Remedial Effect of Costus ,Argel and Emblica Extracts For Hypercholesterolemic Rats .

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ABSTRACT

The ongoing study highlights the effect of some plants which include (Costus speciosus- Solenostemma Argel– Phyllanthus emblica and their mixture) to treat hypercholeaterolemia. Rat's hypercholesterolemia was induced in normal healthy male albino rats by Cholesterol feeding for 2 weeks on diet containing 1.5% cholesterol plus 10% sheep tail fat. It total number of (42) mature male albino rats of Sprague – dawley strain weighting 180-200 g rats were divided into 6 groups (7rats in each group). One of them used as control positive(+ve)group, second as control negative(-ve) and others groups from 3-6 fed on diets and containing plants extracts at dose (400 mg/kg b.w.) for 28 days orally. At the end of experimental period blood samples were collected for serum separation to evaluate liver function (AST,ALT&ALP), kidney function (uric acid , urea & creatinine) , internal organs Weights % of b.w. , lipids profile(TG -TC-LDL-VLDL-HDL),& histopathological change of liver. Results of present investigation revealed that all tested plants improved previous phytochemical parameters, but mixtured extract(Mix) was the best treatment in practicular Histopathological changes for liver, showed remarkable recovery of liver tissues, for all groups especially the mix group which indicated maximum recovery possibly due to phytochemicals compared to livers of control (+)ve group which was showing hyperplasia of epithelial lining bile duct& fibro-plasia in portal triad ,besides sinusoidal leucocytosis. The results of present work suggestes the use of tested plantes for clinical nutrition .

Keywords: hypercholesterolemia – Costus speciosus- Solenostemma argel- Pyllanthus emblica

INTRODUCTION

Cholesterol is the principal sterol synthesized by all animals.it modulates membrane fluidity over the range of physiological temperatures. Cholesterol is required to build and maintain membranes; and modulates membrane fluidity over the range of physiological temperatures allowing animal cells to change shape and animals to move.

Ohvo et al**., (2002)** indicted that cholesterol is needed for absorption of fat molecules as well as the fat-soluble vitamins, A, D, E, and K. Some research indicated that cholesterol may act as an antioxidant**.(** William, christie 2003**). (**Jensen et al., 1978)repoted that there are several types of lipoproteins in the blood. In order of increasing density, they are chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Lower protein/lipid ratios make for less dense lipoproteins.

The 1987 report of 6GV (National Cholesterol Education Program, Adult Treatment Panels) suggests the total blood cholesterol level should be: < 200 mg/dL normal blood cholesterol. 200–239 mg/dL borderline; > 240 mg/dL high cholesterol Given the well-recognized role of cholesterol in cardiovascular disease, some studies have shown an inverse correlation between cholesterol levels and mortality. A 2009 study of patients with acute coronary syndromes found an association of hypercholesterolemia with mortality outcomes.

Wang et al**., (2009)** showed that the methanol costus extract perturbed cell cycle progression, modulated cell cycle and regulated signal molecules, which were involved in induction of apoptosis in HepG2 cells. Their findings indicate that phytochemicals of leaves of *C. speciosus* shows potential for natural therapeutic product development for hepatocellular carcinoma. This is the first report to demonstrate in vitro anticancer activity of leaf extract of *C. speciosus* in relation to liver cancer.

Sandhya et al., (**2014)** stated that K, Ca and P, creatinine, urea, aspartate aminotransferase (AST) and alkaline phosphatase (ALP were found to be significantly higher ($P < 0.05$) in experimental rats group when compared with the control group. In the meantime, total protein, albumin, total bilirubin, alanine aminotransferase (ALT), and Na and Fe did not differ significantly from those of the control group ($P > 0.05$).

Osman et al., (2014**)** Reported that the effects of aqueous fruit extract of Phyllanthus emblica Linn was studied on type-II diabetes rats this study showed that aqueous fruit extract, in a dose of 200 mg/kg

body weight, significantly decreased the blood glucose level in alloxan-induced diabetic rats ($p<0.05$). Almost similar decrease in glucose level was also observed by chlorpropamide, a known antidiabetic drug in a dose of 84 mg/kg. The aqueous extract also induced hypotriglyceridemia by decreasing TG levels at 0, 1, 2 and 4 hours in diabetic rats ($p<0.05$). In addition, the extract was also found to improve liver function by normalizing the activity of liver-specific enzyme alanine transaminase (ALT).

Mani et al., (2010**)** stated that *Phyllanthus emblica* has antidiabetic action and beneficial effects on lipid profile, thus it can be recommended for use as a natural supplementary herbal remedy in patients suffering from diabetes mellitus .This work carried out to study the effect of certain single plants and their mix which were costus (*Costus speciosus*)Branches argel(Solonestemma argel)leaves& emblica fruits (*Phyllanthes emblica*) as, extracted with distilled water and heating , then dried to obtain a powder ; the latter dissolved in distilled water and 1/ml of extract containing 400 mg powder kg B.W was given to in male albino rats (180-200g intial weight).

MATERIALS AND METHODS

4. Materials:

- Plants: The first one was costus **(*Costus speciosus*)** branches the second was argel **(*Salenostemma argel*)** leaves, the third was emblica **(*Phyllanthus.emblica*)** fruit.
- All Plants were purchased as dried material from the local market(spices &herbes shop) cairo ,Egypt ,and milled The mixture prepared of collected dried powders.

