A possible protective effect of *Citrullus colocynthis* Melon against diabetes mellitus type 2 associated with non-alcoholic fatty liver syndrome in rats.

Omnia Ezzat Ali Esmail

Biochemistry Department, Faculty of Pharmacy Egyptian Russaion University
to141999@hotmail.com

Abstract: Recent trends in controlling and treating diseases tend to prefer natural drugs rather than synthetic ones. The medicinal value of these plants lies in its constituents which include alkaloids, glycosides, saponins, flavonoids, volatile oils, steroids and minerals. An alcoholic extract of *Citrulls colocynthis* fruit seeds in a dose of 25 and 5o mg/Kg.B.wt
intraperitonealy have been given daily to the rats exposed to high fat diet for 25 weeks (30% fat instead of 5% fat in normal diet). Sampling has been done every 5 weeks with verifying of blood glucose level, plasma insulin level, liver lipid extraction and plasma leptin level. The results revealed a protective ability of the fruits seeds extract in preventing, to a large extent, the onset of the fatty liver syndrome as well as diabetes mellitus type 2 associated with it. These results could be valuable when discussed at molecular level.

[Omnia Ezzat Ali Esmail. **A possible protective effect of *Citrullus colocynthis* Melon against diabetes mellitus type 2 associated with non-alcoholic fatty liver syndrome in rats.** *J Am Sci* 2012;8(8):1054-1061]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 156

Key words: *Citrullus colocynthis* Melon, diabetes mellitus type 2, non-alcoholic fatty liver, blood glucose, plasma insulin and plasma leptin.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is reported worldwide and the number of affected patients is growing rapidly that the disease has reached epidemic proportions Powell *et al.*, [1] have detected NAFLD in 1.2 to 9% of patients subjected to liver biopsy.

Furthermore, NAFLD is considered a multifactorial disease that involves a complex interaction of genetic, diet and life style, all of which alone or in combination to form NAFLD phenotype [2 & 3]

Obesity and type II diabetes (insulin resistance) are the most recognized as risk factors or associated with NAFLD [4]. Meanwhile, Kershwa and Flier (2004) [5] reported that increase of adiposity as a result of high fat diet predisposes towards the development of insulin resistance and NAFLD.

On the other hand, the melon of *Citrullus Colocynthis* with its seed content revealed a wide range of pharmacological activities including antimicrobial [6], antirheumatic [7], antispasmodic [8], laxative and antihyperglycemic effect [9].

The antidiabetic effect of *C. colocynthis* fruits could be linked to more than one mechanism, it include the stimulation of β- cells with subsequent release of insulin and activation of insulin receptors [10]. Meanwhile Huseini, *et al.*, (2010) [11] reported that administration of *C. colocynthis* fruit extract had a beneficial effect on improving the glycaemic profile without sever adverse effects in type 2 diabetic patients. Furthermore, treatment with *C. colocynthis* fruit extract to diabetic mice also led to an increase in the activities of Hexokinase and phosphofructokinase a key enzymes of the glycolytic pass way as well as stimulate the oxidative phosphorylation [12].

The aim of the present study suspect a possible protective effect of 70% alcoholic extract of *C. colocynthis* melon seeds against type II diabetes and NAFLD progress in rate exposed to high fat diet.

2. Material and Methods

A total of 168 male Sprague-Dawley rats (120 ± 5 g) were used in this study, accommodated at the Animal House in the Faculty of Veterinary Medicine, Zagazig University for two weeks to accommodate before the experiment and kept on basal diet.

Rats under study were randomly divided into four groups as follows:
Group I: fed control diet (48 rats).
Group II: fed isocaloric high fat diet (40 rats).
Group III: fed isocaloric high fat diet and administered (i. p. injection) with alcoholic extract of *C. colocynthis* fruit seeds in a dose of 25 mg/kg B.wt /day (40 rats).
Group IV: fed isocaloric high fat diet and injected (i.p.) with alcoholic extract of *C. colocynthis* fruit seeds in a dose of 50 mg/kg B.wt/daily (40 rats).
Rats under study were fed on diets formulated according to (13) as follows:

	Control diet	Corn oil suppl. Diet
► **Ingredients (g/kg)**		
Casein	200	200
Methionine	4	4
Sucrose	246	146
Wheat starch	295	145
Olive oil	-	-
Corn oil	-	200
Beff tallow	20	20
Cellulose	-	250
Mineral mix.	25	25
Vitamin mix.	10	10
► **Composition by energy**		
Protein	20.64	20.64
Lipids	4.55	50.01
Carbohydrates	74.79	29.25

The experimental diet were freshly Prepared three times weekly and stored at 0 -4 °C to avoid rancidity.

Alcoholic extract of *C. colocynthis* fruit seeds obtained from Halayp region (Egypt) were dried and crushed, then soaked for 2 weeks in a suitable amount of 70% ethyl alcohol and filtrated using filter paper (Wattman No. 1). The obtained filtrate was evaporated under vacuum using Heidolph rotator evaporator (Germany). The extract was weighted, labeled and kept in refrigerator at 4ºC till used [14].

