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Effect of Ambrex (A Herbal Formulation) on Hematological Variables in Hyperlipidemic Rats

Jamuna Devi and Johanna Rajkumar
Department of Biotechnology, Rajalakshmi Engineering College, Thandalam, Chennai, 602105, India

Abstract: Cardiovascular and related disorders are one of the most common disease prevailing all over the world. Hyperlipidemic condition have been largely considered in the treatment of cardiovascular diseases. The present study was carried out to investigate the effect of Ambrex on hematological factors in hyperlipidemic rats and untreated hyperlipidemic rats. In this study, eighteen rats were randomly divided into three groups of six animals each The groups received normal diet (Control Group A) high fat diet (HFD group B) and Ambrex treatment (Group C). After the study period, White Blood Cell (WBC), Red Blood Cell (RBC), hematocrit (HCT), Hemoglobin, platelet (PLT), lymphocytes, monocytes, granulocytes, Mean Corpuscular Hemoglobin Concentration (MCHC), plateletcrit (Pct), Mean Corpuscular Volume (MCV), Platelet Distribution Width (PDW), red cell distribution-standard deviation (RDW-SD), red cell distribution-correlation variance (RDW-CV), micro red blood cell (μRBC), macroRBC were measured using digital cell counter (MS9-3s). Hyperlipidemia increases markedly the PLT count. Administration of Ambrex appeared to significantly increase WBC, Lymphocytes, granulocytes. However, erythrocyte indices does not show statistically significant variations among the test groups and control groups. The findings demonstrated that Ambrex does not cause any significant undesirable alterations in hematological factors in male rats. Ambrex also enhances white blood cell concentration and lymphocytes which probably stimulate the immune defense mechanism.

Key words: Ambrex, hyperlipidemia, hematological parameters

INTRODUCTION

Hyperlipidemia is characterized by elevation of serum cholesterol, triglyceride and LDL cholesterol and decreased level of HDL cholesterol. Presumably, hyperlipidemia leads to atherosclerosis, cardiovascular disorders, myocardial infarction etc. Researches support that there exists a correlation between hematological parameters and atherosclerotic heart diseases (Kesmarky *et al*., 2006). Dr. Maxwell Myer Wintrobe in 1929 introduced Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) to elucidate the size (MCV) and hemoglobin content (MCH, MCHC) of red blood cells. The literature suggests that hyperlipidemia may result in erroneous PLT and WBC counts increase and induce serum turbidity, which may likely augment the MCH and MCHC (Michael and Frederick, 1996; Zandecki *et al*., 2007). A large number of reports have highlighted that consumption of medicines or herbal drugs can alter the normal range of hematological parameters. These changes could be either positive or negative (Ofuya and Ebong, 1996; Ajagbonna *et al*., 1999). In recent years, most automated blood cell counters determine the number of white blood cell, red blood cell, MCV, MCHC, hemoglobin, hematocrit, platelet, PDW, RDW etc. These hematology analyzers are a boon in the improvement of cellular hematology, as the results obtained are quicker and accurate.

Plants have been a major source of medicine for millions of years. The world Health Organisation (WHO) reports that about three-forth of the people depend on herbal medicines for maintaining their health (Farnsworth *et al*., 1985). Ambrex is a polyherbal formulation which consists of *Withania somnifera* (100 mg), *Orchis mascula* (25 mg), *Cycas circinalis* (62.5 mg), *Shorea robusta* (25 mg) with amber (37.5 mg). It is evident from previous studies that ambrex possesses hepatoprotective and antiulcerogenic properties (Devi *et al*., 2003; Jainu and Devi, 2004). It is well known that only few studies have closely evaluated the associations between hyperlipidemia and hematologic parameters. Therefore, investigation was done to determine the effect of ambrex (a herbal formulation) on hematologic variables to bring into limelight the better understanding of cellular implications in hyperlipidemic rats.

Corresponding Author: Jamuna Devi, Department of Biotechnology, Rajalakshmi Engineering College, Thandalam, Chennai, 602105, India Tel: 9840234882

MATERIALS AND METHODS

Polyherbal formulation: Ambrex in capsule form obtained from Care and Cure Herbs Ltd, Chennai. Ambrex is a polyherbal formulation consisting of *Withania somnifera* (100 mg), *Orchis mascula* (25 mg), *Cycas circinalis* (62.5 mg), *Shorea robusta* (25 mg) with amber (37.5 mg).