4.1. Preparation of basal diet:

The basal diet was prepared according to **Reeves et al., (1993).** It was consisted of 20% protein (casein), 10% sucrose, 4.7% corn oil 0. 2% choline chloride, 1% vitamin mixture, 3.5% salt mixture and 5% fiber (cellulose). The remainder from 100 was corn starch The composition of salt mixture was according to **(Hegested, et al., 1941)** and vitamin mixture **(Campbell, 1963)**

5.Methods:

5.1. Preparation of samples:

1) The branches of (*costus speciosus*), leaves of, Solenostemma argl , and *Phyllanthus emblica* fruit were ground using porcelain grinder to pass through sieve mesh pores of 1mm diameter.

2) Twenty g sample of each plant and 1000ml distilled water, were kept in conical flasks provided with glass condensers, then heated under reflux for one hour at 70°C.
3) The heated mixture was cooled and filtered.
4) The filtrate poured in different Petri dishes and dried in a fan oven at 70°C till dried as a film, separated then crushed and the dried powder solubilized in distilled water, each rat was oral administrated with 1 ml litter of liquid. containing 400mg /kg B.W. rat form plant extracts
5) Extracts were kept in dark bottles to prevent oxidation then saved until the experiment **(Nagm, 2002).**
6) The mixture was cooled and filtered before filing in dark bottles.

5.2. Induction of experimental design

Diet containing of 1.5% Cholesterol for 15days body weight, plus 10% sheep tail was given to rats according to the method described by **Ain (1993)**

5.3. Biological Experiment:

Fourty two (42) male albino rats, weighting between 180–200 g were used in the study. The rats were derived from Research Institute farm of Ophthalmology, Medical Analysis Department, Giza, Egypt. Rats were housed in individual stainless steel cages under controlled environmental conditions. Diets were introduced to rats in a special non scattering feeding cups to avoid loss of feed and contamination.Tap water was provided to rats by means of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage. Food and water provided and checked daily. The animals were weighed twice weekly throughout the period of the experiment, while feed consumed weighed each day.

5.4. Grouping design and feeding of rats:

The rats were divided into 6 groups (7 rats each). The groups of rats were as follows:

Group 1- (-ve) Normal rat group fed on a basal diet only, as negative control for 28 days.

Group 2- (+ve) Fed on a basal diet as(hypercholesterolemic) rats, as positive control for 28 days.

Group 3- Hypercholesterolemic group received cousts branches extract, orally given at a dose of 400 mg/kg B.W.and fed on basal diet.

Group 4-Hypercholesterolemic group received argl leaves extract orally, given at a dose of 400mg/kg B. W. and fed on basal diet.

Group 5- Hypercholesterolemic group received emblica fruit extrac orally, given at a dose of 400mg/kg B.W,and fed on basal diet.

Group 6-Hypercholesterolemic group received mix extract diet of all plants equal proportions orally given at a dose of 400mg/kg B.W. and fed on basal diet.

5.5. Biological evaluation:

Biological evaluation of the different diets was carried out by determination of body weight gain (BWG g), fed intake (FI)& feed efficiency ratio (FER) according to **Chapman et al., (1959),**

5.6. Biochemical parameters:

Determination of serum lipids:

Determination of triglycerides (TG):

Serum triglycerides (T.G) were measured using the modified kinetic method according to the method described by **Fossati and Principe (1982).**

- **Determination of total cholesterol (TC):**

Serum cholesterol was measured using the modified kinetic method according to **Richmond (1973).**

- **Determination of high density lipoprotein cholesterol (HDL):**

Serum high density lipoprotein cholesterol (HDL-c) was sing the modified kinetic method according to **Allain (1974)**.

- **Determination of low density lipoprotein cholesterol (LDL):**

Serum low density lipoprotein cholesterol (LDL-c) was calculated as mg/dl according to **Castelli et al., (1977)** equation:

Determination of very low density lipoprotein cholesterol (VLDL):

Serum very low density lipoprotein cholesterol (VLDL-c) was calculated as mg/dl according to Lee and **Nieman (1996)** equation:

$$\text{VLDL-C concentration mg/dl} = \frac{\text{T.G}}{5}$$

- **Determination of liver function:**
- **Determination of alanine transaminase (ALT):**

ALT activities were measured in serum using the modified kinetic method of **Tietz (1976).**

Determination of aspartate transferase(AST):

AST activities were measured in serum using the modified kinetic method of **Henry (1974).**

Determination of alkaline phosphatase (ALP):

ALP activities were measured in serum using the modified kinetic method or liquicolor of **Moss(1982)**by using kit supplied by Human, Germany.

▪ **Determination of total protein and albumin:**

Serum total protein levels were measured using the modified kinetic method of **Ratree et al.,(1991**).Albumin is a protein made specifically by the liver. It is the main constituent of total protein (the remaining from globulins)which was measured using the modified kinetic method of **Young(2001).**

▪ **Determination of kidney function indicators:**

▪ Determination of urea nitrogen:

Urea was determined in serum using the modified kinetic method of Patton and **Crouch(1977).**

▪ **Determination of creatinine:**

Serum creatinine was measured using the modified kinetic method according to **Schirmeister(1964).**

▪ **Determination of uric acid:**

Serum uric acid was measured using the modified kinetic method according to **While et al ., (1970).**

5.7.Histopathological study:

Autopsy samples were taken from the internal organs of rats and fixed in 10% buffered formlin for twenty-four hours. Washing was done in tap water, then serial dilutions of alcohol till absolute ethyl,were used for dehydration. Specimens were cleared in xylene, embedded in paraffin at 56 degrees in hot air oven for twenty-four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns' thickness by sliding microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin **(Banchroft et al., 1996)** for histopathological examinations by the light microscope.

