

Antihyperlipidemic and Haematological Effect of Triherbal Formulation on Triton1339-Induced Hyperlipidemic Rats

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Abstract

Hyperlipidemia is one of the principal factors underlying the risk for cardiovascular disease. Confirmatory evidence from animal, clinical, and epidemiological studies has clearly established the positive correlation. Allopathic antihyperlipidemic drugs are available but the side effects and contraindications. However, this study was designed to determine the haematological and antihyperlipidemic effect of ethanol leaf extract of triherbal formulation (*Mucuna pruriens*, *Phyllanthus amarus*, *Securidaca longipedunculata*) in wistar rat as a cheaper and readily available alternative. 46rats were grouped into normal, diseased, standard drug controls and test groups with seven rats each. quantitative phytochemical, haematological and antihyperlipidemic analysis were carried out according to standard methods. Results revealed presence of saponin, alkaloids, tannin, flavonoids, cardiac glycosides, phenolic with Total phenol exhibiting the highest values haematological results showed improving activities of the formulation on the erythrocytes, haemoglobins, lymphocytes and neutrophils, this confers good effect on haematological and immunological parameters especially at higher dose. In antihyperlipidemic activities, triherbal formulation significantly

($p \leq 0.05$) decrease the level of TC, TAG, LDL and improve upon HDL in both serum and liver homogenate effects thought to be via inhibition of cholesterol biosynthesis, absorption modification of lipogenic and lipolytic enzymes. Conclusively, the studies indicated triherbal formulation contain bioactive compounds with diverse biological activities such as immunomodulatory, inflammatory, haematological and lipid profile regulating activities.

Keywords: Antihyperlipidemic, Haematological, Triherbal, Formulation, Triton1339-, Induced Hyperlipidemic, Rats

INTRODUCTION

Hyperlipidemia is one of the principal factors underlying the risk for cardiovascular disease. Confirmatory evidence from animal, clinical, and epidemiological studies has clearly established the positive correlation of plasma cholesterol to atherosclerosis and its complications, mainly coronary heart disease (CHD) (Talekar *et al.*, 2017; Singh and Avupati, 2017). Although, the predisposition to hyperlipidemia may be purely genetic in association with other influences approximately 50 per cent (Bhinge *et al.*, 2017; Shan *et al.*, 2020). Blood is a very important specialized body fluid that has many functions such as transporting oxygen, nutrients, waste products, antibodies and regulating body homeostasis (ASH, 2021; Qiu *et al.*, 2014; Szklanna, *et al.*, 2021). Full Blood Count (FBC) test is a simple blood test that can provide valuable information about a person's health. It can be used to diagnose conditions such as anemia, leukocytosis, and thrombocytopenia, infection, immunological status, and it can help guide treatment decisions (Neuen *et al.*, 2016; Roy and Pavord, 2018). It uses the coulter principle which states “that particles passing through an orifice (along with an electrical current) will produce an increase in impedance, due to displacement of electrolytes caused by the presence of the particle” (Majoni *et al.*, 2020). This change in impedance is proportional to the volume of the particle (Gilde *et al.*, 2021) and light scattering, fluorescence principles (Adeyemi *et al.*, 2015; Qiu *et al.*, 2014).

Herbal combination have been employed in traditional Ayurvedic, Chinese, Unani, and Peruvian practices for millennia in the management of numerous ailments. This concept Results in a better therapeutic effects with reduced toxicity (Parasuraman *et al.*, 2014). It is also important to establish the active components of these herbal extracts (Ahmed *et al.*,

2013; Dev *et al.*, 2019). The use of a herbal formulation could affect the results of an FBC test either by increasing or decreasing values of red blood cells, white blood cells, or platelets (Tietge, 2014), having beneficial or negative effects on health by altering blood compositions (Talekar *et al.*, 2017; Singh and Avupati, 2017).

Allopathic antihyperlipidemic formulations are available at large in the market, but the side effects and contraindications of these drugs could be deleterious to the body (Joshi *et al.*, 2020).

