Sjournals
Scientific Journals

Scientific Journal of Veterinary Advance (2016) 5(9) 123-133
ISSN 2322-1879
doi: 10.14196/sjva.v5i9.2244

CODEN (USA): SJVAAS

Contents lists available at Sjournals
Scientific Journal of **Veterinary Advances**
Journal homepage: www.Sjournals.com

Original article

Influence of *Cymbopogon proximus* extract on lipid profile, biochemical hematological and coagulation parameters of hyperlipidemic albino rats

Hager Tarek H. Ismail*

Department of Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University , 1 Alzeraa Street, Zagazig City, Sharkia Province, Postal Code 44511, Egypt.

*Corresponding author; Department of Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University, 1 Alzeraa Street, Zagazig City, Sharkia Province, Postal Code 44511, Egypt.

ARTICLE INFO

Article history,
Received 02 August 2016
Accepted 02 September 2016
Available online 08 September 2016
iThenticate screening 04 August 2016
English editing 01 September 2016
Quality control 05 September 2016

Keywords,
Cholestyramine
Cymbopogon proximus
Hypercholesterolemia
Hypolipidemic

ABSTRACT

The objective of this work was to investigate the possible hypolipidemic activity and side effects of the *C. proximus* extract alone and combined with cholestyramine with half therapeutic doses by studying changes in different biochemical, hematological and coagulation parameters beside histopathological evaluation. A total of 50 female albino rats divided into five equal groups: Gp.(1) control, Gp.(2) hyperlipidemic, Gp.(3) hyperlipidemia plus cholestyramine, Gp.(4) hyperlipidemia plus *C.proximus* extract, Gp.(5) hyperlipidemia plus half therapeutic doses of cholestyramine and *C. proximus* extract. At the 29th day of the experiment, blood samples were collected and divided into three portions for biochemical, hematological and coagulation studies. Liver was dissected out for histopathological studies. Treatment of hyperlipidemic rats with *C. proximus* extract alone or combined with cholestyramine showed marked decrease in different lipid profile values with hepatoprotective activity, lowering lipid peroxidation, increasing antioxidant activity in addition to non significant change in erythrogram, platelets count, leukogram and coagulation markers in compare with hyperlipidemic group. It could be concluded that, combining treatment of *C.proximus* extract with cholestyramine as a synthetic drug with half therapeutic doses gave a complementary hypolipidemic effect with a fewer side effects than treatment with each therapeutic agent alone.

1. Introduction

Hyperlipidemia is considered as one of the most serious factors contributing to the prevalence of coronary heart disease, stroke, atherosclerosis, hypertension, type II diabetes mellitus, obesity, myocardial infarction, congestive cardiac failure, gall bladder diseases, degenerative joint diseases and infertility. It is manifested as hypercholesterolemia and/or hypertriglycerdemia (Senecha et al., 2012). Hyperlipidemia control involves diet regime, exercise and administration of hypolipidemic drugs which include statins, bile acid sequestrants (anion-exchange resins) such as cholestyramine and colestipol, fibrates such as clofibrate, gemfibrozil, fenofibrate, ciprofibrate, and bezafibrate, cholesterol absorption inhibitors such as ezetimibe and omega-3-fatty acids and niacin (Lin et al., 2010). Hyperlipidemia treatment without any adverse effects is still a challenge to the medical field beside none drug is effective for treating all lipoprotein disorders (McKenney, 2007). Thus, there is still need for development of better anti-hyperlipidemic therapies. Medicinal plants extracts are frequently considered to be with minimal adverse effects in compare with synthetic drugs. There are a number of medicinal plants recorded to possess hypolipidemic activity in clinical studies (Dhingra et al., 2014).

Cymbopogon proximus, Family Gramineae, is an aromatic densely-tufted grass growing wildly in Upper Egypt and known as Halfa-bar. The herb is highly reputed in folkloric medicine as an effective diuretic, renal or abdominal antispasmodic agent (Abou-Shoer et al., 2011). There were several studies performed to determine the hypoglycemic properties of *C. proximus* in alloxan-induced diabetic rats and the observed results after treatment of diabetic rats showed different hypolipidemic effects of *C. proximus* (Newairy et al., 2002). Further extensive biochemical and pharmacological investigations on *C. proximus* should be conducted to evaluate the use of this herb as an alternative or complementary therapeutic agent for treatment of hyperlipidemia. The present study was designed to assess the possible hypolipidemic activity and side effects of the *C. proximus* extract alone and combined with standard hypolipidemic drug (cholestyramine) with half therapeutic doses by studying changes in different biochemical, hematological and coagulation parameters beside histopathological evaluation.

2. Materials and methods

2.1. Experimental animals

A total of 50 clinically healthy female albino rats (two months old and 130 gm average body weight) were purchased from the laboratory animal housing of Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were kept in metal cages, under hygienic conditions, given a standard pellet diet with water *ad-libitum* and were acclimatized for 7 days before starting of experiment. All procedures of the current experiment were carried out in accordance with the Egyptian laws and university guidelines for the care of experimental animals and have been approved by the Committee of the Faculty of Veterinary Medicine, Zagazig University, Egypt.

2.2. Hyperlipidemic agents

Cholesterol AR and cholic acid were purchased from Alpha Chemika, Mumbai, India as a white powder and coconut oil was obtained from El Captain Company (Cap Pharm), Egypt.

2.3. Standard hypolipidemic drug

Cholestyramine (Cholesteran) was obtained from Pharco Pharmaceuticals, Egypt as a white powder sachet.