5.8. Statistical analysis of data:

Data were statistically analyzed using a computerized program at theScientific Computer Center, Faculty of Home Economics, Menofia University, using Duncan Multiple Range Test (one-way ANOVA test) according to **(Armitage and Berry, 1987).**

RESULT AND DISCUSSION

1-Hypercholesterolemic rats:

a- Biological Parameters:

Data listed in tables (1 & 2) and) show the effect of oral administration with costus branches, argel leaves emblica fruits &their combined extracts on body weight gain (BWG), feed intake (FI), feed efficiency ratio (FER) and relative organs weight of hypercholesterolemic rats

Table (1) Effect of oral administration with costus banches ,argel leaves, and emblica fruits &their combined extracts on body weight gain (BWG), feed intake (FI) feed efficiency ratio (FER) of hypercholesterolemic rats

Parameters		BWG g/28day/rat		FI g/day/rat		FER	
		Mean±SD	Change % of c+	Mean±SD	Change % of c+	Mean±SD	Change % of c+
Control	-ve	35±2.70^{b}	-22.22	23.7±2.60^{b}	-51.43	0.053±0.012^{b}	60.61
	+ve	45±2.00^{a}	-	48.8±1.80^{a}	-	0.033±0.012^{c}	-
Herbs 400/mg/ kg	Costus	21±3.00^{d}	-53.33	16±4.00^{c}	-67.21	0.047±0.01^{b}	42.42
	Argel	25±4.00^{d}	-44.44	19.8±2.30^{bc}	-59.43	0.045±0.01^{b}	36.36
	Emblica	29±3.00cd	-35.55	16.8±4.20^{c}	-65.57	0.062±0.01^{a}	87.88
	mix.	34±4.00bc	-24.44	18.6±2.80^{bc}	-61.89	0.065±0.01^{a}	96.97
L. S. D (0.05)		5.68		5.47		0.008	

Values denote arithmetic means ± standard deviation of the means.

Means with different letters (a, b, c, d) in the same column differ significantly at $p \leq 0.05$

using one-way ANOVA test, while those with similar letters are non-significantlydifferent.

Data presented in table (1) show the effect of oral administration with costus branches, argel leaves emblica fruits and their combined extracts on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of hypercholesterolemic rats. Concerning body weight gain (BWG)and feed efficiency ratio, it is clear that (C+ve) group showed significant increases which were 45±2.00 g and 48.8±1.89g compared to normal rats (C–ve) group which indicated 35±2.70 g and 23.7±2.6g respectively. these results denote that there was significant increase in (BWG) of hypercholesterolemic rats as compared to (c –ve) group rats was possily due to feeding on sheep for tail fat (higher T.Caloris). Rats of hypercholesterolemia and oral administrated with emblica, costus extracts showed the highest decreases in FI and BWG was lowest for costus when compared to control positive group. Meanwhile, it is clear that the best treatment for FER was that of the mix diet showing value of 0.065±0.01. this FER value of mix diet group was high, being 96.97% of the control (-) ve normal rats. This indicated that the best group was that of the mix diet. Data of table (1) are in line with that reported by Saif, Asmaa (2014) who assayed the biological (BWG, FI&FER) of rats in inflicted with hypercholesterolemia. According to same author diets containing same condiment (fennel, dill, leek, coriander) corrected the changes of biological Parameters in hypercholesterolemia rats. **Patel et al., (2016)** observed similar results to the present study, they concluded that dietary addition of *E. officinalis (Amla)* fruit powder had a positive effect on growth performance and net profit per bird in commercial broiler chickens.

Table (2) Effect of oral administration with costus branches ,argel leaves, emblica fruits &their combined extracts on relative organs weight (kidney , liver , heart , spleen and pancrease) of hypercholesterolemic rats

Parameters		Kindy (g/day)		Liver (g/day)		Heart (ratio)		Spleen (row)		Pancrease (row)	
		Mean ±SD	Change%of c+	Mean ±SD	Change%of c+	Mean ±SD	Change % of c+	Mean ±SD	Change% of c+	Mean ±SD	Change% of c+
Control	-ve	0.9±0.40^{b}	- 47.06	5.8±2.30^{a}	- 28.40	0.4±0.20^{b}	- 42.86	0.7±0.40^{b}	- 36.36	0.3±0.10^{b}	-72.37
	+ve	1.7±0.30^{a}	——	8.1±2.00^{a}	——	0.7±0.20^{a}	——	1.1±0.90^{a}	——	1.1±0.10^{a}	——
Herbs 400/mg/kg	Costus	1.4±0.30^{a}	- 17.65	6.6±0.80^{a}	- 18.52	0.5±0.30^{b}	- 28.75	0.7±0.30^{b}	-36.36	0.6±0.30b	- 45.46
	Argel	1.4 ±0.30^{a}	- 17.65	6.8±1.40^{a}	- 16.05	0.4±0.20^{b}	- 42.86	0.8±0.30^{b}	- 27.72	0.6±0.30^{b}	-45.46
	Emblica	0.9±0.40^{b}	- 47.06	5.7±1.40^{a}	- 29.63	0.4±0.20^{b}	- 42.86	0.5±0.30^{b}	-54.55	0.5±0.20^{b}	- 54.55
	mix.	0.9±0.50^{b}	- 47.06	5.6±1.40^{a}	- 30.86	0.4±0.20^{b}	-42.86	0.5±0.30^{b}	-54.55	0.4±0.30^{b}	-63.64
L. S. D (0.05)		0.36	——	2.88	——	0.19	——	0.23	——	0.31	——

Values denote arithmetic means ± standard deviation of the means.