Sampling followed every 5 weeks by obtaining the blood serum for the determination of blood glucose, as well as insulin and leptin and the left lobe of the liver for determination of hepatic glycogen and hepatic lipids.

Hepatic lipids have been determined according to Folch *et al.*, (1957)[15]; blood glucose according to Trinder (1969)[16]; hepatic glycogen according to Johann and Lentini (1971)[17]; insulin according to Kjems *et al.*, (1993)[18] and leptin according to Keiichi *et al.*, (1998)[19].

The obtained data have been statistically analyzed by ANOVA and L.S.D. test calculated according to Kirkwood (1989)[20].

3. Result

High fat diet regime for 25 weeks induced significant increase in hepatic total lipids started after 10 weeks and increased with the time. Administration of ethanolic extract of fruit seeds were able to delay the onset and intensity of the hepatic steatosis and this action was proportional to the dose (Table 1).

Meanwhile, the blood glucose level was significantly increased up to 10th weak in high fat diet group and precede elevation with the time; such hypoglycemia showed delaying of its onset and intensity following oral application of the *C. colocynthis* fruit seeds with superiority of the higher dose. Hepatic glycogen in contrast to blood glucose showed significant dropping after 20 weeks and enhanced to a large extent following the seeds extract administration (Table 2).

Furthermore, blood insulin elevated significantly starting after 10 weeks in high fat diet group and increased more with the time of exposure. Leptin in blood also elevated starting from the 5th weeks after high fat diet regime and both hyperinsulinaemia and hyperleptnaemia have been corrected to large extent with delaying of its onset following the oral administration of the ethanolic extract of *C. colocynthis* fruit seeds and this action was doses related (Table 3).

Table (1): Hepatic total lipids (mg/g) after *Citrullus colocynthis* fruits seeds application in rats exposed to high fat diet

	Normal diet	High fat diet	High fat diet + *Citr. colo.* Fruits	
			25 mg/kg B.wt	50 mg/kg B.wt
Control	**72.5^g ± 3.12**			
After 5 weeks	**72.2^g ± 5.11**	**78.23^g ± 9.55**	**76.88^g ± 11.31**	**70.40^g ± 9.51**
After 10 weeks	**73.6^g ± 6.14**	**98.55^e ± 7.14**	**79.61^g ± 9.98**	**72.40^g ± 12.21**
After 15 weeks	**73.7^g ± 7.25**	**139.31^c ± 11.21**	**122.46^f ± 9.73**	**92.50^f ± 8.33**
After 20 weeks	**75.8^g ± 8.13**	**152.23^b ± 11.66**	**131.20^c ± 10.55**	**114.3^d ± 9.22**
After 25 weeks	**76.10^g ± 7.21**	**168.41^a ± 10.91**	**139.4^c ± 12.21**	**121.20^{cd} ± 10.15**

- Each value represents the mean of 8 animals ± S.D.
- The highest mean value has the symbol (a) and decreasing in value was assigned alphabetically.
- Values which have different letters are different significantly at $p < 0.05$