2.4. Preparation of medicinal plant extract

The aerial parts of *Cymbopogon proximus* (Halfa bar) purchased from a local market in Zagazig city, Sharkia governorate, Egypt. The plant was identified and authenticated in the department of Botany at Zagazig University. It was washed, dried at 37 °C for 24 h and milled well to fine powders then, were suspended in boiled distilled water (5 g/100 ml) (Mansour et al., 2002).

2.5. Experimental design

The animals were divided randomly into five groups of 10 animals/group and kept on a standard pellet diet:

Gp.(1) rats were kept on a standard pellet diet only (control group).

Gp.(2) rats were administered orally 2.5ml/kg b.wt./day suspension of 2% cholesterol and 1% cholic acid in coconut oil for 28 days via stomach tube (hyperlipidemic group) (Dhande et al., 2014).

Gp.(3) rats were treated as hyperlipidemic group plus administration of cholestyramine orally as hypolipidemic standard drug at a dose of 800 mg/kg b.wt./day for 28 days via stomach tube (Van Berge Henegouwen et al., 2011).

Gp.(4) rats were treated as hyperlipidemic group plus administration of *C. proximus* extract orally at a dose of 75 mg/100 g b.wt. (1.5 ml/100 g b.wt.) for 28 days (Mansour et al., 2002).

Gp.(5) rats were treated as hyperlipidemic group plus administration of combination of half therapeutic doses of cholestyramine and *C. proximus* orally for 28 days.

2.6. Sampling

At the 29th day of the experiment, blood samples were collected from the retro orbital plexus after overnight fasted rats under ether anesthesia and were divided into three portions. The first portion was collected into the plain centrifuge tube without anticoagulant for serum separation for biochemical analysis. The second portion was collected into clean Wasserman tubes containing dipotassium salts of ethylenediamine tetraacetic acid for hematological analysis. The third portion was collected into coagulation tubes containing 3.2% sodium citrate then plasma was separated for prothrombin time (PT) and activated partial thromboplastin time (aPTT) estimation. Three rats from each group were sacrificed for collecting samples from the liver for histopathological examination.

2.7. Biochemical studies

Serum was used to determine total proteins, albumin and globulins levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and malondialdhyde (MDA) levels and catalase (CAT) activity. All of these parameters were measured using commercial diagnostic kits purchased from Diamond Diagnostic Company, Spinreact, Vitro and biodiagnostic by Photometer 5010 (Robert Riele GmbH and co-kg, Germany) except globulins level was estimated by subtracting albumin from total proteins. Low density lipoprotein (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) was calculated by using equations (Friedewald et al., 1972; Bauer, 1982).

2.8. Hematological studies

Total erythrocytic and platelets counts, packed cell volume (PCV) value, hemoglobin (Hb) concentration, total and differential leukocytic counts were determined by using an automated blood cell analyzer (Hemascreen 18, Hospitex diagnostic, Italy).

2.9. Coagulation studies

Plasma was used to determine coagulation markers, prothrombin time (PT) and activated partial thromboplastin time (aPTT) by using specific reagent kits purchased from Siemens Healthcare Diagnostics.

2.10. Histopathological studies

Liver of rats was dissected out, then fixed in 10% neutral buffered formalin, dehydrated in a graded ethanol series, cleared in xylene and finally embedded in paraffin wax. Paraffin sections of 5 µ thickness were stained by hematoxylin and eosin (H&E) and examined microscopically (Bancroft et al., 1996).

2.11. Statistical analysis

All data were performed using SPSS software (v.16). Data were analyzed using one-way analysis of variance (ANOVA), Tukey's HSD multiple comparison tests was used to test the significant differences between the mean values. Variability in the data was expressed as the pooled SEM and the alpha level for determination of

significance was 0.05. Means in the same column followed by different letters were significantly different and the highest value was represented by the letter (a).

3. Results and discussion

3.1. Changes in lipid profile

Results (Table 1) in the present study revealed that rats in hyperlipidemic group showed a highly significant increase in serum TG, TC, LDL-C, VLDL-C levels and a significant decrease in the level of HDL-C in compare with the control group. Treatment of hyperlipidemia with cholestyramine, *C. proximus* extract and combination of half therapeutic doses of them revealed a significant decrease in serum TG, TC, LDL-C, VLDL-C levels and a significant increase in the level of HDL-C in compare with hyperlipidemic group.

Treatment of hyperlipidemia with cholestyramine alone showed a highly significant increase in serum TG, TC, HDL-C, VLDL-C levels and non significant changes in the level of LDL-C in compare with control group. Treatment of hyperlipidemia with *C. proximus* extract alone or in combination with cholestyramine in half therapeutic doses returned serum TG, TC, VLDL-C levels towards normal control level with highly significant decrease and increase in serum HDL-C and LDL-C levels respectively in *C. proximus* extract treated group and a highly significant increase and non significant change in serum HDL-C and LDL-C levels, respectively in combined treated group in compare with the control one.

Table 1
Lipid profile of rats in gps. (1-5) after 28 days of hyperlipidemia treatment.