Means with different letters (a, b, c, d) in the same column differ significantly at $p \leq 0.05$

using one-way ANOVA test, while those with similar letters are non-significantly different.

Data listed in table (2) illsterate the effect of oral administration of costus branches, argel leaves and emblica fruits at dose 400mg/kg on relative organs weight, (kidney, liver, heart, spleen and pancreas) of hypercholesterolemic rats. Such data indicated that rats fed on hypercholesterolemic diet (c+ve) group showed possible inflammatory changes in control (+) group; liver weight was 1.7±0.30 g compared to (c-ve) group which was 0.9±0.40 g; it is evident that the best treatment recorded to group to emblica and extract mixture of all plants at dose 400mg/kg which were 0.9±0.40 & 0.9±0.50 g respectively.Also significant increase of liver weight which was 8.1±2.00 g for control (+) rats compared to c-ve group which showed 5.8±2.30g, it is evident that the best treatment group was that of emblica and the mixture of all plants (at dose 400mg/kg) which were 5.7±1.40, 5.6±1.40 g with nonsignificant difference between them

Data indicted that rats fed on hypercholesterolemic diet (c+ve) group showed significant increases in wights of (heart, spleen, and pancreas when compared to normal rats which showed 0.4±020 ,0.7±0.40 and 0.3±0.10g respectively. Rats inflicted with hypercholesterolemia and orally administrated with all plants combiend diet(mix) showed significant decreases which were 0.4±0.20, 0.5±0.30 and 0.4±0.30 g respectively for the mentioned organs being considerably low when compared to control positive group. These results are in line with those reported by **Saif, Asmaa (2014)** who assayed the biological parameters (BWG, FI&FER) of rats as inflicted with

hypercholesterolemia. According to same author diets containing same condiment (fennel, dill, leek, coriander) corrected the changes of biological Parameters in hypercholesterolemia rats. **Kushwaha et al., (2013)** the study on emblica revealed exhibited no significant changes in general behavior, body weight, gross appearance of internal organs, hematological and biochemical parameters, and the histological profile of liver also indicated the nontoxic nature of this plant. Biochemical studies showed no significant change in the levels of ALT, AST, albumin, triglycerides, cholesterol and albumin. There was no evidence found about congestion of sinusoids, hemorrhage, hepatocytes, fatty changes, centrilobular necrosis and the changes in number of Kupffer cells in the liver. There was also no increase of blood pressure

2-Biochemical Parameters:

a-Lipids Profile

Table (3) Effect of oral administration with costus branches ,argel leaves, and emblica fruits & their combined extracts on serum lipid profile total cholesterol " TC" triglycerides " TG" and atherogenic index" AI" of hypercholesterolemic rats

Parameters		TC (Mg/dl)		TG (Mg/dl)		AI (atio)	
		Mean±SD	Change% of c+	Mean±SD	Change% of c+	Mean±SD	Change % of c+
Control	-ve	6.8±3.00^{d}	- 69.09	50±2.00^{c}	- 57.27	0.7±0.20^{b}	- 8.97
	+ve	220±2.00^{a}	____	117±3.50^{a}	____	7.8±1.80^{a}	____
Herbs 400/mg/kg	Costus	77±2.00^{b}	- 60.46	56±3.00^{b}	- 52.14	1.1±0.20^{b}	- 79.49
	Argel	7.9 ±4.00^{c}	- 64.09	56±3.00^{b}	- 52.14	1.3±0.20^{b}	- 83.33
	Emblica	7.5±2.00^{c}	- 65.91	56±3.00^{b}	- 54.70	0.9±0.50^{b}	- 88.46
	mix.	70±2.00^{d}	- 68.18	51±3.00^{b}	- 56.41	0.8±0.10^{b}	-89.74
L. S. D (0.05)		4.65	____	5.24	____	1.38	____

Values denote arithmetic means ± standard deviation of the means.

Means with different letters (a, b, c, d) in the same column differ significantly at $p \leq 0.05$using one-way ANOVA test, while those with similar letters are non-significantly different.