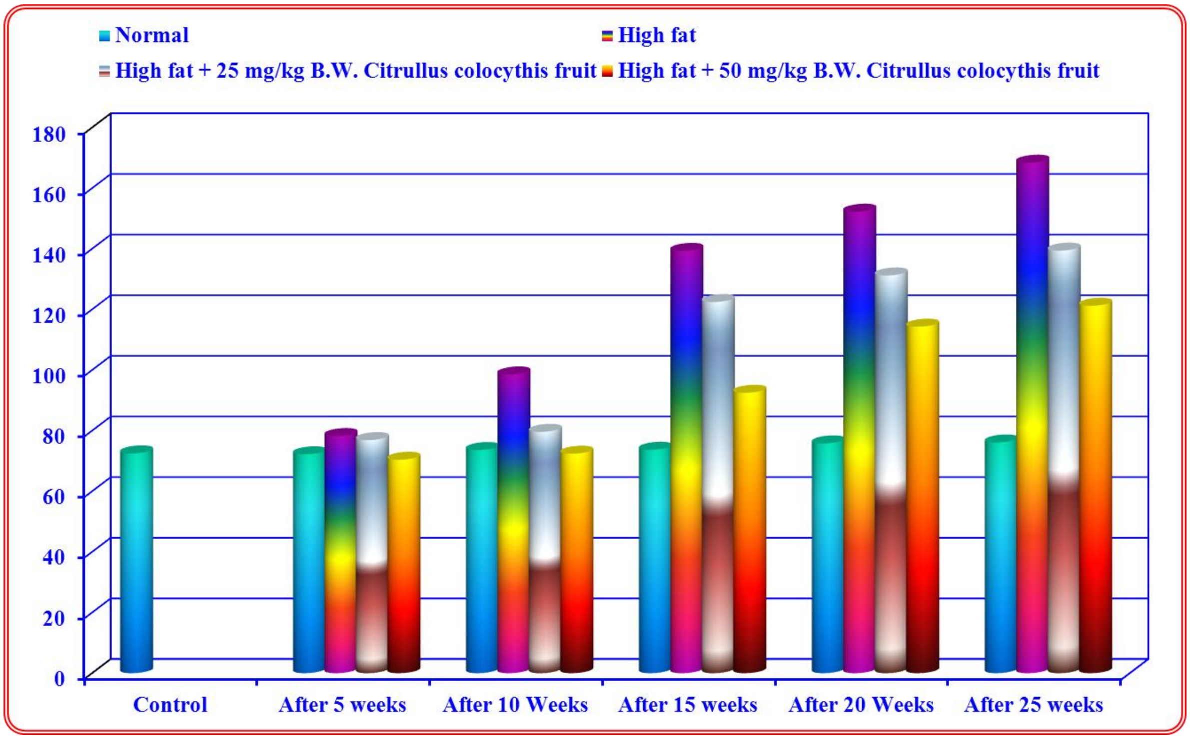

Figure (1): Hepatic total lipids (mg/g) after *Citrullus colocynthis* fruits seeds application in rats exposed to high fat diet

Table (2): Blood glucose (mg/dl) and hepatic glycogen (mg/g) after *Citrullus colocynthis* fruits seeds application in rats exposed to high fat diet.

	Normal diet		High fat diet		High fat diet + Cit. Melon			
					25 mg/kg B.wt		50 mg/kg B.wt	
	Glucose	Glycogen	Glucose	Glycogen	Glucose	Glycogen	Glucose	Glycogen
Control	94.5^{d} ± 11.31	44.3^{c} ± 5.35						
After 5 weeks	95.20^{d} ± 12.42	45.20^{c} ± 6.24	99.80^{d} ± 11.31	45.50^{c} ± 6.55	82.20 ± 9.54	52.7^{B} ± 4.49	86.40^{d} ± 7.81	57.8^{A} ± 4.46
After 10 weeks	95.80^{d} ± 9.95	45.90^{c} ± 7.53	116.40^{d} ± 12.32	42.70^{cD} ± 4.12	94.3 ± 11.21	55.40^{A} ± 3.35	81.50^{A} ± 8.31	$61.^{A}$ ± 3.79
After 15 weeks	97.40^{d} ± 8.55	46.80^{c} ± 6.24	136.80^{c} ± 9.98	40.50^{cD} ± 3.27	102.4 ± 10.63	43.20^{CD} ± 5.29	99.20^{d} ± 11.42	45.20^{c} ± 5.11
After 20 weeks	96.80^{d} ± 11.60	46.80^{c} ± 3.55	198.20^{b} ± 13.55	30.80^{E} ± 4.31	135.40^{c} ± 12.35	33.17^{DE} ± 6.17	112.40^{d} ± 9.65	39.10^{D} ± 4.24
After 25 weeks	97.90^{d} ± 12.73	47.20^{c} ± 4.23	236.40^{a} ± 11.41	22.40^{E} ± 3.41	142.60^{c} ± 12.97	26.44^{E} ± 4.12	121.30^{cd} ± 8.26	29.47^{E} ± 4.66

- Each value represents the mean of 8 animals ± S.D.
- The highest mean value has the symbol (a) for glucose and (A) for glycogen and decreasing in value was assigned alphabetically.
- Values which have different letters are different significantly at $p < 0.05$