	Experimental groups						
Parameters	**Gp.(1)**	**Gp.(2)**	**Gp.(3)**	**Gp.(4)**	**Gp.(5)**	**SEM[1]**	**p-value**
TG (mg/dl)	64.43 ^{cd}	83.97 ^{a}	70.93 ^{b}	59.75 ^{d}	64.99 ^{c}	1.77	0.000
TC (mg/dl)	70.55 ^{d}	110.60 ^{a}	98.79 ^{b}	75.66 ^{cd}	81.30 ^{c}	3.12	0.000
HDL-C (mg/dl)	33.06 ^{c}	23.40 ^{e}	59.28 ^{a}	28.64 ^{d}	43.96 ^{b}	2.61	0.000
LDL-C(mg/dl)	24.61 ^{c}	70.36 ^{a}	25.32 ^{c}	35.07 ^{b}	24.34 ^{c}	3.68	0.000
VLD-C(mg/dl)	12.88 ^{cd}	16.84 ^{a}	14.18 ^{b}	11.94 ^{d}	12.99 ^{c}	0.35	0.000

[1]SEM: Standard error of the mean. Means bearing different superscripts within the same row are significantly different (P<0.05). Gp.(1) control group, Gp.(2) hyperlipidemic group, Gp.(3) hyperlipidemia + cholestyramine, Gp.(4) hyperlipidemia + *C. proximus* extract, Gp.(5) hyperlipidemia + half doses of (cholestyramine and C. proximus extract). TG= Triglycerides , TC= total cholesterol, HDL-C= high-density lipoprotein cholesterol, LDL-C= low-density lipoprotein cholesterol, VLDL-C= Very low-density lipoprotein cholesterol.

3.2. Changes in some liver functions tests

Table 2 shows hyperproteinemia in hyperlipidemic group in compare with control group, non significant changes and highly significant decrease in all treated groups in compare with control and hyperlipidemic groups, respectively. Hyperlipidemic and different treated groups showed hypoalbuminemia in compare with the control group, treated groups represented lower values of albumin in compare with hyperlipidemic group. All groups showed hyperglobulinemia in compare with control group, *C. proximus* extract treated group showed a lower degree of hyperglobulinemia in compare with hyperlipidemic and other treated groups. In this study also, serum ALT and AST activities showed a highly significant increase in hyperlipidemic and cholestyramine treated groups in compare with the control group. Other treated groups revealed a non significant changes in those parameters in compare with the control group and significant reduction in comparison with hyperlipidemic group. Serum ALP activity showed a highly significant increase in all groups in compare with control one, all treated groups showed non significant change in ALP activity in compare with hyperlipidemic group except cholestyramine treated group which showed a higher degree of ALP activity.

3.3. Changes in serum lipid peroxidation level and antioxidant enzyme activity

The results (Table 3) revealed a highly significant increase and non significant change in serum malondialdhyde level in hyperlipidemic and cholestyramine treated groups, respectively in compare with the control group, while *C. proximus* extract and combined treated groups revealed a highly significant decrease in this

parameter in compare with the control group. All treated groups showed highly significant decrease in the same parameter in compare with hyperlipidemic group, lowest value presented in the combined treated group.

Serum catalase activity showed a highly significant decrease in hyperlipidemic group in compare with control one and a highly significant increase in all treated groups in compare with control and hyperlipidemic groups, highest value presented in the combined treated group.

Table 2
Some liver functions of rats in gps. (1-5) after 28 days of hyperlipidemia treatment.

	Experimental groups						
Parameters	**Gp.(1)**	**Gp.(2)**	**Gp.(3)**	**Gp.(4)**	**Gp.(5)**	**SEM[1]**	**p-value**
Total proteins (g/dl)	5.41 ^{bc}	6.02 ^{a}	5.50 ^{b}	5.49 ^{b}	5.33 ^{c}	0.05	0.000
Albumin (g/dl)	4.04 ^{a}	3.85 ^{b}	3.46 ^{d}	3.61 ^{c}	3.21 ^{e}	0.06	0.000
Globulins (g/dl)	1.37 ^{c}	2.16 ^{a}	2.04 ^{a}	1.88 ^{b}	2.11 ^{a}	0.05	0.000
ALT (U/L)	6.28 ^{cd}	12.70 ^{a}	9.80 ^{b}	5.19 ^{d}	7.78 ^{bc}	0.58	0.000
AST (U/L)	32.22 ^{c}	35.41 ^{b}	40.96 ^{a}	32.47 ^{c}	32.72 ^{c}	0.70	0.000
ALP (U/L)	98.22 ^{c}	166.80 ^{b}	176.42 ^{a}	158.84 ^{b}	162.34 ^{b}	5.73	0.000

[1]SEM: Standard error of the mean. Means bearing different superscripts within the same row are significantly different (P<0.05). Gp.(1) control group, Gp.(2) hyperlipidemic group, Gp.(3) hyperlipidemia + cholestyramine, Gp.(4) hyperlipidemia + *C. proximus* extract, Gp.(5) hyperlipidemia + half doses of (cholestyramine and *C. proximus extract).* ALT=Alanine aminotransferase, AST=Aspartate aminotransferase, ALP=Alkaline phosphatase.

Table 3
Serum malondialdhyde (MDA) level and catalase (CAT) activity of rats in gps. (1-5) after 28 days of hyperlipidemia treatment.