Data illustrated in tables (3&4) show the effect of oral administration with costus, argel and emblica exteracts as fed for hypercholesterolemic rats. Tc, TG&VLDL (c+ve group) were 220±2.00 ,117±3.50 and 23.4±2.4mg\dlrespectively indicating significant increase in TC, TG and VLDL when compared to normal group which showed 68±3.00 , 50±2.00 and respectively Rats of hypercholesterolemia and orally administrated with costus, argel and emblica extracts significantly decreased TC, TG and VLDL lowered.The best treatment was that of the mixture of all water plant extracts plants which were 70±2.00 , 51±3.00

and 10.2±50m g/dl respectively compared to c +ve group.Emblica effect followed that of the mix diet, then came costus and argel Regardles of that argel was of least reducing compared to control (+ve group)effect it showed nonsignificant differncein comparison with control(-)rats considering VLDL. Compared to mix diet treatment nonsignificant difference recorded for argel group in concerm to TG&VLDL. **Revathy et al., (2011)** reported that the ethanolic of costus extract shows significant reduction in blood glucose, glycosylated haemoglobin, blood urea, serum uric acid, serum creatinine, triglycerides, total cholesterol, phospholipids, low density lipoprotein (LDL), very low density lipoprotein (VLDL), and indicated the increase in liver glycogen, insulin and lactate dehydrogenase (LDH). Thier experimental findings with respect to the mechanism of costus action on alloxan induced diabetic rats suggested that it enhances insulin secretion by the islets of langerhans, enhances peripheral glucose utilization and increases serum protein levels in animal models.

Table (4) Effect of oral administration with costus branches ,argel leaves, and emblica fruits & their combined extracts on serum lipoprotein fraction (VLDL) , HDL and LDL) of hypercholesterolemic rats

Parameters		Vldl (Mg/dl)		Hdl (Mg/dl)		Ldl (Mg/dl)	
		Mean±SD	Change% of c+	Mean±SD	Change% of c+	Mean±SD	Change % of c+
Control	-ve	10 ± 1.00^{b}	- 75.27	40 ± 2.00^{a}	60	18 ± 2.00^{de}	- 89.51
	+ve	23.4 ± 2.40^{a}	____	25 ± 3.50^{a}	____	171.6 ± 2.10^{a}	____
Herbs 400/mg/kg	Costus	11.2 ± 0.40^{b}	- 52.14	37 ± 2.00^{a}	32	28.8 ± 7.00^{be}	- 75.06
	Argel	11.2 ± 0.40^{b}	- 52.14	35 ± 4.00^{a}	40	32.8 ± 20.30^{e}	- 80.89
	Emblica	10.6 ± 1.40^{b}	- 54.70	39 ± 3.00^{a}	56	25.4 ± 4.20^{d}	- 85.20
	mix.	10.2 ± 1.50^{b}	- 56.41	40 ± 4.00^{a}	60	19.8 ± 3.10^{a}	-88.46
L. S. D (0.05)		2.41	____	5.68	____	6.88	____

Values denote arithmetic means ± standard deviation of the means.

Means with different letters (a, b, c, d) in the same column differ significantly at $p\leq0.05$using one- way ANOVA test, while those with similar letters are non-significanltly different.

It is evident from results in table(3&4))that rats fed on hypercholesterolemic(+vegroup) diet revealed25±3.5mg/dl71.6±2.10mg/dl and0.7 ±0.2for HDL&LDL as compared to control negative group.Rats of hypercholesterolemic and

orally administrated with combiend extract of all plants showed significant decreases of LDL &AI but increases in HDL compared topositive groups.Best treatment was that of the mix group for all three parameters .For HDL &AI mix diet revealed nonsignificant differences in compation with the control (-) rats .Moreover, as for TC ,TG &VLDL(table 3)costus treatment followed that of the mix diet ,then came that of costus and argel (Table4) Even for argel which revealed least effect,percent increase of HDL compared to control (+)group was 40% and percent decrease of LDL and AI were 80.89% and 83.33% indicating efficient desirable effect of argel diet **Mahdy and Fayza (2015)** indicated that the ethanolic extract of doum & leek show significant reduction in blood glucose, glycosylated haemoglobin, blood urea, serum uric acid, serum creatinine, triglycerides, total cholesterol, phospholipids, low density lipoprotein (LDL), very low density lipoprotein (VLDL), and increase in liver glycogen, insulin and lactate dehydrogenase (LDH). thier experimental findings with respect to the mechanism of action of extract on alloxan induced diabetic rats suggest that it enhances insulin secretion by the islets of langerhans, enhances peripheral glucose utilization and increases serum protein levels **revathy et al.,(2011)** came to same conclusion . Enzymes **El-Tayeb, Laila et al., (2014)** results supported the idea that both blood glucose level and α-amylase activity can be ameliorated in diabetic rats by administration of *Solenostemma argel* aqueous extract.

b- Liver function parameters

Table (5) Effect of oral administration with costus branches ,argel leaves, and emblica fruits & their combined extracts on liver enzymes (AST, and ALP) in hypercholesterolemic rats

Parameters		AST (U/L)		ALT (U/L)		AST/ALT		ALP (U/L)	
		Mean±SD	Change% of c+	Mean±SD	Change% of c+	Mean±SD	Change % ofc+	Mean±SD	Change% of c+
Control	-ve	72.4±2.40^{b}	- 52.02	21.2±1.30^{c}	- 20.00	3.2±1.00^{b}	- 43.86	139.3±0.80^{b}c	- 34.45
	+ve	150.9±2.70^{a}	____	26.5±240^{a}	____	5.7±1.50^{a}	____	212.5±2.50^{a}	____
Herbs 400/mg/kg	Costus	65.3±5.20^{c}	- 56.73	25.1±2.80^{b}	- 5.28	2.6. ±0.50^{b}	- 54.39	141.2±3.00^{b}	-33.55
	Argel	68.1 ±2.50^{b}	- 54.87	25.4±2.20^{b}	- 4.15	2.7±0.20^{b}	- 52.63	143.5±10.00^{b}	- 32.47
	Emblica	60.7±2.50^{c}	- 59.78	24.2±3.05^{b}	- 8.68	2.5±0.60^{b}	- 56.14	135.4±3.20^{bc}	-36.28
	mix.	53.5±2.70^{d}	- 74.48	22.1±3.50^{c}	- 16.60	2.4±0.60^{b}	-57.90	131±3.00^{c}	-38.35
L. S. D (0.05)		5.62	____	0.98	____	1.49	____	8.44	____

Values denote arithmetic means ± standard deviation of the means.

