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HAEMATOLOGICAL STUDY OF INDIAN BEES WAX IN EXPERIMENTALLY INDUCED HYPERLPIDEMIC MALE ALBINO RABBITS

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ABSTRACT

Bees wax is a substance obtained from honey combs. It consists of esters of straight chain monohydric alcohols due to which it shows hypolipidemic activity. The hematological study of bees wax treated animals shows dose dependent antiplatelet activity. Four groups of 6 male albino rabbits in each, were used for study. To Intact control group no drug was given, to hyperlipidemic control group atherogenic diet with cholesterol powder (500 mg/kg body weight) mixed in 5ml coconut oil was given. To group 3 & 4 bees wax and statin was given as drug by oral administration, the drug treatment was carried out for complete 60 days. Animals were sacrificed by prolonged ether anesthesia, after the 24 hours of last dose of drug blood sample was collected for the hematological and serum assays. The hematological parameters like erythrocyte count, leucocyte count, hemoglobin count etc. were evaluated, the platelet count showed variation from its normal range. Other parameters like sugar, urea, creatinin etc. remain unaltered owing to the nontoxic nature of bees wax.

Keywords: Antiplatelet, Atherosclerosis, Bees wax, Cholesterol, Other serum parameters, Nontoxic nature.

INTRODUCTION

Assessment of hematological and serum parameters is a prerequisite to understand the normal functioning of the system and to confirm the nontoxic nature of the administered crude drug. A large number of allopathic hypolipidemic drugs are currently available in the market but these lag behind the desired properties such as cost effectiveness and nontoxic nature. A number of medicinal plants have shown their beneficial effect on the cardiovascular disease (CVD) by virtue of their lipid lowering, antioxidant and cardioprotective effects [1,2]. Bees wax is one such hypolipidemic drug. Coronary heart disease resulting from progressive atherosclerosis remains the most common cause of morbidity and mortality all over the world [3]. In developing countries, the incidence of cardiovascular disease is increasing alarmingly. India is on the urge of a cardiovascular epidemics [4,5]. The circulatory system disorders are going to be the greatest killer in India by the end of year 2015 [6]. The derivatives of bees wax have shown certain therapeutic properties, particularly in lowering blood cholesterol. Policosanol is a natural mixture of long chain primary aliphatic saturated alcohols that is isolated from bees wax, sugarcane wax etc [7]. A meta-analysis of studies has shown that policosanol, derived from bees wax, can effectively lower both total and LDL (bad) cholesterol while raising HDL (good) cholesterol. Bees wax are the main source of commercial very long chain fatty alcohols, Unhydrolyzed beeswax produced by the honey bee contains about 23% hydrocarbons, 45% wax monoesters, 6% diesters of long chain alcohols with palmitic acid, 1% free alcohols and 12% free acids. Approximately 6% of bees wax is not identifiable [8]. Palmitic acid is the major acid found in the ester fraction. The Wax also contains about 3% diols and 13% hydroxy acids. Evidence suggests that bees synthesize these constitents rather than acquiring them from plants [9].

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The present study was designed to investigate the antiplatelet activity of crude bees wax and its comparative status with that of synthetic drug statin (atorvastatin) currently present in market with certain side effects like rhabdomylysis, muscle weakness etc., the study will also analyse serum parameters like sugar, urea, creatinin, AST,ALT etc. to further prove the nontoxicity of herbal drug.

MATERIALS AND METHODS

Collection of bees wax

Indian whitish grey beeswax was obtained from Apiculture centre, Department of Zoology, Jiwaji University, Gwalior, it was collected by general scrapping method of the commercial rearing chambers.

Animals

Healthy adult male New Zealand rabbits were procured from Forest Department, Jodhpur (Rajasthan). Weights and age of animals were 1.25-1.75 kg and 10-12 month respectively. Animals were housed in well-lighted air-conditioned room in metallic wire gauge cages, under controlled environmental conditions with 12 hours illumination and 12 hours darkness cycle. Animals were fed on standard rabbit chow supplied by Hindustan lever ltd., India. The food was supplemented with green leafy and seasonal vegetables and water *ad libitum.* Ethical approval was taken by ethical committee of the host institute.

Induction of hyperlipidemia

The hyperlipidemic condition was induced by cholesterol feeding to rabbits. The cholesterol powder (500 mg/kg body weight) was mixed in 5ml of coconut oil mixture and administered to the animals orally. In addition animals were fed with atherogenic diet. The atherogenic diet was comprised of wheat flour base with addition of milk powder, dried egg yolk, hydrogenated fat, butter, dried yeast, salt, sugar and vitamin mixture to produce the following nutrients in the given proportion as recommended by WHO protocol. The average consumption of diet was 200g/rabbit per day.