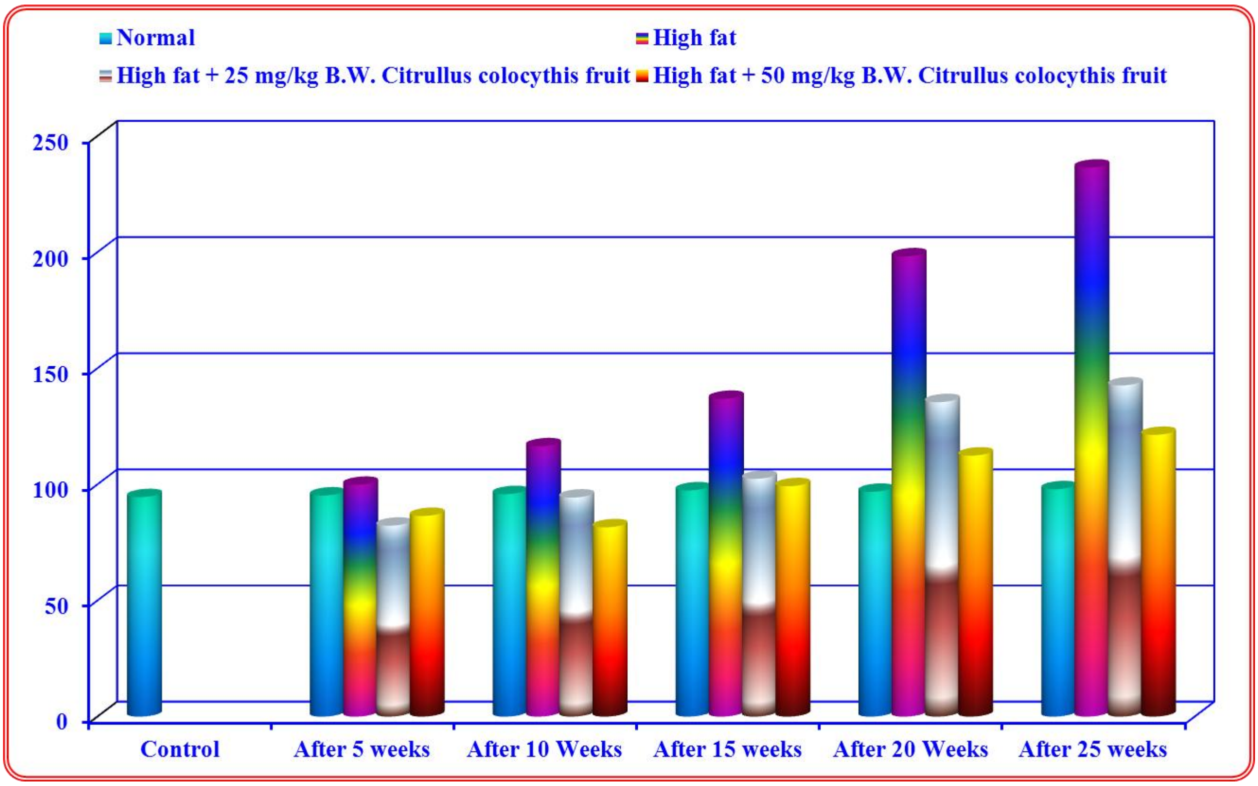

Figure (2): Blood glucose (mg/dl)

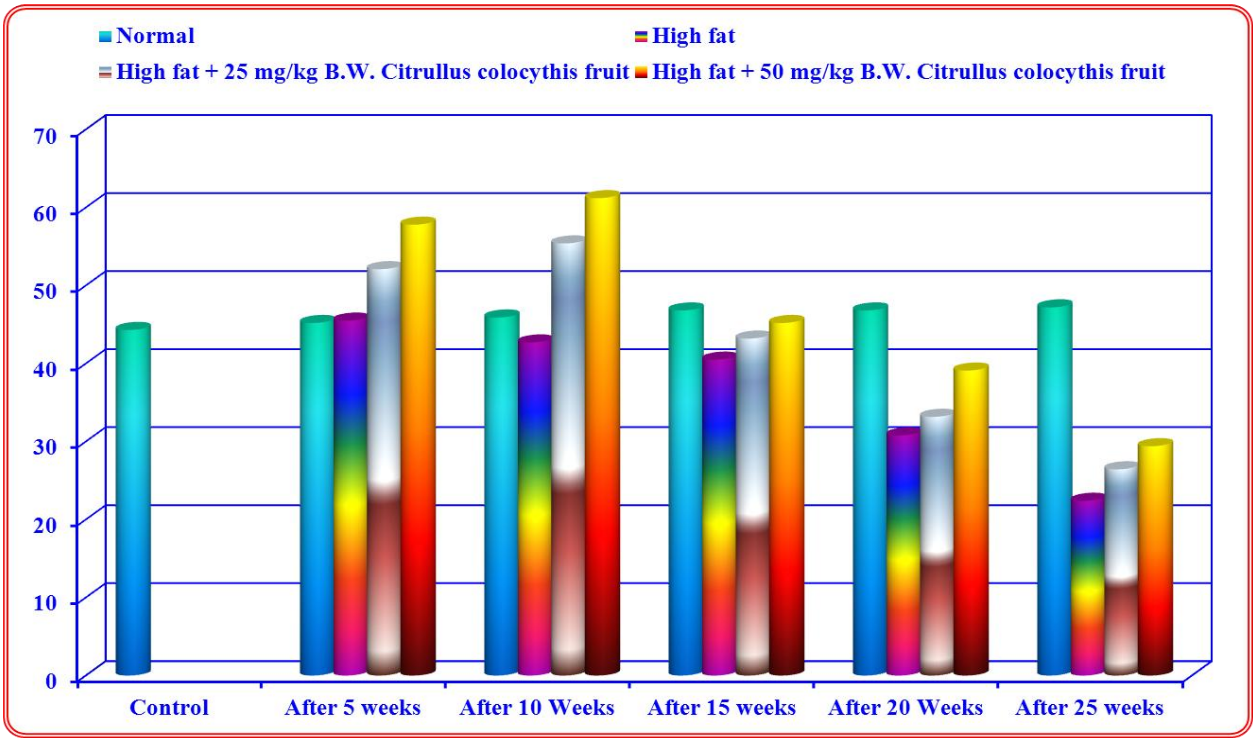

Figure (3): Blood glycogen (mg/dl)

Table (3): Plasma insulin and leptin (pg/ml) after *Citrullus colocynthis* fruits seeds application in rats exposed to high fat diet