	Experimental groups						
Parameters	**Gp.(1)**	**Gp.(2)**	**Gp.(3)**	**Gp.(4)**	**Gp.(5)**	**SEM[1]**	**p-value**
MDA (nmol/ ml)	39.82 ^{b}	62.45 ^{a}	39.45 ^{b}	29.60 ^{c}	23.46 ^{d}	2.72	0.000
CAT (U/L)	113.23 ^{d}	79.22 ^{e}	223.70 ^{b}	192.16 ^{c}	284.60 ^{a}	15.20	0.000

[1]SEM: Standard error of the mean. Means bearing different superscripts within the same row are significantly different (P<0.05). Gp.(1) control group, Gp.(2) hyperlipidemic group, Gp.(3) hyperlipidemia + cholestyramine, Gp.(4) hyperlipidemia + *C. proximus* extract, Gp.(5) hyperlipidemia + half doses of (cholestyramine and *C. proximus* extract).

3.4. Changes in erythrogram and platelets count

As shown in Table 4, red blood cells and platelet counts, packed cell volume and hemoglobin concentration revealed non significant change in all groups.

Table 4
Erythrogram and platelets count of rats in gps. (1-5) after 28 days of hyperlipidemia treatment.

	Experimental groups						
Parameters	**Gp.(1)**	**Gp.(2)**	**Gp.(3)**	**Gp.(4)**	**Gp.(5)**	**SEM[1]**	**p-value**
RBCs ($\times 10^6$/µl)	8.32	7.67	7.91	8.66	7.57	0.15	0.132
PVC(%)	45.04	43.11	42.74	46.68	40.43	0.83	0.156
Hb (g%)	19.22 ^{ab}	19.26 ^{ab}	18.38 ^{ab}	19.56 ^{a}	16.54 ^{b}	0.36	0.038
Platelets ($\times 10^3$ / µl)	607	518.20	553.40	559	536.20	35.30	0.96

[1]SEM: Standard error of the mean. Means bearing different superscripts within the same row are significantly different (P<0.05). Gp.(1) control group, Gp.(2) hyperlipidemic group, Gp.(3) hyperlipidemia + cholestyramine, Gp.(4) hyperlipidemia + C. proximus extract, Gp.(5) hyperlipidemia + half doses of (cholestyramine and C. proximus extract). RBCs=Red blood corpuscles, PCV=Packed cell volume, Hb=Hemoglobin.

3.5. Changes in leukogram

The results (Table 5) revealed non significant changes in total leukocytic count in all groups. Lymphocytic count showed significant decrease in hyperlipidemic group in compare with control and non significant change in

other treated groups in compare with control and hyperlipidemic groups. Granulocytic and monocytic counts showed non significant change in all groups in compare with control one. *C. proximus* extract showed highly significant increase in monocytic count in compare with hyperlipidemic group.

Table 5
Leukogram ($\times 10^3/\mu l$) of rats in gps. (1-5) after 28 days of hyperlipidemia treatment.

	Experimental groups						
Parameters	**Gp.(1)**	**Gp.(2)**	**Gp.(3)**	**Gp.(4)**	**Gp.(5)**	**SEM[1]**	**p-value**
T.L.C	12.96	10.45	11.12	12.18	12.14	0.31	0.075
Lymphocytes	9.36 ^{a}	6.94 ^{b}	7.88 ^{ab}	8.12 ^{ab}	8.55 ^{ab}	0.26	0.046
Granulocytes	2.14	2.19	1.96	2.21	2.03	0.06	0.748
Monocytes	1.46 ^{ab}	1.32 ^{b}	1.28 ^{b}	1.85 ^{a}	1.56 ^{ab}	0.05	0.006

[1]SEM: Standard error of the mean. Means bearing different superscripts within the same row are significantly different (P<0.05). Gp.(1) control group, Gp.(2) hyperlipidemic group, Gp.(3) hyperlipidemia + cholestyramine, Gp.(4) hyperlipidemia + *C. proximus* extract, Gp.(5) hyperlipidemia + half doses of (cholestyramine and *C. proximus* extract). T.L.C.=Total leukocytic count.

3.6. Changes in some coagulation markers

In the present study, Table 6 shows a highly significant decrease in prothrombin time in hyperlipidemic and cholestyramine treated groups and non significant changes in other treated groups in compare with control group, *C. proximus* extract and combined treated groups showed a highly significant increase in same parameter in compare with hyperlipidemic group. Activated partial thromboplastin time showed a highly significant decrease and non significant change in hyperlipidemic group and all treated groups respectively in compare with control group, treatment of hyperlipidemia with various therapeutic agents showed non significant change in same parameter in compare with the hyperlipidemic non treated group.

Table 6
Some coagulation markers of rats in gps. (1-5) after 28 days of hyperlipidemia treatment.

	Experimental groups						
Parameters	**Gp.(1)**	**Gp.(2)**	**Gp.(3)**	**Gp.(4)**	**Gp.(5)**	**SEM[1]**	**p-value**
PT (sec)	10.84 ^{ab}	10.01 ^{cd}	9.94 ^{d}	11.10 ^{a}	10.52 ^{bc}	0.10	0.000
aPTT (sec)	35.96 ^{a}	28.74 ^{b}	31.40 ^{ab}	32.06 ^{ab}	31.99 ^{ab}	0.67	0.000

[1]SEM: Standard error of the mean. Means bearing different superscripts within the same row are significantly different (P<0.05). Gp.(1) control group, Gp.(2) hyperlipidemic group, Gp.(3) hyperlipidemia + cholestyramine, Gp.(4) hyperlipidemia + *C. proximus* extract, Gp.(5) hyperlipidemia + half doses of (cholestyramine and *C. proximus* extract). PT= prothrombin time, aPTT= activated partial thromboplastin time.