Therapeutic application of herbal formulation could provide a relatively cheaper alternative to synthetic medicines. Herbal medicines are apparently considered relatively safer and readily available compared to conventional therapy. However, these Problems informed this study designed to combine *Mucuna pruriens*, *Phyllanthus amarus*, *Securidaca longipedunculata* in triherbal formulation and to determine the anti hyperlipidemic effect of herbal mixture in wistar rats.

METHODOLOGY

Quantitative determination of secondary metabolites (Tannins, Phenols, Saponnins, Flavonoid Alkaloids) was carried out according to the methods described by AOAC (1995) Ushie *et al.*, (2019).

Hematological parameters were determined using DYMIN D automated haematological analyzer (DYMIN D DH76 BIOTECH, China). method described (Allexander and Griffiths 1993)

Determination of Cholesterol, Triglyceride, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL): were determined using Randox kit according to the protocol as described by (Tietz, 1986).

Data Analysis

Analysis of variance (ANOVA) of statistical Package for the social Sciences (SPSS) version 26.0 will be used. Results of different treatment groups will be expressed as mean $\pm$ Standard deviation. The difference between various treatments groups will be compare using the Duncan Multiple Range Test. P values less that 0.05 ($p < 0.05$) were taken as significant. Results will be presented in Tables and charts.