Standard drug

Atorvastatin was used as standard hypolipidemic drug and it was given to the animals at the dose of 0.25mg/kg body weight dissolved in 5ml distilled water.

Feeding of bees wax

For administration to the animals, the bees wax was given in a dose of 50mg/kg body weight suspended in 5ml of distilled water. The dose of the drug was determined by LD_{50} test.

Experimental groups

Twenty four male albino rabbits were divided into four groups the control and experimental groups, usually consisted of six animals each.

Group 1 – Vehicle treated control or intact control (60 days)

Group 2 – Atherodiet + cholesterol feeding (500mg/kg body weight) for 60 days

Group 3 – Cholesterol feeding (500mg/kg body weight) for 15 days + bees wax (50mg/kg body weight) for 45 days.

Group 4 – Cholesterol feeding (500mg/kg body weight) for 15 days + statin (0.25mg/kg body weight) for 45 days

Criteria of observation

At the end of experimental period, animals were autopsized under prolonged ether anesthesia. Blood was collected through cardiac puncture in E.D.T.A vial for blood parameters determinations and centrifuged in plane vials for serum analysis.

Hematological observation

Hemoglobin concentration, WBC, RBC, Platelet Counts, MCV, MCH, MCHC, Lymphocytes, Monocytes, Granulocytes, RDW, PCT, MPV, PDW and Hematocrit were all determined on a Celltac- α Hematology analyzer (Nihon Kohden Japan).

Other parameters

Blood sugar, urea, creatinin, SGOT and SGPT were determined by serum analysis in Nex-Gen Semi Autoanalyzer USA. The analysis were performed by commercial test kits.

RESULTS

The results of all the haematological parameters of vehicle treated control group (Gr. 1) and all other experimental groups (Gr.2-4) were found to be within the normal range except platelet count .

Platelet Count

Slightly significant reduction was observed in bees wax and statin treated groups when compared with group 1 and group 2. All other hematological parameters showed nonsignificant changes when there comparative study was done (Table-1).

Other parameters

High cholesterol leads to increase in sugar level . Bees wax and statin cannot bring sugar level to normal range. The concentration of urea and creatinin did not show any significant change in rabbits treated with atherodiet and drug treated groups (Gr.1-2).With increase in cholesterol SGOT increases. Bees wax reduces it, but statin do not show much change. SGPT shows non significant change with increase in cholesterol, statin had no impact on SGPT, while bees wax showed reduction (Table-2).

Table 1. Hematology of various drug (biowaxes) treated intact rabbits (Mean of 5 Values ± SEM)

Hematology Parameters	Control (Gr.1)	Hyperlipidemia (Gr.2)	Bees Wax (Gr.3)	Statin (Gr.4)
TLC/cu.mm.	7090.0 ± 378.0	7033.3 ± 388.0^{d}	7750.0 ± 250.0^{d,h}	7400.00 ±208.16^{d,h}
RBC.ml/dl	6.34 ± 0.30	5.32 ± 0.37^{d}	5.20 ± 0.24^{d,h}	5.00 ± 0.24^{d,h}
HGB.gm/dl	11.46± 0.42	10.46 ± 0.24^{d}	10.25± 0.28^{d,h}	10.53 ± 0.29^{d,h}
HCT.%	35.33± 0.598	36.00±3.732^{d}	31.650.± 3.12^{d,h}	30.00± 3.08^{d,h}
MCV.fl.	64.19± 2.70	64.466± 2.90^{d}	62.75± 3.00^{d,h}	66.26± 2.74^{d,h}
MCH.pg.	17.620±0.434	18.666±0.66^{d}	18.15± 0.60^{d,h}	17.76± 0.47^{d,h}
MCHC.gm/dl	28.610±2.309	28.00±2.08^{d}	28.95± 2.00^{d,h}	27.43± 2.41^{d,h}
PLT.lacs/cu.mm.	2.909±0.900	3.75±0.19^{c}	1.94± 0.50^{c,g}	1.0± 0.52^{c,g}
LYM.%	39.290±3.057	39.680±3.48^{d}	45.65± 4.450^{d,h}	44.23± 2.98^{d,h}
MO.%	10.700±.0.651	11.666±0.88^{d}	10.70± 0.70^{d,h}	9.99 ± 1.10^{d,h}
GRN.%	50.710±3.315	48.666±3.05^{d}	43.50±3.15^{d,h}	45.78 ± 4.761^{d,h}
RDW.%	14.84 ± 0.59	14.26 ± 0.40^{d}	12.50 ± 0.50^{d,h}	12.66± 0.24^{d,h}
PCT.	0.02± 0.002	0.02± 0.003^{d}	0.02 ± 0.003^{d,h}	0.02 ± 0.003^{d,h}
MPV.fl.	7.12 ± 0.23	7.76± 0.05^{d}	7.5 ± 0.5^{d,h}	7.00 ± 0.5^{d,h}
PDW.%	18.22 ± 0.47	17.66 ± 0.88^{d}	17.50 ± 0.50^{d,h}	18.93± 0.29^{d,h}

