the hemoglobin (Hb), were determined in Mindray 2800 hematology analyzer.

Statistical analysis.

Statistical analysis was done by Duncan's Methods **(SAS, 1996).**

Results and discussion

Data reported in Table (1) show the chemical composition of carob**.**

Table 1. Chemical composition of carob.

Constituents	carob based on dried weight (%)
Moisture	11.8
Crude fiber	10.03
Ash	3.42
Crude protein	3.80
Total lipid	2.80
Total carbohydrates	70.97

Data in Table (1) show that carob contained 11.8% moisture, 10.03% crude fiber, 3.42% ash, 3.80% crude protein, 2.80% total lipid and 70.97 total carbohydrates.

Khlifa *et al.* (2013) reported that the protein content in carob was 2.74% while, **Avallon *et al.* (1997)** reported that carob contained was 3% protein. **Hussein *et al.* (2011)** stated that carob contained 10.1% moisture, 5.48% total lipid, 73.14 % total carbohydrate.

Extraction.

Table (2) presents the effect of extraction methods on the total phenols, total tannins, total flavonoids and antioxidants activity of carob extract. The results indicate that water extract at 50^{o}C for 20 min recorded the highest value in total polyphenols, followed by methanol extract at 50^{o}C for 20 min. Total tannins recorded the highest value in water extract at 50^{o}C for 20 min, followed by methanol extract at 50^{o}C for 20 min, water and methanol extracts at 25^{o}C for 24 h. Water extract at 50^{o}C gave the highest yield of total flavonoids, followed by

water extract at 25 ºC for 24h. Antioxidant activity was significantly highest in methanol extract at 50ºC for 20 min followed by methanol extract at 25ºC for 24h. Increasing water boiling time cause significant decrement in total polyphenols, total tannins, total flavonoids and antioxidants. Extraction by Soxhlet showed the lowest efficiency.

Al-Rawahi *et al.* (2013) indicated that water (as the highest polar solvent), extracted highest phenolic compounds followed by methanol then ethanol (due to decreasing polarity).

The obtained results are in agreement with those reported by **Yim *et al.* (2009)**, **Sebai *et al.* (2013), Zam *et al.* (2013), Abugri and McElhenney (2013)** and **Yaser *et al*. (2014)**.

Table 2. Effect of different methods of extraction of carob on total phenolic, total tannins, total flavonoids and antioxidantes activity.

Extraction methods	Total phenols (g/100g)	Total tannins (g/100g)	Total flavonoids (mg/100g)	Antioxidants activity %
Water extract at 25°C for 24h	2.103d ± 0.08	0.85b ± 0.25	24.20b ± 1.26	63.89e ± 1.2
Methanol extract at 25°C for 24h	1.75e ± 0.11	0.80b ± 0.21	19.01d ± 1.87	82.99b ± 2.73
Ethanol extract at 25°C for 24h	1.437f ± 0.07	0.396c ± 0.15	16.73e ± 0.66	69.32d ± 1.79
Water extract at 50°C for 20 min	3.65a ± 0.22	1.20a ± 0.19	27.05a ± 1.73	69.13d ± 1.16
Methanol extract at 50°C for 20 min	3.297b ± 0.20	0.91b ± 0.29	21.57c±0.64	86.00a ±1.51
Ethanol extract at 50°C for 20 min	2.670c ± 0.09	0.42c ±0.15	14.05f ± 1.45	74.28c ± 1.66
Boiling water for 5min	1.12g ± 0.12	0.37dg ± 0.02	18.84d ± 0.94	64.16e ± 3.56
Boiling water for10 min	0.87h ± 0.11	0.32cd ± 0.02	15.83ef ± 0.87	59.46f ± 1.26
Boiling water for 20min	0.63i ± 0.10	0. 253de ±0.02	12.03g ± 1.52	51.13g ± 0.27
water extract in Soxhlet	0.11j ±0.01	0.056f ± 0.01	7.10i ± 0.42	39.86i ± 1.88
Methanol Soxhlet extract	0.22j ± 0.01	0.46ef ± 0.03	10.15gh ± 1.32	44.01h ± 3.96
Ethanol Soxhlet extract	0.12 j ±0.01	0.25de ±0.02	8.31hi ± 1.22	43.41h ± 2.80

a,b,c,….. means with column with differ letters different significantly ($p \leq 0.05$) from each other means followed by the same letter don't differ at 0.05 probability level.