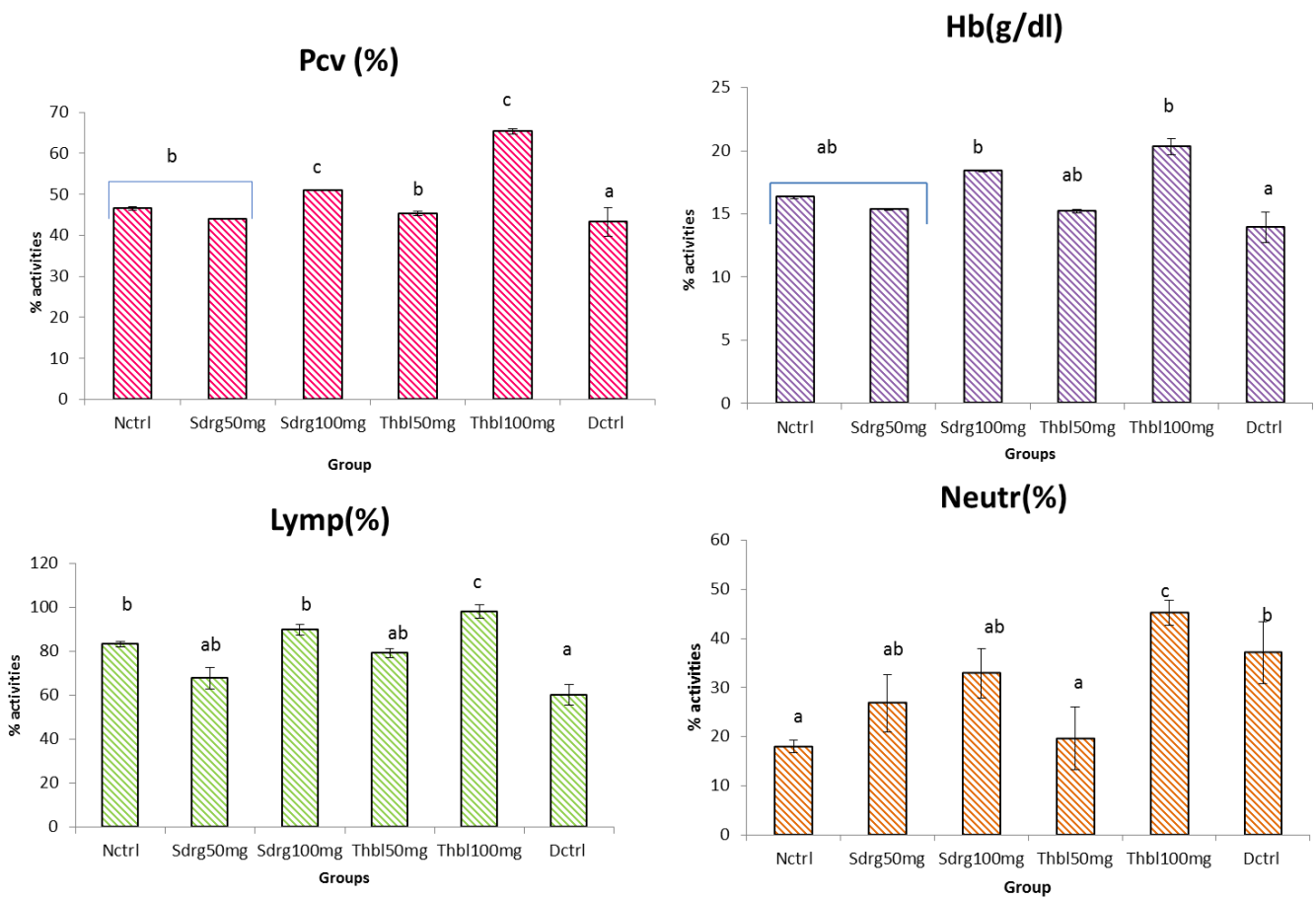
RESULTS

Quantitative phytochemical result of Triherbal formulation revealed presence of saponin, alkaloids, tannin, flavonoids, cardiac glycosides, phenolic with Total phenol exhibiting the highest values (111.67 ± 0.23 mg/GAE/g) as shown on the table below

Table 3.1: Phytochemical composition of Triherbal formulation

PHYTOCHEMICALS	QUANTITIES
Alkaloids (%)	8.75 ± 0.35
Cardiac glycoside(mg/sec/g)	2.40 ± 0.00
Tannins(mg/GAE/g)	92.50 ± 0.14
Sapponin(mg/DE/g)	5.88 ± 0.00
Total Phenol(mg/GAE/g)	111.67 ± 0.23
Flavonoids(mg/QE/g)	20.06 ± 0.08

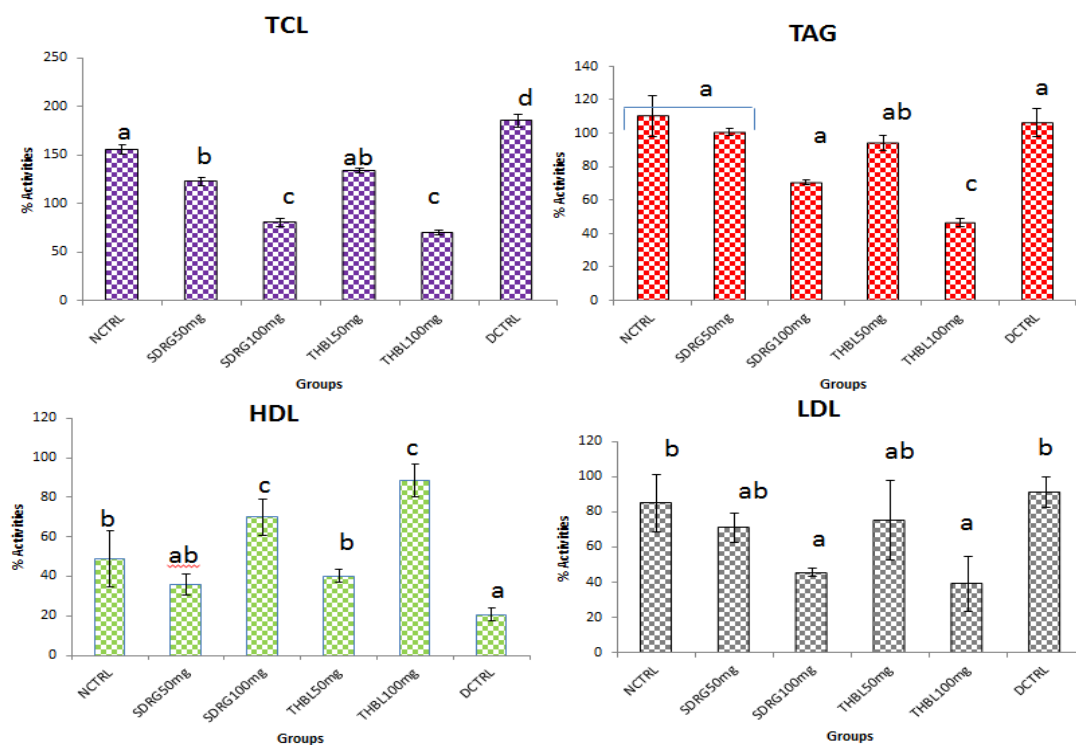
Triherbal formulation indicates improving activities on the erythrocyte, heamoglobin, lymphocytes and neutrophiles, this confer good effect on some immonological and no negative anemic effect on the heamatological parameters but rather inprove level of blood especially at higher dose.



