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# Effects of *Vitex Negundo* Aqueous Extracts on Serum Lipid Variables and Haematological Profile of Albino Rats

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Abstract. The effect of leaf extract of *Vitex negundo* on some haematological and serum lipid parameters in rats during a seven day administration of doses, 250mg/kg and 500mg/kg body weight was investigated. The extracts were fed orally. The parameters evaluated include serum lipids, red and white blood cell indices. The extract showed significant ($p < 0.05$) decrease in serum total cholesterol concentration, while it had no effect on serum HDL cholesterol concentration at all doses administered when compared with control. However, the extract significantly decreased ($p < 0.05$) serum triglycerol concentration at dose of 250mg/kg as well as at dose of 500mg/kg when compared with control. The leaf extract of Vitex negundo had significant effect on RBC, Hb, MCHC, PCV, MCV, neutrophils, basophils, monocytes, lymphocytes and eosinophil. The WBC level was significantly elevated ($p >0.05$) in the group treated with 500 mg/kg body weight of extract. The platelets also showed significant increase ($p>0.05$) in both the groups. Vitex negundo leaf extract showed positive haematological activities in rats and can be recommended to be used in medicinal formulations to cure atherosclerosis, anaemia and hypercholesterolemia and high-tryglyceride related cardiac diseases.

*Keywords:* Hypercholesterolemia, Atherosclerosis, Anaemia, LDL, HDL

## 1. Introduction

Millions of people in sundry traditional societies including India have turned to use of medicinal plants for ailment treatment. This dependency on plant products has its merits, but care must be taken while use of inimical plants or high dose of plant extracts, which could have deleterious effects on vital organs of the body. There seems to be lack of information on safety evaluations of medicinal plants. Plants play a major role in the health care desiderata for treatment of diseases and to improve the immunological response [1].

*Vitex negundo* Linn. Belongs to family Verbenaceae, it is commonly known as Nirgundi

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(Hindi), Five-Leaf chaste tree (English) [2]. It is aromatic shrub; with typical five foliate leave pattern, found throughout the greater part of India at warmer zones and ascending to an altitude of 1500 m in outer Western Himalayas. The shrub is one of common plants used in Indian medicines. It has been claimed to possess many medicinal properties. It contains various chemical compounds of various classes such as alkaloids, tannins, flavonoids, phenolics and saponins[3]. These phytochemicals are responsible for antibacterial and antioxidant properties of the plant leaves [4]. The plant is known to possess elements potassium, calcium, iron, copper, zinc and [4]. Cardiovascular diseases such as coronary heart diseases, stroke and hypertension and elevated plasma lipids are risk factors in heart related problems [5].

Obstruction of blood supply to the heart, brain, liver or kidney cause coronary heart diseases, stroke or kidney failure, as the case may be [6]. Hypercholesterolemia is a risk factor for cardiovascular diseases such as atherosclerosis and myocardial infarction which are common causes of cardiac diseases related mortality [6].

Blood is a vital fluid, containing Red Blood Cell (RBC), White Blood Cell (WBC) and suspended platelets in the serum in homeostatic conditions. The blood is important for pulmonary tissue respiration, as a medium for endocrine and neurohormonal transmissions, biotransformations and metabolic excretions [7].

Despite of several studies on the different pharmacological activities of *Vitex negundo*, not much work has been done on inter-relationship of lipid and haematological profile. Therefore the present study is aimed to investigate the impact of leaf extract of *Vitex negundo* on lipid and haematological indices on mammal animal model.

## 2. Materials and Methods

### 2.1 *Collection of Plant materials*

the fresh tender leaves of *Vitex negundo* were collected, dried in shade under 28 ± 2ºC, for 6-7 days and then crushed into coarse powdery substance by using electric grinder. The coarse powdery substance was again dried in shade, sieved to get fine powder using the fine plastic sieve, which was then stored in air tight dark bottles until required [8].

Extract preparation

50 g of sieved powder was weighed accurately and subjected to extraction in Soxhlet apparatus at room temperature using ~350 mL distilled water. The extract obtained was filtered, concentrated in rotary flash evaporator and maintained at 45$^{o}$C, the percentage yield of extract were calculated and the dried extract was stored in air tight containers at room temperature for further studies[8].

Animals

Albino rats weighing about 175-200 g were used in the study. They were maintained under standard laboraty conditions at ambient temperature of 25 ± 2$^{o}$C and relative humidity at 50 ± 15%, with 12h dark-light cycle. Animals were fed with a commercial pellet diet and water *ad libitum*. The experiment was performed after prior approval of Ethics Committee of Ranchi University, Ranchi.

Experimental design

The animals were randomly assigned into three groups of six rats each as follows. Group 1 (control) was fed with 1mL of distilled water orally. Group 2 was fed with 250 mg/Kg body

weight of *Vitex negundo* leaf extract. Group 3 was fed with 500 mg/kg body weight of *Vitex negundo* orally.

2.2 *Sample collection*

By the end of each experiment, the rats were weighed, starved for 24h and sacrificed under chloroform anesthesia. 5 ml of blood was collected from each animal by cardiac puncture using sterile needle and syringe. Part of the blood sample was put into test tubes and allowed to clot for 30 mints before centrifuging at 800 g (Wisperfuge, 1384, Samson, Holland) for 5 minutes. The supernatant was used for lipid analysis. The remaining blood sample was put in EDTA tubes for haematological determinations.