Nowadays, tend to utilize synthetic hyperlipidemic medications is gradually diminished because of their related adverse effects, as well as a progression of drug resistance. So it has increased the use of medicinal herbs in recent times. Using a combination of synthetic drugs and medicinal herbs become a necessary as synergistic action of them may also increase pharmacological therapeutic effect that is more important in drugs with low safety and narrow therapeutic indices (Rafieian-Kopaei et al., 2014). Although active compounds which were isolated from medicinal herbs have been shown to have important pharmacological activities, but a little information on safety, effectiveness and adverse effects of these medicinal herbs require further study (Rouhi-Boroujeni et al., 2015).

The obtained lipid profile results revealed that, serum TG, TC, LDL-C and VLDL-C concentrations were markedly increased while HDL-C was markedly decreased in rats after induction of hyperlipidemia by cholesterol suspension in comparison with the control group may be due to high cholesterol and saturated fatty acids contents increase the availability of acetyl- CoA, a precursor for cholesterol biosynthesis which lead to increases the activity of HMG-CoA reductase enzyme, thus increasing the synthesis of cholesterol in the body. Also, changing hepatic LDL receptor activity or LDL-C production rate or both by the high fat suspension cause down regulation of LDL receptors. This lead to raising of serum LDL-C level. In addition to increaseing the cholesteryl ester transfer protein (CETP), an enzyme responsible of reverse cholesterol transport and HDL-C metabolism, which lead to increase the

transfer of cholesteryl esters from HDL-C to triglyceride rich particles in exchange for triglycerides. This leads to increased serum concentration of triglycerides and decrease in serum concentration of HDL-C (Rang et al., 2007). Treatment of hyperlipidemia with cholestyramine alone showed highly significant increase in serum TG,TC, HDL-C and VLDL-C levels in compare with the control group but with lower values than hyperlipidemic group except HDL-C and highly significant decrease in serum LDL-C in compare with control and hyperlipidemic groups may be due to the mode of action of cholestyramine which binds to bile acids in the gut, and thus interrupt the enterohepatic recirculation of bile acids which has high impact on metabolism of different lipoproteins types in the liver and level of them in serum. Interruption of bile acid recirculation by the drug lead to activation of phosphatidic acid phosphatase which is a basic enzyme in synthesis of TG in liver and consequently increasing TG synthesis and level. Size, composition and level of serum VLDL-C effected by increasing TG synthesis. Marked reduction in serum LDL-C by drug may be due to activation of 7-hydroxylase by interruption of enterohepatic recirculation which leading to increase conversion of intracellular cholesterol to its 7-hydroxy derivative, hence to bile acids. The immediate response is reduction of intracellular cholesterol stores that in turn increase the number of LDL-C receptors and increase the clearance of LDL-C from the circulation. Also, cholestyramine treatment is associated with an increase of synthesis of apo AI which lead to inflow of small, dense discoidal HDL-C particles rich with high concentration of Apo AI (Shepherd, 1989). On the other hand, according to experimental results, serum TG, TC and VLDL-C levels in rats treated with *C. proximus* extract returned towards the normal control level and consequently were lower than hyperlipidemic group may be due to action of (phytochemicals, nutritionals and essential oils) components of *C. proximus* such as terpenes, tannins, flavonoids, saponins, alkaloids, and phenolic glycosides (Ibrahim and El-Khateeb, 2013), tannins are reported to increase the endothelium bound lipoprotein lipase activity which hydrolyzes triglycerides so it might be involved in triglyceride lowering level, while saponins might have contributed in increasing faecal cholesterol excretion, it also reduces uptake of cholesterol from gastrointestinal via intraluminal physiochemical reaction leading to hypocholesterolemia (Kothari et al., 2011; Shanmugasundaran et al., 2011). Combined treated group showed the lipid profile results near from control group may be due to the complementary action of active principles of both synthetic drug and natural herbal plant on different serum lipid fractions, so the combination of different active principles may be more effective than a single one on lipid profile.

Estimation of serum total proteins, albumin and globulins status may be helpful in the assessment of general health conditions and different organs functions. The present study revealed significant hyperproteinemia, hypoalbuminemia and hyperglobulinemia in hyperlipidemic group in compare with the control group may be due to high calorie lipid suspension reduced the protein intake from intestine beside diminishing synthetic function of the liver which resulting probably from hepatocellular damage by hyperlipidemia. In addition to, stress which resulting from increased the metabolic need for tissue repair and free radical neutralization all of previous causes lead to hypoalbuminemia (Olorunnisola et al., 2012). While, hyperproteinemia may be resulted from observed hyperglobulinemia which may be due to alpha and beta globulins carry lipids and this combination is called lipoproteins so any increase in any type of lipoproteins lead to increase alpha and beta fractions of globulins. Also, hyperlipidemia mainly associated with inflammatory conditions which lead to hyperglobulinemia (Ramakrishnan et al., 2001). Treatment of hyperlipidemia with cholestyramine, *C. proximus* extract and combination of them with half therapeutic doses lead to hypoalbuminemia and hyperglobulinemia in compare with control group may be due to impact of hyperlipidemia on protein profile which explained previously in addition to several adverse effects of treatments which will explain in this study which may lead to decrease synthesis of albumin or increasing its loss and finally lead to marked hypoalbuminemia which in some extent more significant than hyperlipidemic group.