Antimicrobial effects:-

Antimicrobial effects of carob extracts on some Gram positive bacteria.

Data in Table(3) show the effect of carob extracts against *Gram* positive bacteria; *Staph.aeureus, Listeria monocytogenase*, *Bacillus cereus* and *Bacillus megaterium*.

Staph. aeureus

Ethanol extract at 50°C for 20 min, boiling water for 5min and methanol extract at 50°C for 20 min had the highest antimicrobial effect. Boiling water for 20min and water, methanol and ethanol shoxhlet extracts showed no antimicrobial effects.

Listeria monocytogenase

Ethanol extract at 50°C for 20 min had the highest antimicrobial effect followed by methanol extracts at 50 °C for 20 min and at 25 °C for 24 h. Other extracts had no effect.

Bacillus cereus.

Water extract at 50°C for 20 min, boiling water for 5 min and methanol extract at 50°C for 20 min had the highest inhibition zone (13.5, 13.33 and 12.33mm).

Bacillus megaterium.

Methanol extract at 50 °C for 20 min and methanol soxhelt extract had the highest inhibition zones (11.17 and 10.17 mm), followed by boiling water for 5 min and ethanol soxhelt extract.

The obtained results are in agreement with those reported **Hussein *et al.* (2011), Abd Razik *et al.* (2012)** and **Hsouna *et al* .(2012).**

E .coli.

Only water extract at 50°C for 20 nim and 25 °C for 24 h showed antimicrobial effect against *E.coli* (10.17 and 8.33 mm).

Klebsila pneumonia

Boiling water for 5min and methanol Soxhlet extract had the highest antimicrobial effects. Boiling water for 20 min had no antimicrobial effect.

Salmonella typi.

All carob extracts had no antimicrobial effect against *Salmonella typi*.

The obtained results are in agreement with those reported by **Kivcak *et al* (2002), Hussein *et al* (2011), Abd Razik *et al* (2012), Aissani *et al* (2012)** and **Hsouna *et al* (2012)**.

Table 3. Effect of carob extracts on some Gram positive bacteria.

Treatments	*Staph. aeureus*			*Listeria monocytogenase*			*Bacillus cereus*			*Bacillus megaterium*		
	inhibition zone (mm)											
	20µ	40µ	mean	20µ	40µ	Mean	20µ	40µ	mean	20µ	40µ	mean
Water extract at 25°C for 24h	7.33g ± 1.25	9.66cf ± 0.8	8.5B	0d	0d	0C	0f	0f	0D	0g	0g	0G
ethanol extract at 25°C for 24h	0h	10.33ce ± 2.3	5.17C	0d	0d	0C	0f	0f	0D	0g	8.33ce ± 2.11	4.17EF
Methanol extract at 25°C for 24h	0h	8.67eg ± 1.5	4.33C	7.0bc ± 1.10	8.33b ± 0.54	7.67B	0f	0f	0D	0g	11.67ab ± 1.40	5.83DE
Water extract at 50°C for 20 min	8.33fg ± 0.90	8.67eg ± 0.7	8.5B	0d	0d	0C	11.33de ± 1.76	15.67a ± 2.70	13.5A	0g	0g	0G
Ethanol extract at 50°C for 20 min	11.0cd ± 1.30	13.00ab ± 1.8	12.0A	8.33b ± 1.00	10.33a ± 1.20	9.33A	9.0de ± 1.8	13.67ac ± 3.10	11.33AB	0g	12.67ab ± 1.15	6.33CD
Methanol extract at 50°C for 20 min	10.67cd ± 1.50	11.33bc ± 2.1	11.0A	6.0c ± 0.50	10.33a ± 1.51	8.17AB	10.67be ± 1.60	14.0ab ± 2.00	12.33A	8.67ce ± 0.65	13.67a ± 0.86	11.17A
Boiling water for 5min	10.0cf ± 1.20	13.67a ± 0.76	11.83A	0d	0d	0C	10.33ce ± 0.87	16.33a ± 3.00	13.33A	7.67df ± 1.15	11.00ac ± 1.73	9.33AB
Boiling water for10 min	7.33g ± 1.15	9.33df ± 0.54	9.33DF	0d	0d	0C	Of	12.0bd ± 1.20	6.00C	0g	6.33ef ± 0.63	3.17F
Boiling water for 20min	0h	0h	0D	0d	0d	0C	0f	0f	0D	0g	0g	0g
Water Soxhlet extract	0h	Oh	0D	0d	0d	0C	9.0de ± 0.54	10.33ce ± 1.4	9.67B	5.33f ± 1.12	11.00ac ± 1.76	8.17BC
Ethanol Soxhlet extract	0h	0h	0D	0d	0d	0C	8.33e ± 1.3	10.67be ± 2.00	9.50B	8.33ce ± 0.54	10.33bd ± 0.67	9.33AB
Methanol Soxhlet extract	0h	0h	0D	0d	0d	0C	11.33be ± 1.8	11.67be ± 0.9	11.5AB	8.67ce ± 0.86	11.67ab ± 1.54	10.17A
Mean conc	4.56B	7.05A		1.68B	2.42A		6.02B	8.5A		3.22B	8.05A	