Means with different letters (a, b, c, d) in the same column differ significantly at p≤0.05 using one- way ANOVA test, while those with similar letters are non-significanltly different.

It is clear from the results of table (5) that rats fed on hypercholesterolemic diet (control +ve group) showed significant increase in liver enzymes activities which were 150.9±2.70, 26.5±2.40 and 212.5±2.50 U/L respectively, while for normal rats value were 72.4±2.40, 21.2±1.30 and 139.3±0.80 U/L respectively. All tested plant diets extracts and their combined improved mean values as compared to C+ve group. The highest decrease in the liver enzymes activitics when compared to control +ve group recorded for the mix diet where mean values were 53.5±2.7, 22.1±3.50 and 131.00±3.00 U/L respectively.

Hypercholesterolemia raised considerably the AST/ALT ratio to 5.7±1.50 while for control –ve group was only 3.2±1.00. Also, the herbal diets decreased pronouncedly the AST/ALT ratio to 2.4±0.60- 2.7±0.2, provided that least ratio was found for the mix diet. Argel diet which revealed the lowest improvement chacharacterised by pronounced decreases of AST, ALT, AST/ALT&ALP, percent decreases were -54.87%, -4.15%, -52.63% & -32.47%, respectively compared to control +ve group AST and AST/ALT values of argel diet showed non-significant differences in comparison with that of the normal rats. **Mahdy and Fayza (2015)** found that Hypercholesterolemia raised the liver enzymes activities (AST, ALT& ALP), while feeding on doum palm and leek seeds diets revesed such changes. **Osman et al., (2014)** revealed however, to have adverse effect on liver and kidneys of albino rats.

3-Serum protein fraction

Table (6) Effect of oral administration with costus branches ,argel leaves, emblica fruits & their combined extracts on Serum proteins (T.P, ALP and GLB) of hypercholesterolemic rats

Parameters		TP (g/dl)		ALB (g/dl)		GLB (g/dl)	
		Mean±SD	Change% of c+	Mean±SD	Change% of c+	Mean±SD	Change % ofc+
Control	-ve	8.9±1.10^{a}	+ 53.45	6.8±1.01^{a}	+ 233.81	2.1±0.15^{bc}	- 43.24
	+ve	58±1.10^{c}	____	2.1±0.10^{c}	____	3.7±0.40^{a}	____
Herbs 400/mg/kg	Costus	8.5±1.20^{a}	+ 32.76	6.5±0.10^{a}	+ 114.29	2±0.31b	- 13.51
	Argel	7.2 ±1.00^{b}	+ 24.14	4.8±0.07^{b}	+ 128.57	2.4±0.20^{c}	- 35.14
	Emblica	8±2.20^{ab}	+ 37.93	6.1±0.9^{a}	+ 19.48	1.9±0.05^{cd}	- 48.65
	mix.	8.5±0.50^{ab}	+ 46.55	6.7±1.0^{a}	+ 209.52	1.8±0.12^{cd}	-45.95
L. S. D (0.05)		1.19	____	0.95	____	0.31	____

Values denote arithmetic means ± standard deviation of the means.

Means with different letters (a, b, c, d) in the same column differ significantly at p≤0.05

using one-way ANOVA test, while those with similar letters are non-significantly different.

Data listed in table (6) show the effect of oral administration with water extracts of costus, argel and emblica extracts at dose 400mg/kg on T.P,ALB&GLB of serum in rats inflicted with by hypercholesterolemia It is clear that rats of the control(+ve)group revealed 5.8 ±1.10g /dl, &2.1±3.7g/dl for TP&ALP respectively . There were significant changes for control +ve compared to control - ve group in case of T. P, ALB &GLB when feeding with herbals extract: T. P & A/B increased while G/B decreased It is evident that the best treatment was recorded for the mix extract diet, which showed 8.5±0.50, 6.7±2.0 and 1.8±0.12 for T. P, ALB &GLB g/dl respectively. Argel showed the least effect; best increase of T.P & ALB and maximum decrease of GLB recorded for the mix group . Never the less, argel diet revealed also remarkable improvement of serum protein fractions which were +24.14%for T.P, -128.5%and -35.14% for T.P, ALB & GLB respectively. Results of table (6) were in line with that reported by **Mohamed et al., (2013)**; These trials were performed for the evaluation of the safety and effectiveness of *Solenostemma argel* tablets. They study were reported**. Charoenteeraboon et al., (2010)** showed that the fruits of P. *emblica* are potential sources of natural antioxidants, which have free radical scavenging activity and might be useful for hepato-, cyto-, and radio-protection, as well as reducing oxidative stress in many pathological condition.