Liver enzymes are considered as the biochemical markers for estimating liver functions. In the current study, results showed a highly significant increase in the activities of liver enzymes (ALT and AST) in hyperlipidemic group in compare with the control one may be due to excessive release of such enzymes from the damaged liver cells as a result of hyperlipidemia in the blood circulation (Rezq, 2012). The results were confirmed by histopathological changes of liver, which showing clearly underwent fatty change as evident by the presence of fat vacuoles within the cytoplasm, which in many instances have pushed the nuclei to the periphery of the cells giving rise to the characteristic signet ring appearance (Figure 1). Cholestyramine treated group also showed a significant increase in the activities of these enzymes in comparison with the control group may be due to interruption of essential fat soluble molecules and/or bile acids enterohepatic cycling which lead to alterations in hepatocytes membrane integrity. According to another theory increasing bile acid synthesis by liver as compensatory mechanism may indirectly result in transient necrosis and activation of apoptotic pathway (Singhal et al., 2014). The results were

confirmed by histopathological changes of liver, which showing mild stetiohepatitis, as represented by the foamy and vacuolated cytoplasm, which is associated with the presence of mild leukocytic infiltration adjacently (Figure 2).

In contrast, treatment hyperlipidemic rats with *C. proximus* extract improved these enzymes and returned it towards normal control level may be due to the antioxidant properties of *C. proximus* which contains several types of polyphenols and flavonoids (Ibrahim and El-Khateeb, 2013) that is known as antioxidants and had strong free radical scavenging which have the ability to preserve normal liver function. The results were confirmed by histopathological findings of liver, which showing normal hepatocytes morphology, with rare incidences of foamy or vacuolated cytoplasm (Figure 3). Treatment hyperlipidemia with cholestyramine by half therapeutic doses reduced its adverse effects beside hepatoprotective action of combined *C. proximus* lead to improvement of liver condition which reflected by improving liver enzymes activities values. The results were confirmed by histopathological changes of liver, which showing normal hepatic cells morphology, with presence of some foamy or vacuolated cytoplasm (Figure 4). Serum ALP activity showed highly significant increase in all groups in compare with control group may be due to ALP involved in lipid absorption, so intestinal ALP activity may increase after a high fat meal. So, increased ALP activity considers a marker of an atherogenic diet (Webber et al., 2010). In addition to the previous explanation increased activity of serum ALP activity in hyperlipidemic and different treated groups with different degrees may be due to increasing non specific ALP isoenzymes which release from different tissues such as (liver) in response to organ injury which induced by hypolipidemic agents.

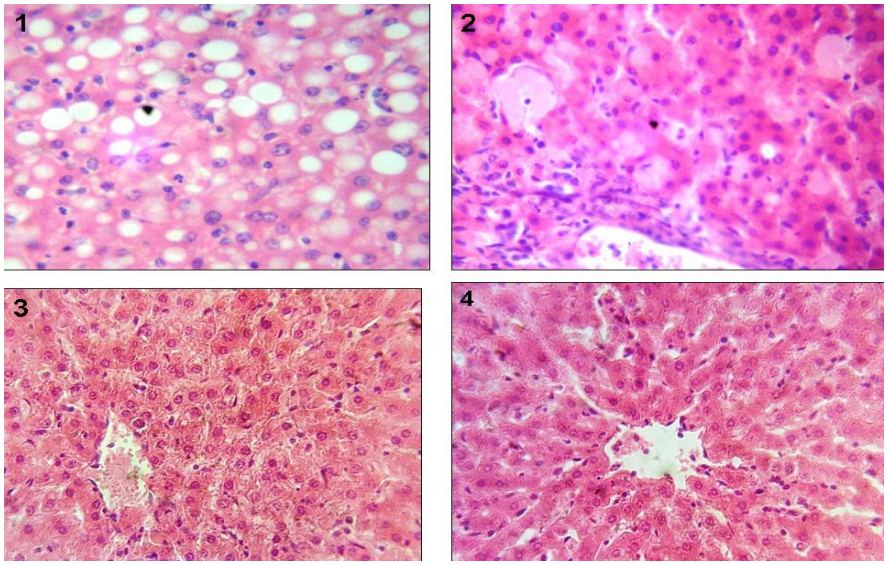

Fig. 1. Liver of rat in gp.(2) showing clearly underwent fatty change in hepatocytes as evident by the presence of fat vacuoles within the cytoplasm, which in many instances have pushed the nuclei to the periphery of the cells giving rise to the characteristic signet ring appearance, H and E, Bar: 500 µm.

Fig. 2. Liver of rat in gp.(3) showing mild stetiohepatitis, as represented by the foamy and vacuolated cytoplasm, which is associated with the presence of mild leukocytic infiltration adjacently, H and E, Bar: 500 µm.

Fig. 3. Liver of rat in gp.(4) showing normal hepatocytes morphology, with rare incidences of foamy or vacuolated cytoplasm, H and E, Bar: 500 µm.

Fig. 4. Liver of rat in gp.(5) showing normal hepatic cells morphology, with presence of some foamy or vacuolated cytoplasm, H and E, Bar: 500 µ.