	Normal diet		High fat diet		High fat diet + Cit. Melon			
					25 mg/kg B.wt		50 mg/kg B.wt	
	Insulin	Leptin	Insulin	Leptin	Insulin	Leptin	Insulin	Leptin
Control	**131.81^{h} ± 11.54**	**251.52^{E} ± 16.37**						
After 5 weeks	**133.64^{h} ± 14.99**	**246.97^{E} ± 19.29**	**142.15^{gh} ± 12.52**	**282.48^{D} ± 19.77**	**136.30^{gh} ± 15.23**	**252.4 ± 12.17**	**137.40^{gh} ± 13.20**	**228.30^{E} ± 19.18**
After 10 weeks	**135.22^{gh} ± 12.25**	**251.29^{E} ± 11.74**	**166.42^{g} ± 14.83**	**289.25^{cD} ± 19.45**	**139.16^{gh} ± 20.55**	**256.6 ± 17.63**	**137.30^{gh} ± 14.16**	**244.20^{E} ± 18.55**
After 15 weeks	**136.12^{gh} ± 11.88**	**259.32^{E} ± 16.58**	**210.25^{e} ± 17.44**	**318.20^{dD} ± 19.39**	**166.41^{g} ± 16.74**	**271.3 ± 19.54**	**155.31^{g} ± 15.52**	**262.30^{D} ± 19.63**
After 20 weeks	**141.15^{gh} ± 15.49**	**260.12^{E} ± 16.64**	**481.22^{b} ± 22.28**	**405.11^{B} ± 18.25**	**298.6^{d} ± 22.34**	**299.4^{cD} ± 35.55**	**212.40^{fg} ± 19.43**	**281.90^{D} ± 29.97**
After 25 weeks	**140.15^{gh} ± 9.98**	**256.25^{E} ± 13.58**	**599.4^{a} ± 17.23**	**451.6^{A} ± 14.97**	**342.32^{cd} ± 22.11**	**388.3^{Bc} ± 29.14**	**235.42^{f} ± 18.88**	**361.80^{c} ± 19.15**

- Each value represents the mean of 8 animals ± S.D.
- The highest mean value has the symbol (a) for insulin and (A) for leptin and decreasing in value was assigned alphabetically.
- Values which have different letters are different significantly at $p < 0.05$

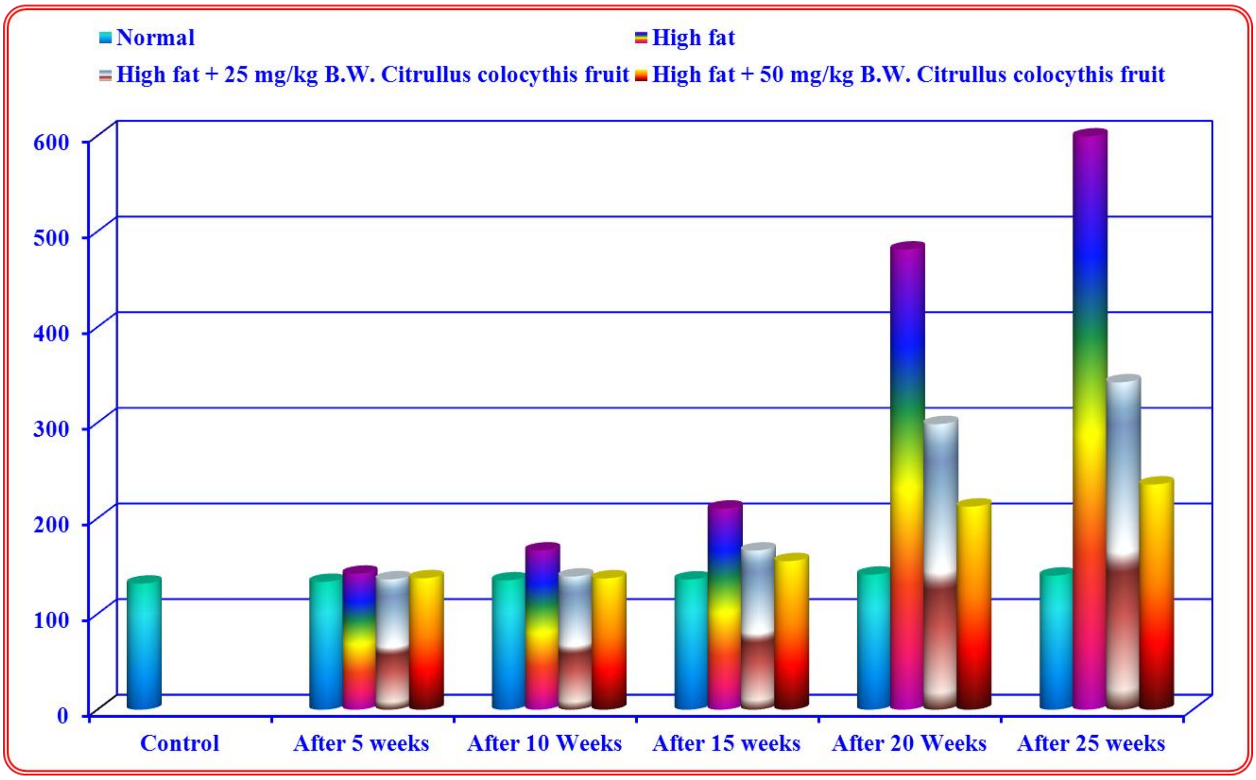

Figure (4): Plasma insulin (pg/ml)