4-Kideny fraction

Table (7) Effect of oral administration with costus branches ,argel leaves, emblica fruits & their combined extracts on Kidney function markers (urea , creatinine and uric acid) of hypercholesterolemic rats

Parameters		UREA (Mg/dl)		CREATININE (Mg/dl)		UREA ACID (Mg/dl)	
		Mean±SD	Change% of c+	Mean±SD	Change% of c+	Mean±SD	Change % ofc+
Control	-ve	21.1±2.9^{b}	- 42.19	0.32±0.02^{b}	- 86.09	1.36±0.94^{b}	- 62.43
	+ve	36.5±1.5^{a}	____	2.3±3.20^{a}	____	3.62±1.62^{a}	____
Herbs 400/mg/kg	Costus	19±2.00^{bc}	- 47.45	0.34±0.03^{b}	- 85.22	1.5±0.30^{b}	- 58.56
	Argel	22 ±4.00^{b}	- 39.73	0.36±0.04^{b}	- 84.35	1.6±0.40^{b}	- 55.80
	Emblica	15±3.00^{c}	- 58.90	0.3±0.20^{b}	- 86.96	1.3±0.20^{b}	- 64.09
	mix.	13±2.00^{c}	- 64.38	0.3±0.10^{b}	- 86.96	1.1±0.20^{b}	- 69.61
L. S. D (0.05)		4.79	____	1.33	____	1.09	____

Values denote arithmetic means ± standard deviation of the means.

Means with different letters (a, b, c, d) in the same column differ significantly at p≤0.05

using one-way ANOVA test, while those with similar letters are non-significantly different.

Table (7) show the effect of oral administration with costus ,Argel , emblica and their mix of plant extracts on kidney function (urea ,creatinine and uric acid) Of hypercholesterolemic rats .It is clear from the table results that rats fed on hypercholesterolemic diet(c+ve groups)and basal diet showed 36.5±1.5, 2.3±3.20 and 3.62±1,62 mg/dl for (urea ,creatinine and uric acid) respectively ,indicating significant increases when compared to normal rats which revealed 21.1±2.9, 0.32±0.02 and 1.36±0.94 mg/dl respectively. Rats of hypercholesterolemia and administrated with water extracts of tested plants showed significant decreases in kidney function parameters when compared to positive groups .It is evidence that the best treatments was for the mixure of plants diet which revealed 13±2.00 , 0.3±0.10 and 1.1±0.20mg/dl respectively (urea ,creatinine and uric acid) when compared to control to positive groups .Argel diet which indicated lowest improvement of kidney function markers , seems to be of potent desirable effect showing pronounced percent decreases of (urea ,creatinine and uric acid) being 39.73% , 84.35% and 55.80%of control + respectively. **Sherif and Neven (2015)** reported that hypercholesterolemia raised (urea, creatinine and uric acid) in serum, while feeding of decenopathic rats on black mulberry diets reversed these changes. **Abou-Hashem et al., (2013)** came to same conclusion .

3-**Histopathological results**:

2- Histological structure of liver from groups hypercholesterolemic rats

Normal histological structure for central vein and surrounding hepatocytes have been shown (Fig 1)for liver of rat from group 1 indicating the normal histological structure of hepatic lobule but liver of rat from group 2 (Fig 2) C+ve hypercholesterolemic fed on diet containing 1.5% cholesterol plus 10% sheep tail fat was showing hyperplasia of epithelial lining bile duct and fibroplasia in portal triad (Fig 3) and sinusoidal leucocytosis

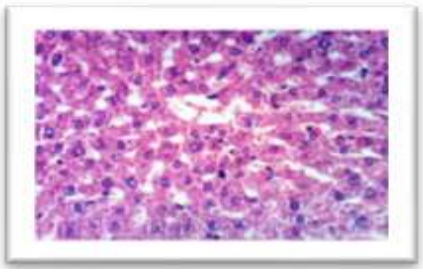

Fig. (1): Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H & E X 400).

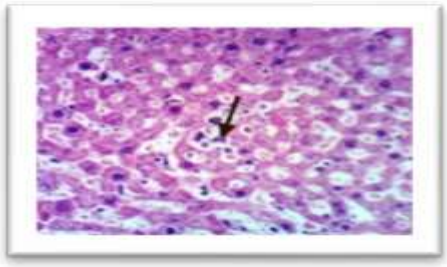

Fig. (2): Liver of rat from group 2 showing hyperplasia of epithelial lining bile duct and fibroplasia in portal triad (H & E X 400).

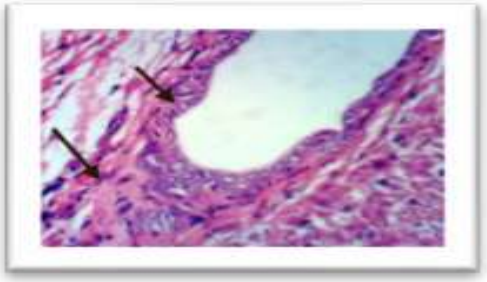

Fig. (3): Liver of rat from group 2 showing sinusoidal leucocytosis (H & E X 400).