Gr. 2, 3 and 4 were compared with Gr.1

$P \leq 0.05$ = a
$P \leq 0.01$ = b
$P \leq 0.001$ = c
Nonsignificant = d

Gr. 3 and 4 were compared with Gr.2

$P \leq 0.05$ = e
$P \leq 0.01$ = f
$P \leq 0.001$ = g
Nonsignificant = h

Table 2. Other parameters of various drug (Biowaxes) treated intact rabbits (Mean of 5 Values ± SEM)

Treatment Groups	B.Sugar mg/dL	B.Urea mg/dL	S.Creatinin mg/dL	SGOT IU/mL	SGPT IU/mL	T.Protein mg/dL
Control (Gr. 1)	113.00±14.00	32.50±3.50	1.10±0.01	62.85±6.85	87.50±9.50	7.55±0.02
Hyperlipidemic (Gr. 2)	154.00±5.65^{c}	29.70±0.98^{d}	1.01±0.02^{d}	105.00±10.21^{c}	110.00±7.07^{d}	7.60±0.14^{d}
Bees Wax (Gr. 3)	190.25±8.75^{c,f}	30.70±3.60^{d,h}	1.20±0.02^{d,h}	36.55±1.15^{c,g}	86.95±5.45^{d,g}	7.10±0.01^{d,h}
Statin (Gr. 4)	188.66±7.44^{c,f}	32.16±4.01^{d,h}	1.23±0.19^{d,h}	108.90±12.44^{b,h}	96.63±1.93^{d,h}	7.60±0.01^{d,h}

Gr. 2, 3 and 4 were compared with Gr.1

$P \leq 0.05$ = a
$P \leq 0.01$ = b
$P \leq 0.001$ = c
Non-significant = d

Gr. 3 and 4 were compared with Gr.2

$P \leq 0.05$ = e
$P \leq 0.01$ = f
$P \leq 0.001$ = g
Non-significant = h

DISCUSSION

No significant variation in AST,ALT, sugar, urea, creatinin were seen after statin and bees wax treatment. Similar results were observed by researchers in policosanol study [10]. Thus there is no adverse effect after the administration of drug. When the endothelial cells found in arterial walls become dysfunctional or denuded allows blood products and macrophages to adhere on the surface of vascular wall and penetrate which leads to platelet clumping, the macrophagic cells oxidize LDL cholesterol present in blood resulting in atheromatous plaque. Platelets and coagulation factors are involved in thrombotic process also that can lead to myocardial infarction. Hence the platelet cells, once thought to be solely involved in clot formation, is now known to be a key mediator of inflammation, thrombosis and atherosclerosis. Owing to this fact, antiplatelet agents have paramount importance in cardiovascular diseases. Normal unperturbed endothelial cells exhibit anticoagulant properties that include the release of prostacyclin, which inhibits platelet aggregation. However, exposure to inflammation and atherogenic factors induces procoagulant activity [11]. More over apoptosis of endothelial cells increases the expression of phosphatidylserine and the loss of anticoagulant components of the endothelial cell membrane, as phosphatidylserine exposure enhances tissue factor activity, which is highly thrombogenic. Certainly, extra-cellular tissue factor expression is increased in and around apoptotic monocyte/lymphocyte cells in necrotic basis for the generation of microparticles within the circulation, which act as potent pro-coagulant substrates both locally and systemically [12]. These particles are increased in patients with unstable coronary disease and account for the

vast proportion of the procoagulant activity of the plaque. Thus prostacyclin reduces atherogenic cholesteryl ester accumulation in macrophages and vessel cell, inhibits platelet activation and mitogen release. Policosanol decreases thromboxane A_2 levels and increase prostacyclin levels [13]. The researchers believes that bees wax may have policosanol like component. Studies states that products from bees wax sources have higher aliphatic primary alcohols which had more anti-inflammatory, anti-ulcer and antiplatelet effect [10].

CONCLUSION

In the present investigation the hematological parameters in all experimental groups remain unaltered except platelet count. The normal range of hematological parameters suggests non-toxic nature of the drug and indicates no drug related side effects on the animal models and the reduction in platelet count show antiplatelet activity of the crude bees wax similar to that of statin. The nontoxic nature is further confirmed by other parameters analysis like sugar, urea, creatinin, total protein, AST and ALT which remains unaltered.

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