a,b,c,….h means with column with differ letters different significantly ($p \leq 0.05$) from each other means followed by the same letter don't differ at 0.05 probability level.

Table 4. Effect of carob extracts on some Gram negative bacteria.

Treatments	*E .coli*			*Klebsila pneumonia*.		
	inhibition zone (mm)					
	20µ	40µ	mean	20µ	40µ	mean
Water extract at 25°C for 24h	8.00b ± 1.00	8.667b ± 1.50	8.33B	8.00df ± 1.9	8.66cf ± 1.50	8.83CE
Ethanol extract at 25°C for 24h	0c	0c	0C	6.667ef ± 1.33	12.00ac ± 2.00	9.33CE
Methanol extract at 25°C for 24h	0c	0c	0C	6.33f ± 0.40	9.667bf ± 1.00	8.00E
Water extract at 50°C for 20 min	9.00b ± 0.50	11.33a ± 1.40	10.17A	6.33ef ± 1.00	11.33bd ± 1.73	8.83CE
Ethanol extract at 50°C for 20 min	0c	0c	0c	10.33be ± 1.1	20.33ac ± 3.7	11.33BC
Methanol extract at 50°C for 20 min	0c	0c	0C	10.33bc ± 1.00	12.33ac ± 1.51	11.33BC
Boiling water for 5min	0c	0c	0C	12.67a b ± 1.15	15.67a ± 2.08	14.17A
Boiling water for10 min	0c	0c	0 C	10.00bf ± 1.25	11.33bd ± 0.86	10.67BD
Boiling water for 20min	0c	0c	0 C	0g	0g	0F
Water Soxhlet extract	0c	0c	0 C	8.66cf ± 0.62	10.67bd ± 1.1	9.66CE
Ethanol Soxhlet extract	0c	0c	0 C	12.67ab ± 1.06	13.00ab ± 1.41	12.83AB
Methanol Soxhlet extract	0c	0c	0 C	12.67ab ± 2.5	15.67a ± 3.46	14.17A
Mean conc	8.72B	11.06A		1.41A	1.66A	

a,b,c,..f means with column with differ letters differ significantly ($p \leq 0.05$) from each other means followed by the same letter don't differ at 0.05 probability level.

Data in Table (5) presents the effect of various carob extracts against *Candida albicans* and *Aspergillus niger*.

Candida albicans

Methanol Soxhelt extract and at 50 °C for 20 min had the highest antimicrobial effects. Water, ethanol and methanol extracts at 25 °C for 24hr and boiling water for 20min had no antimicrobial effect.

Aspergillus niger.

Water , ethanol and methanol extracts at 50 °C for 20 min had the highest inhibition activity. Methanol extract at25 °C for 24h gave the lowest antimicrobial effect. The other extracts had no effect. The obtained results are in agreement with those of **Kicvak *et al* (2002)** and **Hsouna *et al* (2012).**