NCTRL: Normal Control; SDRG: Standard Drug Control; THBL: Triherbal Formulation;
DCTRL: Diseased Control

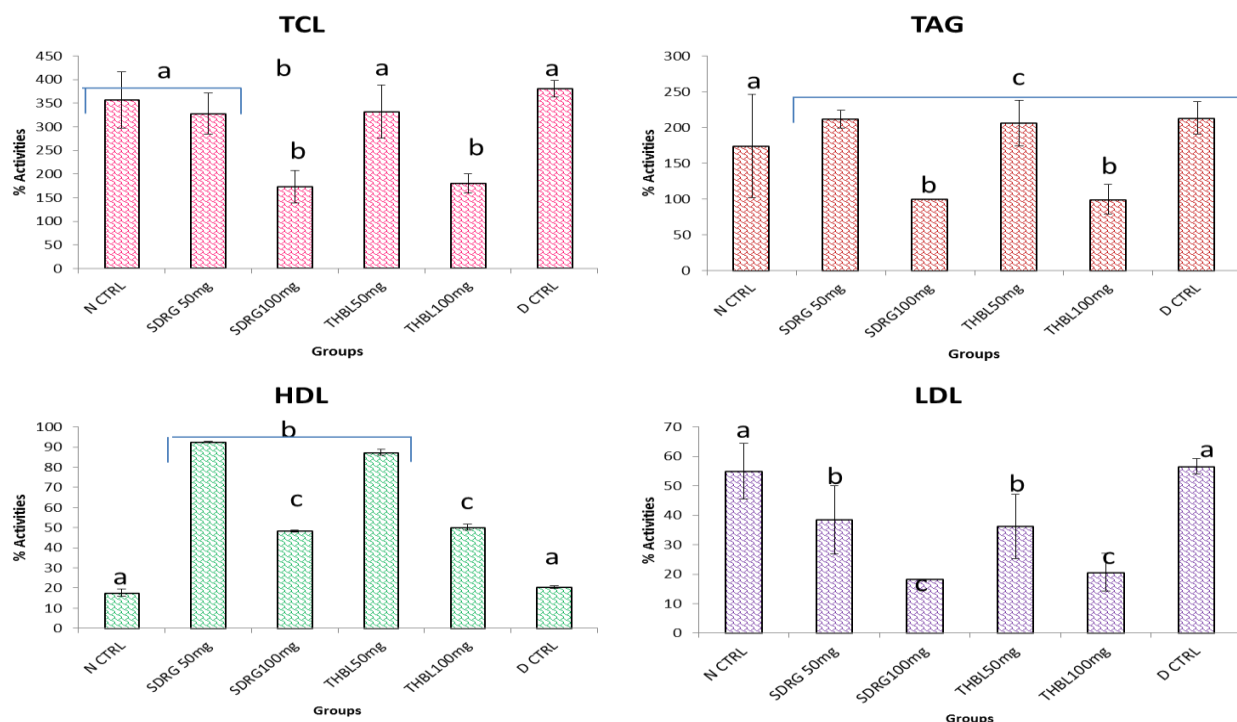
Figure. Effect of Triherbal combinations on haematological parameters

In antihyperlipidemic effect, triherbal formulation significantly ($p \leq 0.05$) decrease the level of TC, TAG, LDL and improve upon HDL in both serum and liver homogenate via inhibition of cholesterol biosynthesis, absorption modification of lipogenic and lipolytic enzymes as shown in the figure below



NCTRL: Normal Control; SDRG: Standard Drug Control; THBL: Triherbal Formulation;
DCTRL: Diseased Control