Animals: Eighteen male Wistar rats weighing about 130-150 g were maintained under standard husbandry conditions 25±5°C temperature, light/dark cycle with standard rat feed (Hindustan Lever Ltd.) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC No. Biotech REC.001/10) and the experimental work was carried out as per Committee for the Purpose of Control and Supervision on Experiments on Animal guidelines.

Experimental protocol: The experiment was divided into two periods, the former was the induction of hypercholesterolemia and the latter was treatment period (15 days). Induction of hypercholesterolemia continued for 30 days by feeding the rats with 10% egg yolk powder, 5% lard, 1% cholesterol and 0.5% cholic acid (Zhao *et al*., 2009).

Then, rats were divided into three groups of six animals each as follows:

- **Group A:** Normal basal diet receiving rats (Control group)
- **Group B:** Untreated hyperlipidemic rats
- **Group C:** Ambrex treated hyperlipidemic rats (40 mg kg^{-1} b.wt.). Dosage was arrived from previous studies (Jainu and Devi, 2004)

Blood samples taken from the abdominal aorta were collected in sample bottles containing EDTA as anticoagulant. Hematological assessments of RBC (million mm^{-3}), HGB (gm dL^{-1}), HCT (%), MCV (μmm^3), MCH (pg), MCHC (gm dL^{-1}), LYM (%), MO (%), GRN (%), RDW (%), PCT., MPV (μmm^3) and PDW (%) were examined by using digital cell counter (MS9-3s).

Statistical analysis: The experimental results were expressed as mean of six replicates±SD. Significance of the differences among the control and treatment groups was determined using analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. The $p<0.05$ was considered as a statistically significant difference.

RESULTS AND DISCUSSION

In the present study, evaluation of hematological parameters is carried out to determine whether Ambrex has any influence on cellular pathogenesis in hyperlipidemic state.

The leukocyte parameters of the control and study groups are shown in Table 1. In this study, hyperlipidemic group shows statistically significant elevation in WBC (20.7 m mm^{-3}) ($p<0.05$). There was observed no significant difference in lymphocytes granulocytes and monocytes in hyperlipidemic rats. Furthermore, Ambrex treated rats show significant increase in WBC count (33.05 m mm^{-3}) ($p<0.001$), lymphocytes (77.96%) ($p<0.05$) and granulocytes (8.85 m mm^{-3}) ($p<0.05$).

Table 2 show details of erythrocyte parameters namely, RBC, MCH, MCV, Hct, Hgb, RDW-SD, RDW-CV, µRBC and macro RBC of the control and treated groups after the 45 days study period. The results indicates that there was no significant changes observed in erythrocyte indices between control and treated animals.

The results of the thrombocyte indices are shown in Table 3. There is significant increase in PLT (1601 m mm^{-3}), PcT (1.36%), PDW (13.43), µPLT (18.35%) in untreated hyperlipidemic rats. Administration of Ambrex markedly decreased the PLT (1037 m mm^{-3}), Pct (0.86%), PDW (11.65), µPLT (15.75%) when compared to hyperlipidemic rats.

The above findings report that hypercholesterolemic animals has elevated leukocyte count and are in accordance with the results observed by Kostis *et al*. (1984). From Table 1, Ambrex treated animals were found to have significant elevated levels of white blood cell counts. The result of this study agrees with that of

Table 1: Effect of Ambrex on leukocyte parameters in hyperlipidemic rats

Group	A	B	C
WBC (m mm^{-3})	12.57±4.07	20.7±5.530*	33.05±4.03***
Lym (%)	59.55±4.78	66.61±6.18	77.96±5.67*
Mon (%)	2.78±0.60	3.8±0.580	3.58±1.34
N/Gr (%)	27.75±4.32	24.35±3.26	18.28±3.41
$Lym^{\#}$	29.85±3.61	28.36±3.38	40.78±4.19**
$Mon^{\#}$	1.45±0.58	1.2±0.200	1.63±0.49
$N/Gr^{\#}$	4.15±1.63	5.21±1.57	8.85±2.46*

WBC: White blood cell, Lym%: Lymphocytes percentage, Mon%: Monocytes percentage, N/Gr%: Neutrophil/granulocytes percentage, $Lym^{\#}$: Lymphocytes number, $Mon^{\#}$: Monocytes number, $N/Gr^{\#}$: Neutrophil/granulocytes number, Group A-Normal basal diet receiving rats (Control group), Group B-Untreated hyperlipidemic rats ; Group C-Ambrex treated hyperlipidemic rats (40 mg kg^{-1} b.wt.), Values are expressed as Mean±SD for 6 animals in each group, p-values: *$p<0.05$, **$p<0.01$, ***$p<0.001$ statistically significant when compared with control group A. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ statistically significant when compared with hyperlipidemic group B