Oxidative stress is an equilibrium defect in the production of free radicals and the antioxidant protective system inside the organism. In the present study, lipid peroxidation was highly significant increased in hyperlipidemic group and indicated by increasing serum MDA level which considers the major product of lipid peroxidation while serum CAT activity was reduced significantly in compare with control group may be due to hyperlipidemia enhances the free radical generation which attack the polyunsaturated fatty acids in the membrane lipids causing lipid peroxidation which may lead to disorganization of cell structure and function

(Dhande et al., 2014), these free radicals reduced the activity of the antioxidant enzyme reserve and lead to reducing serum CAT activity. Treatment of hyperlipidemia by *C.proximus* extract reduced serum MDA level and increased serum CAT activity significantly in compare with control and hyperlipidemic groups may be due to maintenance of free radical levels by herbal extract due to the presence of phenolic compounds which are known to be good scavengers for free radicals so it reduced oxidative stress and improve antioxidant defense system (Sheweita et al., 2002). Combination of *C.proximus* extract and cholestyramine to treat hyperlipidemia give the lowest and highest values of serum MDA and CAT, respectively among all groups may be due to combining benefits of *C.proximus* extract as good free radicals scavengers and cholestyramine as a hypolipidemic drug has high reducing effect on LDL-C which has ability to convert to oxidized LDL (oxLDL) which increasing oxidative stress and generation of reactive oxygen species (ROS) and subsequently lipid peroxidation (LPO) (Shuang et al., 2015).

Concerning the results of erythrogram, there were non significant differences in mean values of different parameters (RBCs count, PCV value and Hb concentration) between different groups and control one. Also, platelets count showed no significant differences in mean values between different groups and control one**.** These data support that hyperlipidemia and different treatment agents have no adverse effects on erythrocytic parameters and platelets count. Regarding to leukogram, lymphopenia observed in the hyperlipidemic group in compare with control group may be due to the positive correlations between hyperlipidemia and inflammation (Kim et al., 2008) which lead to margination and redistribution of lymphocytes inside lymphatic tissues with marked increasing in apoptosis (Zahorec, 2001). Differential leukocytic count in groups treated with different hypolipidemic agents returned towards normal value may be due to the lowering inflammatory conditions induced by hyperlipidemia under influence of hypolipidemic agents.

Concerning the coagulation markers, this study showed that highly significant shorting of plasma PT and aPTT in hyperlipidemic group in compare with control group may be due to an accumulation of circulating activated coagulation factors (II, VII, VIII and XII mainly) in plasma under influence of hyperlipidemia, this factor mainly share in intrinsic, extrinsic and common coagulation pathway which tested by PT and aPPT (Cleuren et al., 2015). Treatment of hyperlipidemia with cholestyramine showed a highly significant shorting of plasma PT in compare with control group with value near from a hyperlipidemic group may be due to PT is most sensitive to factor VII level and fail of cholestyramine to reduce serum triglycerides significantly which correlate positively with factor VII lead to hypercoagulability and shorting PT (Silveira et al., 1994). On the other hand, treatment of hyperlipidemia with *C.proximus* extract alone or in combination with cholestyramine returned plasma PT and aPPT towards the normal value which means the benefits of their use as hypolipidemic and anticoagulant agent.

4. Conclusion

It was concluded that phytochemical constituents of *C.proximus* extract has effective hypolipidemic activity besides antioxidant and hepatoprotective properties. Combining treatment of *C.proximus* extract with cholestyramine as a synthetic drug with half therapeutic doses gave a complementary hypolipidemic effect with fewer side effects than treatment with each therapeutic compound alone. Further studies are necessary to estimate proper dose of *C.proximus* extract as hypolipidemic agent without any side effects and the possibility of using it with different synthetic hypolipidemic drugs.

Acknowledgments

The author would like to thank Dr. Haytham Ali lecturer of Pathology, Faculty of Veterinary Medicine at Zagazig University for his valuable help in examining and reading of histopathological slides.

References

Abou-Shoer, M.I., Fathy, H.M., Omar, A.A., 2011. Extract-template modeling and pattern recognition in the assessment of (Cymbopogon proximus). Am. J. Anal. Chem., 2, 500-510.

Bancroft, J.D., Stevens, A., Turner, D.R., 1996. Theory and Practice of Histological Technique. 4th ed. Churchill Livingstone, New York, USA.

Bauer, J.D., 1982. Clinical laboratory methods. 9th ed. C.V. Mosby Co., Missouri, 555.

Cleuren, A.C.A., Blankevoort, V.T., van Diepen, J.A., 2015. Changes in dietary fat content rapidly alter the mouse plasma coagulation profile without affecting relative transcript levels of coagulation factors. Plose One. 10(7), 1-15.

Dhande, S.R., Gursahani, M.S., Kadam, V.J., 2014. Standardization of cholesterol suspension induced hyperlipidemia in rats. World J. Pharm. Pharmaceut. Sci., 3(12), 1107-1123.

Dhingra, D., Lamba, D., Kumar, R., Nath, P., Gauttam, S., 2014. Antihyperlipidemic activity of Aloe succotrina in rats: Possibly mediated by inhibition of HMG-CoA reductase. I. S. R. N. Pharmacol., 1-9.

Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18(6), 499–502.

Ibrahim, F.Y., El-Khateeb, A.Y., 2013. Effect of herbal beverages of Foeniculum vulgare and Cymbopogon proximus on inhibition of calcium oxalate renal crystals formation in rats. Ann. Agric. Sci., 58(2), 221–229.

Kim, E., Tolhurst, A.T., Qin, L.Y., Chen, X., Febbraio, M., Cho1, S., 2008. CD36/Fatty acid translocase, an inflammatory mediator, is involved in hyperlipidemia-induced exacerbation in ischemic brain injury. J. Neurosci., 28(18), 4661– 4670.