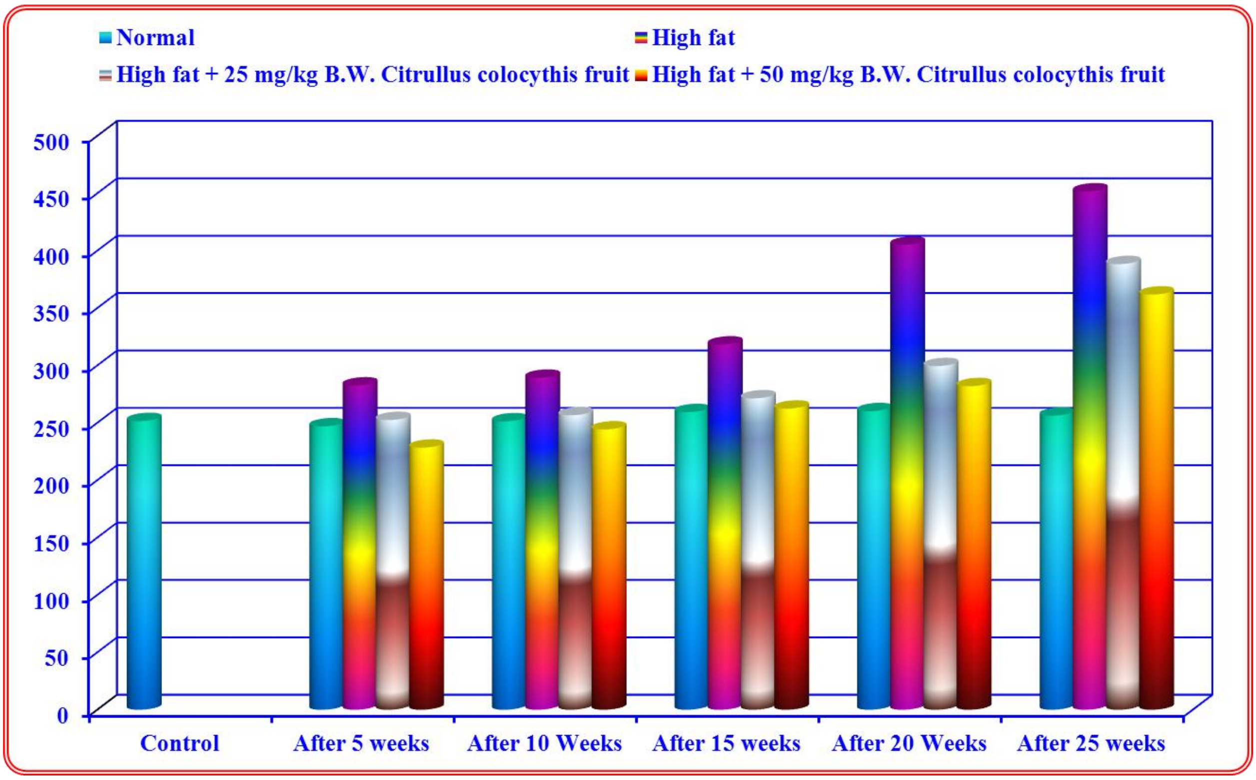

Figure (5): Plasma leptin (pg/ml)

4. Discusion

Traditional plant medicines are used throughout the world of such medicines might after a natural key to unlock a diabetologist's pharmacy for the future [(21)]. Meanwhile a *Citrullus colocynthis* fruits is commonly consumed as a vegetable in India and other Asian countries and recognized to be of medicinal value in the Indian system of medicine that the fruit juice is considered a tonic for the stomach with application for diseases of diabetes mellitus [(22)]. It may also have antiviral activity [(23)], antitumor [(24)] and immune-potentiating quality [(25)]. Such pharmacological activities of bitter Mellon could be very expected that it is very rich in chemical constituents which include glycosides, saponins, alkaloids, resins, phenols, fixed oils and free acids [(26)]. In this study we used the ethanolic extract of the *C. colocynthis* fruit seeds in two different doses 25 & 50 mg/ kg B.wt. applied daily and orally along the experiment.

On the other hand, Masek and Fabry (1999)[(27)] have stated the first description of a "high-fat diet" that induces obesity by a nutritional interconvertion. Further studies have revealed that high- fat diet promote hyperglycemia and whole body insulin resistance[28)]. In this work we used high- fat diet that the fat represents 22% instead of 2% in weight in normal diet, corresponding to 50% instead of about 5% as composition by energy, respectively