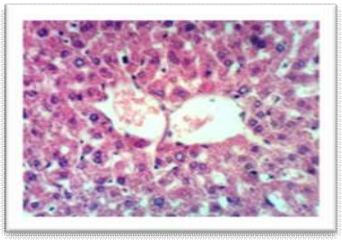

Fig 4

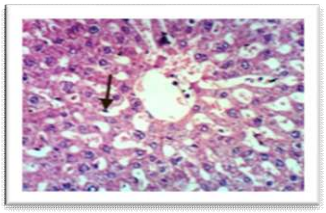

Fig 5

Liver of rat from group 3(Fig 4)rats administrered with extract of *costus* at dose(400mg/kg/day)was showing activation of Kupffer cells and showing no histopathological changes indications potential recovery due to bioactive molecules. Due to the remarkable biological activity of *costu speciousus* it may be appropriate to develop them as a medicine. Meanwhile Liver of rat from group 4(Fig5) during dministration with extract of *argel* at dose (400mg/kg/day)the section was showing no histopathological changes. ***Godswill* et al.,(2014)** stated that the bioactive compounds identified in *the n-butanol fractions of C.* afer leaves and stem may explain *the folkloric use of C.* afer plant in the treatment of chronic inflammatory and oxidative stress related diseases

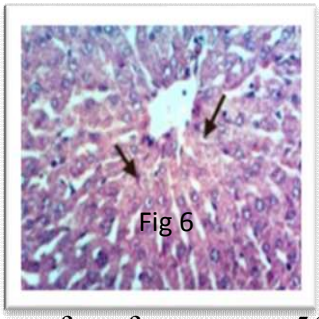
Fig 6

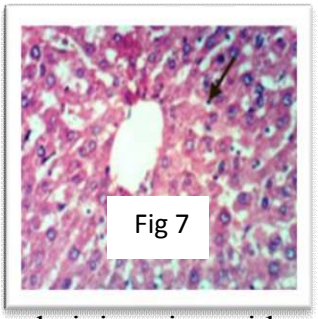
Fig 7

Liver of rat from group 5(Fig 6)during administration with extract of *emblica* at dose(400mg/kg/day)was showing Kupffer cells activation hence this study indicates that water embilica extracts possess hepatoprotective activity that is most likely because of the isolated chemical constituents during extraction . Meanwhile liver of rat from group 6(Fig 7) treated with mixture of all plants extrect at dose(400mg/kg/day) was showing slight activation of Kupffer cells. Our findings suggest that the test material may potentially ameliorate the hyperthyroidism with an additional hepatoprotective benefit **Jaiswal et al., (2010)** studied the effect of fruit of *Phyllanthus emblica L*. mixed with the basal rat feed at the concentration of 50 g/kg (w/w) along with lead acetate @ 1000 mg/kg feed daily. The treatments were continued for sixty consecutive days. The Analysis of haematological (haemoglobin concentration and total erythrocyte count) and biochemicall parameters (blood urea nitrogen, serum aspartate transaminase and serum alanine transaminase) reflected that *Phyllanthus emblica L*. protected the haematic, renal and hepatic systems of Wistar rats from lead toxicity.

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التأثير العلاجى لمستخلصات القسط والحرجل والأملج في إرتفاع الكوليسترول لدى فئران التجارب

مستخلص البحث

ألقت هذه الدراسة الضوء لمعرفة تأثير بعض النباتات المشتملة على (فروع القسط الهندى – أوراق الحرجل – فاكهة الاملج – ومخلوطهم) لعلاج أرتفاع كوليسترول الدم وقد تم أستخدام (٤٢ فأر أبيض من ذكور الالبينو) ذات أوزان ١٨٠-٢٠٠ جرام من بداية التجربة تم إعطائهم ١.٥%كوليسترول +١٠%دهن (لية خروف) وذلك لمدة أسبوعين وقد قسمت لستة مجموعات (٧فئران في كل مجموعة) حيث تركت الأولى كمجموعة ضابطة سالبة والثانية كمجموعة ضابطة موجبة ومن المجموعة ٣-٦ تم معالجتهم بمستخلصات النباتات ومخلوطهم بجرعة ٤٠٠ ملليجرام /كجم عن طريق الحقن الفموى يومياً من مستخلصات النباتات لمدة ٢٨ يوم أي طول مدة التجربة تم تجميع عينات الدم وتقدير الكوليسترول الكلى TC ، الجليسريدات الثلاثية TG، والليبوبروتينات والدهون منخفضة الكثافة جدا ومنخفضه الكثافه (LDL,VLDL) وعالية الكثافة (HDL) ووظائف الكلى"الكرياتينين،اليوريا، حامض اليورك ووظائف الكبد(AST,ALT,ALP)الفحص الهستوباثولوجى لعضو (الكبد) وقد أظهرت النتائج ان كل مستخلصات النباتات المختبرة أحدثت تحسناً معنويا في الاختبارات الكيمائية بالإضافة الى الفحص الهستوباثولوجى وقد أظهرت المجموعة الخليط أنها أفضل وأعلى في التأثير العلاجي وأعلي تعافيا مقارنا بباقى المجموعات من الناحية الكيمائية الحيويه والتشريحية للاعضاء وقد أرجع ذلك التعافي الى زيادة محتوى النباتات من المركبات الكيمائية الغنية وجاء ذلك التعافي مقارنة بمجموعة الضابطة الموجبة والتي أظهرت وجود ارتشاح وتليف وأيضا ظهور كمية هائلة من الالتهابات في المدخل الوريدى ولذلك أقترح اتخاذ الاليات الممكنة لهذه الإجراءات وتوفير التوجيهات المستقبلية التي يمكن ان تتخذ وتستفيد من النباتات المدروسه في التغذيه العلاجيه.

الكلمات المفتاحية التسمم الكبد – ارتفاع ليبيدات الدم – القسط –الحرجل –الأملج .