Table 5. Effect of carob extracts on some fungi

Treatments	*Candida albicans*			*Aspergillus niger*		
	inhibition zone (mm)					
	20µ	40µ	mean	20µ	40µ	mean
Water extract at 25°C for 24h	0i	0i	0F	0e	0e	0D
Ethanol extract at 25°C for 24h	0i	0i	0F	0e	0e	0D
Methanol extract at 25°C for 24h	0i	0i	0F	0e	3.66d ± 1.00	1.83C
Water extract at 50°C for 20 min	11.0fg ± 1.52	17.67ac ± 2.0	14.33B	11.00b ±2.5	18.00a ±1.51	14.50A
Ethanol extract at 50°C for 20 min	13.00ef ± 1.67	15.33d ± 3.10	14.17B	10.33bc ± 1.40	17.67a ± 0.89	14.00A
Methanol extract at 50°C for 20 min	14.33de ± 2.10	19.33a ± 2.40	16.83A	8.00c ± 1.15	10.33bc ± 2.08	9.16B
Boiling water for 5min	9.0gh ± 1.00	10.00gh ± 1.50	9.50D	0e	0e	0D
Boiling water for10 min	0i	8.67h ± 0.54	4.33E	0e	0e	0D
Boiling water for 20min	0i	0i	0F	0e	0e	0D
Water Soxhlet extract	9.67gh ± 1.52	12.67ef ± 1.94	11.17C	0e	0e	0D
Ethanol Soxhlet extract	13.0 ef ± 0.57	16.00cd ± 2.09	14.50B	0e	0e	0D
Methanol Soxhlet extract	16.33bd ± 1.52	18.33ab ± 2.50	17.33A	0e	0e	0D
Mean	7.19B	9.80A		2.44B	4.13A	

a,b,c,…..i means within column with different letters different significantly ($p \leq 0.05$) from each other means followed by the same letter don't differ at 0.05 probability level.

Biological effects:-

Data concerning the effect of carob fiber and water extract on blood serum glucose and lipid profile are shown in (Table, 6). Data reported in Table (6) indicate that glucose levels in diabetic and hypercholesterolemia groups had significant increment compared with control. Carob fiber and water extract administration caused significant decrement in glucose level comparing with diabetic and hypercholesterolemia control groups.

The obtained results are in agreement with those reported by **Tabatabai and Li (2000), Forestieri *et al.* (2006)**, **and** Mokhtari ***et al.* (2011).**

Diabetic and hypercholesterolemia groups administrated with carob fiber and water extract showed significant decrement in triglyceride, total cholesterol and LDL levels and significant increment in HDL level in diabetic group comparing to diabetic and hypercholesterolemia control groups.

The obtained results are in agreement with those reported by **Zunft *et al.* (2001), Zunft *et al.* (2003)**, **Roso *et al.* (2010), Mokhtari *et al.* (2011) and Ali *et al.* (2012).** Table (7) show the effect of orally intake carob fiber and water extract of carob on liver functions.

Diabetic group administrated with carob fiber and carob water extract showed non-significant difference in total bilirubin, total protein and albumin. Carob fiber administration caused significant decrement in ALT comparing to diabetic control group. Carob water extract showed significant decrement in AST, ALT and ALP comparing with diabetic control group. Hypercholesterolemia groups administrated with carob fiber and water extract showed no significant difference in total bilrubin and significant decrement in total protein, albumin, AST, ALT and ALP comparing with hypercholesterolemia control group. The obtained results are in a agreement with those

reported by **Roso *et al* (2010).** Data in Table (8) show the effect of orally intake carob fiber and water extract on kidney functions.

Table 6. Effect of orally intake carob fiber and carob water extract on glucose and lipid profile.

Groups	Blood serum glucose mg/dl	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Control Basal diet	81.09ce ±11.00	61.46df ± 4.0	100.30e ± 5.9	39.25f ± 1.60	45.45bd ± 1.12
Basal diet + Carob fiber	61.84e ± 12.76	54.44eg ± 3.13	78.27h ± 3.66	25.99g ± 0.54	46.94bd ±1.84
Basal diet + Carob water extract	63.85e ± 10.38	49.61g ± 2.14	79.98gh ± 3.41	34.58fg ± 2.15	37.65f ± 3.30
Diabetic control group	420.10a ± 86.32	91.87b ± 7.69	131.60b ± 7.5	68.24c ± 1.76	29.03g ± 0.97
Basal diet + carob fiber	116.30b ± 14.73	57.44eg ± 9.43	120.00c ± 5.61	55.3de ± 1.52	48.94bd ± 3.33
Basal diet + carob water extract	123.8b ± 13.27	59.86eg ± 5.19	116.40cd ±6.64	58.67d ± 2.00	43.43ce ± 1.73
Hypercholesterolemia control group	102.00bd ± 16.40	121.60a ± 16.67	157.85a ± 4.78	109.00a ± 3.40	29.77g ± 1.90
Basal diet + carob fiber	73.72be ± 4.50	72.25cd ± 6.36	139.50b ± 4.31	85.62b ± 0.76	35.31fg ± 2.65
Basal diet + carob water extract	68.41e ± 4.13	61.99de ± 5.01	135.00b ± 4.77	89.45b ± 0.58	30.31g ± 0.58

a,b,c,….h means column with different letters differ significantly ($p \leq 0.05$) from each other means followed by the same letter don't differ the 0.05 probability level.
Each value presents the mean of 5 rats ± S.E.