Table 2: Effect of Ambrex on erythrocyte indices in hyperlipidemic rats

Group	A	B	C
RBC (m mm^{-3})	5.49±0.31	6.13±0.68	6.16±0.58
MCV fl	51.05±2.83	50.83±1.15	51.05±2.08
Hct (%)	28.1±2.750	34.76±3.83	31.41±2.81
MCH pg	23.65±3.10	22.86±2.97	26.3±1.710
MCHC (g dL^{-1})	48.78±4.66	44.98±5.63	51.48±1.76
Hgb (g dL^{-1})	14.58±0.47	16.88±3.14	16.2±1.890
RDW-SD fl	30.85±2.24	30.7±1.400	30.81±1.13
RDW-CV (%)	16.1±0.830	16.41±0.33	15.78±0.61
Ìrbc (%)	13.85±3.34	12.01±1.57	11.91±3.97
MRBC (%)	1.48±0.48	1.48±0.21	1.38±0.44

RBC: Red blood cell, MCV: Mean corpuscular volume, HcT: Hematocrit MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, Hgb: Hemoglobin, RDW-SD: Red cell distribution-standard deviation, RDW-CV: Red cell distribution-correlation variance, µRBC: Micro red blood cell, MRBC: Macro red blood cell, Group A-Normal basal diet receiving rats (Control group), Group B-Untreated hyperlipidemic rats, Group C-Ambrex treated hyperlipidemic rats (40 mg kg^{-1} b.wt.,), Values are expressed as Mean±SD for 6 animals in each group, p-values: *p<0.05, **p<0.01, ***p<0.001 statistically significant when compared with control group A. #p<0.05, ##p<0.01, ###p<0.001 statistically significant when compared with hyperlipidemic group B

Table 3: Effect of Ambrex on thrombocyte parameters in hyperlipidemic rats

Group	A	B	C
PLT (m mm^{-3})	977.33±294.93	16.01±298.2**	103.7±142.2##
MPV fl	6.43±0.7200	10.73±3.05**	8.33±1.84
Pct (%)	0.83±0.1700	1.36±0.37*	0.86±0.32#
Mod fl	3.13±0.7000	3.2±0.61	3.08±0.20
Medn fl	4.2±0.74000	5.93±1.46	5.28±1.25
PDW	9.38±2.1100	13.43±0.92**	11.65±1.98
µplt (%)	17.4±3.05000	15.61±3.46	15.66±3.96
MPLT (%)	11.25±3.1100	18.3562.23**	15.75±4.12

PLT: Platelet, MPV: Mean platelet volume, Pct: Plateletcrit, Mod fl: Mode flow, Medn fl: Median flow, PDW: Platelet distribution width, Mplt: Micro platelet and MPLT: Macroplatelet, Group A-Normal basal diet receiving rats (Control group), Group B-Untreated hyperlipidemic rats, Group C-Ambrex treated hyperlipidemic rats (40 mg kg^{-1} b.wt.,), Values are expressed as Mean±SD for 6 animals in each group, p-values: *p<0.05, **p<0.01, ***p<0.001 statistically significant when compared with control group A. #p<0.05, ##p<0.01, ###p<0.001 statistically significant when compared with hyperlipidemic group B

Kashinath (1990) and Abdulrahman *et al.* (2010) who observed increased WBC production in hyperlipidemic rats which may lead to possible stimulation of the immune defense system.

It was observed that there was no effect of dietary fat on the erythrocyte indices since there are no significant differences in the mean values of red cell indices between the control and study groups. The findings are in agreement with Choi and Pai (2004) and Lee *et al.* (2004) that erythrocyte indices does not alter significantly between the subjects with and without hyperlipidemia. The insignificant variations in the erythrocyte indices suggested that Ambrex did not induce anemia in the experimental rats.

The present study described that there is a significant decrease in PLT, PcT, PDW, µPLT in Ambrex treated group. The primary physiological function of platelets is to mediate the haemostatic response. An elevation in platelet count is considered as an indicator of vascular disease like microangiopathy and macro angiopathy (Kwaan, 1992). It is therefore possible that Ambrex administration may not contribute to the risk for Cardiovascular disease.

From the foregoing, Ambrex have no adverse effects on hematological parameters. Furthermore, Ambrex improved the levels of lymphocytes and WBC significantly indicating the stimulation of immune defense mechanism. Ambrex treated group also exhibited low count of blood platelets which improves blood haemostasis by alleviating thrombosis-inducing platelets.

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