The obtained results concerned the effect of the two tested doses of ethanolic extract of *C. colocynthis* fruit seeds on the hepatic total lipids in high- fat diet rats (Table 1) were very positive 10 weeks, that the hepatic lipids which elevated to about 9.2% after 15 weeks following high- fat diet instead of 7% after normal diet which stayed in the same range after the higher dose of ethanolic extract (50 mg/kg. B. wt) while the 25 mg/kg. B. wt was less effective. These results are inconsistence with that of Abd El-Baky *et al.*, (2009)[(29)] who reported a hypoglycaemic effect of *C. coloncynthis* seeds in normoglycaemic and hyperglycaemic diabetic rats. Concerning blood glucose and hepatic glycogen, (Table 2) revealed that high- fat diet induced hyperglycemic effect starting after 10 weeks and reached its maximum after 25 weeks, in contrast to the hepatic glycogen which correlated negatively with that of blood glucose level that hepatic glycogen% decrease from about 4.5% in control to 2.24% after 25 weeks exposure to high-fat diet. These results are in consistence with that of Day and James (1998)[(30)] who stated that obesity and diabetes type 2 are frequent in aging population and usually associated with heptin steatosis. Furthermore the result concerned insulin and heptin in blood (Table 3) confirmed the above mentioned statement that the high fat diets induce elevation of both insulin (up the 5 weeks) and leptin in spite the obtained hyperglycemia. Multiple meachanisms have been proposed as the cause of bitter malons hypoglycaemic effect, Meir and Yaniv (1985) [(31)] reposted an inhibitory effect on glucose absorption, an increased of circulating glucose as hepatic glycogen [(32)] and/or an enhanced secretion of insulin [(33)].

The obtained hyperinsulinaemia in a ccomparried with hyperglycemia following high fats diet revealed typical diabetes type 2 which matches with the results of Cresceno, *et al.,* (2003) [(34)] who reported that feeding of adult male rats with a high fat diet for 7 weeks induced alterations in the mitochondrial component which associated with development of insulin resistance and ectopic fat storage in the liver. Meanwhile, table (3) revealed that the hyperisulinemia and increase of leptin level precedes the hepatic steatosis (Table 1) could leads to the acceptance that the sequence of events leading to hepatocyt fatty degeneration begins with insulin resistance which precedes fat accumulation that both of them act as a powerful oxidative stress which activate different sources of reactive oxygen species as reported by Grattagliano, *et al.,* (2008)[(35)].

Thus our results could reveal that the ethanolic extract of *Citrullus colocynthis* fruit seeds with its high content of pharmacologically active ingredient including tannin, saponin and flavanoids were able to correct for a large extent, the fatty liver syndrome and the hyperglycemic state associated with hyperinsulnemia (type 2 diabetes) induced by exposure to high-fat diet and this action was doses dependant.

Referances

1- **Powell, E.E., W.G. Coksly, R. Hanson, J. Searle, J.W. Hallday and L.W. Powell (1990):** The national history of nonalcoholic steatohepatits: a follow-up study of forty-two patients for up to 21 years. *Hepatology, 11: 74-80.*

2- **McCullough, A. J. (2004):** The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin. Liver. Dis., 8, 521-33.*

3- **Angulo, 2005 P. (2006):** NAFLD, Obesity and bariatric surgery. *Gastroenterol., 130: 1848-1852.*

4- **Adam, L.A. and K.D. Linder (2007):** Nonalcoholic fatty liver disease. *Ann. Epidemoil., 17(11): 863-869.*

5- **Kershaw E.E. and J.S. Flier (2004):** Adipose tissue as an endocrine organ. *J. Clin. Endocrinol. Metab., 89: 2548-2556.*

6- **Offonary, S.U. and O.K. Achi (1998):** microbial population associated with the retting of melon pods (*Citrullus colocynthis*) during seed recovery. *Plant food for Human Nutrition Dordrech, 52, 37-47.*

7- **Agrawal, V.K. and Gamblir, S.S., and A.K. Wahi (1994):** preliminary study on anti-inflammatory activity of some medicinal plants. *Indian J. Nat. Prod., 10 (1), 14-15.*

8- **Wasfi, I.A., A.K. Bashir, A.A. Abdalla, N.R. Danna and M.M. Tanira (1995):** Anti-inflammotry activity of some medicinal plants of the United Arab Emirates. *International J. of Pharmacognosy, 33(2), 124-128.*

9- **Burkill, H.M. (1985):** The useful plants of west tropical Africa. Vol. I Families A.D. *Royal Botanic Garden, Kew, UK.*

10- **Kaleem, M. Asif, O.U. Ahmed, B. Bano (2006):** antidiabetic and antioxidant activity of *Annona squamosa* extract in STZ induced diabetic rats. *Singapore Med. J., 47, 670-675.*

11- **Huseini, H.F., F. Darvistizadeh, R. Heshmat, Jafariazar, M. Raza and B. Larijani (2010).** The clinical investigation of *Citrullus colocynthis* (L.) shrad fruit in treatment of type II diabetes: a randomized, double blind, placebo-controlled clinical trial. *Phototherapy Research, 23(8), 1180-1189.*

12- **Rathi, S.S., O.K. Grover and V. Vats (2002):** the effect of *Momordi cachanti* and *Mecuna pruriens* in experimental diabetes and their effect on key metabolic enzymes involved in carbohydrate metabolism. *Phytotherapy Research, 16, 351-357.*

13- **Portillo, M.P., M-Chavarr, D. Duran and M., (2001):** Differential effects of diets that provide different lipids sources on hepatic lipogenic activities in rats under *ad libitum* or restrical feeding. *Nutrition, 17(6), 467-473.*