Table 7. Effect of orally intake carob fiber and carob water extract on liver functions**.**

Groups	Total Bilirubin (mg/dl)	Total protein (g\l)	Albumin (g/dl)	AST U L–1	ALT U L–1	ALP U L–1
Control Basal diet	0.90b ± 0.14	6.95d ± 0.91	2.78e ± 0.26	24.85fg ±2.93	34.09gh ± 1.19	84.06df ± 4.60
Basal diet + Carob fiber	1.76ab ± 0.09	6.74d ± 0.32	2.96de ± 0.23	26.71f ±2.87	36.24g ± 1.76	81.01ef ± 4.95
Basal diet + Carob water extract	1.67ab ± 0.16	6.62d ± 0.43	2.97de ± 0.57	26.98f ± 1.50	32.91gi ± 3.40	75.59f ± 2.14
Diabetic control group + Basal diet	2.10a ± 0.08	6.93d ± 0.91	3.51be ± 0.41	43.53b ± 4.93	66.83b ± 3.19	98.69c ± 5.67
Diabetic + Basal diet + Carob fiber	1.62ab ± 0.11	6.30d ± 0.20	3.53be ± 0.77	43.34b ± 1.00	58.29cd ± 2.89	90.54cd ± 4.60
Diabetic + Basal diet + carob water extract	1.57ab ± 0.04	6.79d ± 0.63	3.8ab ± 0.29	38.38cd ± 2.99	56.39de ± 2.00	86.97de ± 5.30
Hypercholesterolemia control group	2.53a ± 0.23	9.60a ± 0.42	4.53a ± 1.11	56.26a ± 1.40	77.26a ±4.90	147.30a ± 8.40
Hypercholesterolemia + Basal diet + Carob fiber	1.82ab ± 0.15	8.25b ± 0.97	3.78bc ± 0.60	44.60b ± 1.75	68.83b ± 3.01	120.25b ± 2.90
Hypercholesterolemia + Basal diet + carob water extract	1.77ab ± 0.08	6.81b ± o.32	3.75bc ± 0.70	41.65bc ± 4.50	61.92c ± 2.00	118.85b ± 2.50

a,b,c,…g means within column with different letters differ significantly ($p \leq 0.05$) from each other means followed by the same letter don't differ at 0.05 probability level.
Each value represents the mean of 5 rats ± S.E.

Table 8. Effect of orally intake carob fiber and carob water extract on kidney functions.

Groups	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl
Control Basal diet	50.97fg ± 3.75	2.81de ± 0.15	0.28cd ± 0.10
Basal diet + Carob fiber	49.87fg ± 4.25	2.29d ± 0.08	0.18ji ± 0.06
Basal diet + Carob water extract	45.44gh ± 3.60	2.23ef ± 0.07	0.23eg ± 0.1
Diabetic control group + Basal diet	80.61a ± 6.11	4.64ab ± 0.39	o.37b ± 0.12
Diabetic + Basal diet + Carob fiber	72.01b ± 3.20	3.01c ± 0.14	0.19fh ± 0.04
Diabetic + Basal diet + carob water extract	69.00bc ± 2.82	3.20d ± 0.29	0.21fg ± 0.09
Hypercholesterolemia control group	83.63a ± 5.50	5.05a ± 0.23	0.52a ± 0.18
Hypercholesterolemia + Basal diet + Carob fiber	60.68de ± 2.21	4.64ab ± 0.36	0.32bc ± 0.08
Hypercholesterolemia + Basal diet + carob water extract	63.06cd ± 1.63	4.75ab ± 0.20	0.23dg ± 0.08

a,b,c,…g means within column with different letters differ significant ($p \leq 0.05$) from each other means followed by the same letter don't differ the 0.05 probability level.
Each value represent the mean of 5 rats ± S.E.

In diabetic and hypercholesterolemia groups there were significant increment in urea, uric acid and creatinine comparing to control (basal diet). Administration of carob fiber and water extract induced significant decrement in urea, uric acid and creatinine in diabetic group and significant decrement in urea and creatinine in hypercholesterolemia groups comparing with control groups. The obtained results are in agreement with those reported by **Mahgoub (2010)**, **Ali *et al.* (2012)** and **Shalby *et al.* (2012)**. The effect of carob fiber and water extract on hematological parameters of the rats are shown in Table (9). Administration of carob fiber and water extract induced significant increment in WBC in diabetic groups. In hypercholesterolemia group administrated with carob fiber showed significant increment in RBC count while, carob water extract showed increment in RBC count and decrement in WBC count. **Gulay *et al.* (2012)** reported that hematological parameters showed no significant differences between control and treated animals with carob bean extract.