Analytical procedure

Estimation of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides was done by cholesterol oxidase-phenol aminoantipyrine method [9]. The Haemoglobin (Hb) level was measured by the cyanomethaemoblobin method. The Red Blood Cell (RBC) and Reticulocyte counts were determined by visual method [10]. Packed cell volume (PCV) was measured using microhaematocrit method and total White Blood Cell (WBC) count was estimated by visual method **[11].** The RBC indices were calculated from the RBC count, Hb level and PCV estimation[10, 11]

## 3. Results and Discussion

The effect of the oral administration of aqueous extract of *Vitex negundo* leaves on some serum lipid indices is presented in Table 1. The extract showed significant ($p < 0.05$) decrease in serum total cholesterol concentration, while it had no effect on serum HDL cholesterol concentration at all doses administered when compared with control. However, the extract significantly decreased ($p < 0.05$) serum triglycerol concentration at dose of 250 mg/kg as well as at dose of 500mg/kg when compared with control.

The total cholesterol is strongly associated with the risk of coronary heart disease (CHD), and rising levels of cholesterol is expected to contribute to substantial increase in the overall burden of cardiovascular diseases [12]. Few years back it was reported [13]. That increase in cholesterol level leads to production and accumulation of β-amyloid, which gets deposited within neurons in brain of non-demented individuals with heart disease leading to Alzheimer's disease, and a decrease in the cholesterol level thus prevents the production of β-amyloid. Triglyceride level is a risk factor in cardiovascular disease [14]. Thus the reduction in serum

**Table 1.** Effect of aqueous leaf extract of Vitex negundo on lipid profile of albino rats

| Parameters | Group 1 | Group 2 | | Group 3 | |
|---|---|---|---|---|---|
| | Value | Value | % Diff from Group 1 | Value | % Diff from Group 1 |
| Total cholesterol (mg%) | 59.62 ± 0.29 | 61.17 ± 0.50$^{ns}$ | 0.015 | 52.30 ± 0.36$^{\#\#}$ | 0.073 |
| HDL cholesterol (mg%) | 32.26 ± 0.20 | 30.13 ± 0.05$^{\#\#}$ | 0.021 | 32.2 ± 0.12$^{ns}$ | 0.006 |
| LDL cholesterol (mg%) | 19.97 ± 0.004 | 20.78 ± 0.71$^{\#}$ | 0.008 | 20.06 ± 0.04$^{\#}$ | 0.001 |
| Triglycerides (mg%) | 116.42 ± 0.26 | 97.51 ± 0.25$^{\#\#}$ | 0.189 | 89.56 ± 0.26$^{\#\#}$ | 0.269 |

#p > 0.05, ##p < 0.05

**Table 2.** effect of aqueous leaf extract of Vitex negundo on haematological profile of albino rats

| Parameters | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| Total Leucocytes (µL) | 6.79 | 7.26# | 8.82# |
| Lymphocytes (%) | 32.88 | 34.53# | 33.43# |
| Total Rbc (µL) | 4.21 | 4.21ns | 4.59# |
| Haemoglobin (g/dL) | 11.6 | 11.6ns | 12.5# |
| Haematocrit (PCV) (%) | 39.03 | 39.16ns | 43.99# |
| Mean Corpuscular Volume (MCV) | 93.33 | 92.33## | 98.29# |
| Mean Corpuscular Haemoglobin (MCH) | 30.46 | 27.88# | 29.56# |
| Mean Corpuscular Haemoglobin Concentration (MCHC) | 31.04 | 29.55# | 31.66ns |
| Platelet (µL) | 338.93 | 344.66# | 351.626# |

#p > 0.05, ##p < 0.05

total cholesterol concentration effected by the extract is beneficial and may reduce the risk of cardiovascular and related diseases; since the agents that decrease the blood cholesterol level have been reported to reduce vascular resistance by improving endothelial function [7]. Similar results were reported by Yakabu and Afloyayan[5] and Abebayo *et al.* [7] in *Bougainvillea spectabilies* (leaves) and *Fadogia agrestis* (stem) extracts.

Assessment of haematological parameters can be used to determine the deleterious effect of extracts on the blood of the animal model, and can also be used to explain blood related functions of the plant extract [5]. The extract of *Vitex negundo* had significant effect on RBC, Hb, MCHC, PCV, MCV, neutrophils, basophils, monocytes, lymphocytes and eosinophil (table 2). The WBC level was significantly elevated (p >0.05) in the group treated with 500 mg/kg body weight of extract. The platelets also showed significant increase (p>0.05) in both the groups. The significant increase in the WBC level is due to reaction of rat's immune system towards the foreign substance (extract). Thrombocytopenia is a condition in which the blood platelets count falls below normal. The extract in both the groups, significantly increased the blood platelet count as compared to control. Thus the extract can be used in medicinal formulations to cure Thrombocytopenia like conditions.

*Vitex negundo* leaf extract showed positive haematological activities in rats and can be recommended to be used in medicinal formulations to cure atherosclerosis, anaemia and hypercholesterolemia and high-tryglyceride related cardiac diseases.

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