14- **Shan, A.H. and Z. A. kham (1997):** Gastroprotective effects of pretreatment with *Zizyphus sativa* fruits against toxic damage in rats. *Fitoterapia, 3, 226-234.*

15- **Folch, J., N. Lees and G.H. Sloane-Stanly (1957):** A simple method for the isolation and purification of total lipids from animal tissues. *J. Biolog. Chem., 226(1): 497-509.*

16- **Tinder, P. (1969):** Enzymatic method for glucose estimation. *Ann. Clin. Biochem, 6, 24-29.*

17- **John, A. and S. Lentini (1971):** Stimultaneous determination of glycogen and lipid from heart and muscles. *Analytical biochem., 43, 183-187.*

18- **Kjems, L. L., M. E. Roder, B. Dincsen, S. G. Hartling, P.N. Jorgensen and C. Binder**

(1993): Highly sensitive enzyme immunoassay of proinsulin immunoreactivity with use of two monoclonal antibodies. *Clin. Chem., 39(10): 2146-2150.*

19- **Keiichi, I, M. Yayoi, N. Yoshito, M. Rtsuchi, K. Mikio, H. Chie, T. Tetsuo, S. Kazuyuk., T. Hiroshi, M Hiroaki, H. Kiminori, O. Yoshihiro and N. Kazuwa (1998):** Development of a sensitive ELISA for Ruman Leptin USING monoclonal antibodies. *Clin. Chemistry, 44: 2165-2171.*

20- **Kirkwood, B.R. (1989):** Essentials of medical statistics. *Blacwell Scientific publication 2nd ed., p. 151.*

21- **Baily, C. J. and Flatt, P. R. (1986):** Antidiabetic drugs, new developments. *Indian Biochemistry, 6, 139-144.*

22- **Platel, K. and K. Srinivasan (1995):** Effect of dietary intake of freeze dried bitter gourd (*Momordica charantia*) in streptozotocin in induced diabetic rats. *Nahrung, 39, 262-268.*

23- **Bourinbaiar, A.S. and S. Lee-Huany (1996):** The activity of plant- derived antirctrovital proteins MAP30 and GAP31 against herpes simplex virus in vitro. *Biochem Biophys Res Commun, 219: 923-929.*

24- **Jilke, C., B. Strikler, G. W. Fortner, E. F. Hays and D. J. Takemoto(1983):** In vitro antitumor activity of the bitter melon (*Momordico charantia*). *Cancer Res, 43, 5151-5155.*

25- **Cunnick, J. E., K. Sakamoto, S. K. Chapes, G. W. Fortner and D. I. Takemoto (1990):** Induction of tumor cytotoxic immune cells using a protein from the better melon (*Momordica charantia*). *Cell immunol, 126, 278-289.*

26- **Dhalla,** N.S., Gupta, K.C. and M. S. Sastry (1961): Chemical composition of the fruit of momordica charantia (*Citrullus colocnthis*). *Ind. J. Pharm., 23, 128-133.*

27- **Mask, J. and P. Fary (1999):** High fat diet and the development of obesity in albino rats. *Experientia, 15: 444-445.*

28- **Lingohr, M. K, Buettner, Rhoders (2002):** Pancreatic beta-cell growth and survival- a role in obesity- linked type 2 diabetes. *Trends Molec. Med., 8 (8): 375-384.*

29- **Abd-El Baky, A., A. Abdulla, Abd El-Mawgoud and E. Abd-Hay (2009):** hypoglycaemic and hypolipidaemic action of Bitter Melon on normoglycaemic and hypoglyceaemic diabetic rats. *Research J. Medicine and Medical Sci. 4(2), 519-525.*

30- **Day, C. P. and O. F. James (1998):** Steatohepatitis, a tale of two "hits"? *Gastroenterol., 114, 842-845.*

31- **Meir, P. and Z. Yaniva (1985):** An *in vitro* study on the effects of *Momoridica charantia* on glucose uptake and glucose metabolism in rats. *Planta Medica, 33, 12-16.*

32- **Welihinda, J. and E. H. Karunanayka (1986):** Extra pancreatic effects of *Momoridica charantia* in rats. *J. of Ethnopharmacology, 17, 242-255.*

33- **Welihinda, J. and E. H. Karunanayka**, M. H. R. Sheriff and K. S. A. Jaya- Singhi (1986): Effect of *Momoridica charantia* on the glucose tolerance in maturity diabetes. *J. of Ethnopharmacology, 17, 277-282.*

34- **Crescenzo, R., F. Bianco, I. Falcone, P. Marina, G. Liverini and S. Iossa (2008):** Alteration in hepatic mitochondrial component in a model of obesity and insulin resistance. *Obesity (Silver spring), 16(5): 968-964.*

35- **Grattaliano, I., V. O. Balmieri, P. Portineasa, A. Moschetta and G. Palasciamo (2008):** factors associated with the metabolic syndrome: a unifying hypothesis. *J. Nutr. Biochem., 19 (8): 491-504.*