Table 9. Effect of orally intake carob fiber and water extract of carob on hematological parameters.

Groups	RBC ($X10^6$/µl)	WBC ($X10^3$/µl)	Hb (g/dl)
Control Basal diet	6.7ab ± 0.31	15.78eg ± 2.39	14.34ac ± 0.61
Basal diet + Carob fiber	7.03ab ± 0.30	15.70fg ± 0.70	13.98bd ± 0.95
Basal diet + Carob water extract	7.49a ± 0.14	17.06de ± 1.95	15.1a ± 0.67
Diabetic control group + Basal diet	6.54ac ±0.39	14.6g ± 2.41	13.34df ± 1.36
Diabetic + Basal diet + Carob fiber	6.33bc ± 0.22	16.34df ± 3.20	12.66f ± 0.31
Diabetic + Basal diet + carob water extract	7.03ab ± 0.23	19.16b ± 3.6	14.02bd ± 0.72
Hypercholesterolemia control group	5.76c ± 0.55	17.60cd ± 1.64	12.86ef ± 0.06
Hypercholesterolemia + Basal diet + Carob fiber	7.07ab ± 0.06	18.85bc ± 1.82	13.58cf ± 0.12
Hypercholesterolemia + Basal diet + carob water extract	7.01ab ± 0.18	15.64fg ± 1.79	13.8be ± 0.57

a,b,c,…f means within column with different letters differ significantly ($p \leq 0.05$)from each other means followed by the same letter don't differ at 0.05 probability level.
Each value represents the mean of 5 rats ± S.E

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الملخص

تهدف هذه الدراسة إلي دراسة أفضل طريقه لاستخلاص البولي فينول و التانينات والفلافونويدات ومضادات الأكسدة من الخروب و قد تم استخدام الماء و الميثانول والايثانول علي درجات حرارة 25م لمده 24ساعة و50م لمده 20 دقيقه وكذلك الاستخلاص بجهاز سوكسلت و الغليان لمده 5و 10و20 دقيقه. أيضا تم دراسة تأثير هذه المستخلصات كمضاد للميكروبات وقد تم استخدام بعض الميكروبات الموجبة لجرام مثل (*Staphylococcus aureus, Listeria monocytogenese, Bacillus megaterium* and *Bacillus cereus)*

والسالبة لجرام مثل

(*Escherichia coli*, *Klebsiella pneumonia* and *Salmonella typi*)

والفطريات مثل (*Aspergillus niger* and *Candida albicans*) *كما تم دراسة تأثير* الياف الخروب والمستخلص المائي للخروب علي الفئران المصابة بارتفاع سكر الدم و الفئران التي تعاني من ارتفاع نسه الكوليستيرول .

أوضحت النتائج إن المستخلص المائي علي درجه حرارة 50م لمده 20 دقيقه أعطي اعلي كفاءة في استخلاص البولي فينول و التانينات و الفلافونويدات بينما مستخلص الميثانول علي 50م لمده 20 دقيقه أوضح اعلي كفاءة في استخلاص مضادات الأكسدة.

أظهرت جميع المستخلصات تأثير مضاد لجميع الميكروبات موضع الدراسة ما عدا *Salmonella typi*. حدث انخفاض في سكر الدم والجلسريدات الثلاثية و الكوليستيرول منخفض الكثافة و اليوريا و الكرياتينين بينما حدث ارتفاع في الكوليستيرول عالي الكثافة في الفئران المصابة بارتفاع سكر الدم و الفئران التي تعاني من ارتفاع نسبه الكوليستيرول .

أدت المعاملة بألياف الخروب إلي زيادة عدد كرات الدم البيضاء في الفئران التي تعاني من ارتفاع سكر الدم ، بينما الفئران التي تعاني من ارتفاع نسبه الكوليستيرول أدت المعاملة بألياف الخروب إلي زيادة عدد كرات الدم الحمراء و كذلك أدت المعاملة بمستخلص الخروب إلي زيادة عدد كرات الدم الحمراء والبيضاء.