Kothari, S., Jain, A.K., Mehta, S.C., Tonpay, S.D., 2011. Hypolipidemic effect of fresh Triticum aestivum (wheat) grass juice in hypercholesterolemic rats. Acta. Pol. Pharm., 68(2), 291-294.

Lin, Y., Mousa, S.S., Elshourbagy, N., Mousa, S.A., 2010. Current status and future directions in lipid management: Emphasizing low-density lipoproteins, high-density lipoproteins and triglycerides as targets for therapy. Vasc. Health. Risk. Manag., 6(1), 73–85.

Mansour, H.A., Newairy, A.A., Yousef, M.I., Sheweita, S.A., 2002. Biochemical study on the effects of some Egyptian herbs in alloxan-induced diabetic rats. Toxicol., 170(3), 221–228.

McKenney, J.M., 2007. Report of the national lipid association's safety task force: the nonstatins. Am. J. Cardiol., 99 (6 A), 1C-58C.

Newairy, A.A., Mansour, H.A., Yousef, M.I., Sheweita, S.A., 2002. Alterations of lipid profile in plasma and liver of diabetic rats: Effect of hypoglycemic herbs. J. Environ. Sci. Health. B., 37(5), 475-484.

Olorunnisola, O.S., Bradley, G., Afolayan, A.J., 2012. Protective effect of T. violacea rhizome extract against hypercholesterolemia-induced oxidative stress in wistar rats. Molecules., 17(5), 6033-6045.

Rafieian-Kopaei, M., Shahinfard, N., Rouhi-Boroujeni, H., Gharipour, M., Darvishzadeh-Boroujeni, P., 2014. Effects of Ferulago angulata extract on serum lipids and lipid peroxidation. Evid. Base. Compl. Alternat. Med., 1-4.

Ramakrishnan, S., Prasannan, K.G., Rajan, R., 2001. Textbook of Medical Biochemistry.3rd ed. Orient Longman, India.

Rang, H.P., Dale, M.M., Ritter, J.M., Flower, R.J., 2007. Rang and Dale's Pharmacology. 6th ed. Churchill Livingstone, Elsevier, Edinburgh, 321.

Rezq, A.A., 2012. Beneficial health effects of fennel seeds (shamar) on male rats feeding high fat-diet. Med. J. Cairo Univ., 80(2), 101-111.

Rouhi-Boroujeni, H., Rouhi-Boroujeni, H., Heidarian, E., Mohammadizadeh, F., Rafieian-Kopaei, M., 2015. Herbs with anti-lipid effects and their interactions with statins as a chemical anti- hyperlipidemia group drugs: A systematic review. A.R.Y.A. Atheroscler. 11(4), 244-251.

Senecha, C., Shama, P.K., D'Souza, U.P., Shastry, C.S., 2012. Anticholesteremic and antilipidemic activity of stem bark extracts of Moringa oleifera in diet induced hyperlipidemia model in rats. I. J. P. C. S., 1(3), 567-574**.**

Shanmugasundaran, R., Kalpana Devi, V., Tresina Spris, P., Maruthupandian, A., Mohan, V.R., 2011. Antidiabetic, antihyperlipidemic and antioxidant activity of Senna auriculat (L.) roxb leaves in alloxan induced diabetic rats. Int. J. Pharm. Tech. Res., 3(2), 747 - 756.

Shepherd, 1989. Mechanism of action of bile acid sequestrans and other lipid-lowering drugs. Cardiol., 76(1), 65-74.

Sheweita, S.A., Newairy, A.A., Mansour, H.A., Yousef, M.I., 2002. Effect of some hypoglycemic herbs on the activity of phase I and II drug-metabolizing enzymes in alloxan-induced diabetic rats. Toxicol., 174(2), 131–139.

Shuang, L.I.U., Wei, H.O.U., Hua, Q.I.N., Ying, W.A.N.G., 2015. Oxidized LDL stimulates lipid peroxidation-derived DNA and protein adducts in human vascular endothelial and smooth muscle cells. J. Huazhong Univ. Sci. Technol., 35(2), 200-205.

Silveira, A., Karpe, F., Blombäck, M., Steiner, G., Walldius, G., Hamsten, A., 1994. Activation of coagulation factor VII during alimentary lipemia. J. Am. Heart. Assoc., 14(1), 60-69.

Singhal, R., Harrill, A.H., Menguy-Vacheron, F., Jayyosi, Z., Benzerdjeb, H., Watkins, P.B., 2014. Benign elevations in serum aminotransferases and biomarkers of hepatotoxicity in healthy volunteers treated with cholestyramine. B.M.C. Pharmacol. Toxicol., 15(42), 1-7.

Van Berge Henegouwen, G.P., Keppler, D., Leuschner, U., Paumgartner, G., Stiehl, A., 2011. Biology of Bile Acids in Health and Disease. Falk Symposium. Kluwer Publishers, Netherlands.

Webber, M., Krishnan, A., Thomas, N.G., Cheung, B.M.Y., 2010. Association between serum alkaline phosphatase and C-reactive protein in the United States national health and nutrition examination survey 2005–2006. Clin. Chem. Lab. Med., 48(2), 167–173.

Zahorec, R., 2001. Ratio of neutrophil to lymphocyte counts rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl. Lek. Listy., 102(1), 5-14.

How to cite this article: Ismail, H.T.H., 2016. Influence of *Cymbopogon proximus* extract on lipid profile, biochemical hematological and coagulation parameters of hyperlipidemic albino rats. Scientific Journal of Veterinary Advance, 5(9), 123-133.