Figure: Effect of Triherbal formulation on serum Lipid profile



NCTRL: Normal Control; SDRG: Standard Drug Control; THBL: Triherbal Formulation; DCTRL: Diseased Control

Figure: Effect of Triherbal formulation on Liver homogenate lipid profile

RESULTS AND DISCUSSION

Triherbal formulation indicates improving activities on the erythrocyte, hemoglobin, lymphocytes and neutrophils, this confer good effect on some immunological and no negative anemic effect on the hematological parameters but rather improve level of blood especially at higher dose suggestive of the formulation activities in line with the Patel *et al.*, (2010) who say Treatments of Sudarshanam oil at all the doses shows normal hematology (RBC, WBC, HCT, MCV, MCH, MCHC, hemoglobin etc.) as compared to control group. Immune activation of immunomodulatory activities of Triphala. Also similar to the conclusion of Udom *et al* (2023), which says polyherbal mixture may contain intrinsic biological actives responsible for stimulating haematopoietins (erythropoietin, thrombopoietin) synthesis and/or release.

In antihyperlipidemic effect, triherbal formulation significantly ($p \leq 0.05$) decrease the level of TC, TAG, LDL and improve upon HDL in both serum and liver homogenate via

inhibition of cholesterol biosynthesis, absorption modification of lypogenic and lipolitic enzymes as seen in this work, It is believed that if levels of lipids, especially LDL-cholesterol and triglycerides, are controlled, it can markedly prevent many chronic inflammatory and coronary diseases (Lu *et al.*, 2012. These results are generally in line with the results from previous studies that showed improvements in TC and LDL cholesterol concentration with respect to consumption of these herbs (Elmahdi *et al.*, 2008; Lu *et al.*, 2012; Mukthamba and Srinivasan. 2016; Sil *et al.*, 2020)

A study of Nazir and. Chauhan (2019). reported that phytochemical constituents (steroids, flavonoids, saponins) possess lipid lowering activity. The steroids reduce the absorption of cholesterol and thus increase its faecal excretion, while saponins cause a reduction in blood cholesterol by binding with the cholesterol in the intestinal lumen, so that cholesterol is less readily absorbed [Singh and Kumar. 2017].

CONCLUSION

In conclusion, the studies indicated that triherbal formulation of ethanolic leaf extract (*Mucuna pruriens* (velvet beans), *Phyllanthus amarus* (stone breaker), *Securidaca longipedunculata* (violet tree) contain bioactive compounds such as alkaloids, flavonoids and polyphenols, tannin, saponnin and cardiacglycoside that exhibit diverse biological activities, through multiple mechanisms of action such as inflammatory, immunomodulatory activities and hepatocytes regeneration by improving activities on the erythrocyte, heamoglobin, lymphocytes and neutrophiles, indicating good effect on some immonological on the heamatological parameters especially at higher dose.

REFERENCES

- Ahmed, M., & Hussain, F. (2013). Chemical composition and biochemical activity of Aloe vera (*Aloe barbadensis* Miller) leaves. *International Journal of chemical and biochemical sciences*, 3(5), 29-33.
- Akter, R., Khan, S. S., Kabir, M. T., & Halder, S. (2022). GC-MS-employed phytochemical characterization, synergistic antioxidant, and cytotoxic potential of Triphala methanol extract at non-equivalent ratios of its constituents. *Saudi Journal of Biological Sciences*, 29(6), 103287.
- AOAC (1995). Official methods of analysis (16th edn) Association of Official Analytical Chemists. Arlington, V. A. USA

- Bhinge, S. D., Bhutkar, M. A., Randive, D. S., Wadkar, G. H., Todkar, S. S., Kakade, P. M., & Kadam, P. M. (2017, September). Formulation development and evaluation of antimicrobial polyherbal gel. In *Annales pharmaceutiques francaises* (Vol. 75, No. 5, pp. 349-358). Elsevier Masson.
- Dev, B., Babu, K. S., Denton, P., Machado, P., Argüelles, C. A., Barrow, J. L., ... & Xu, X. J. (2019). Neutrino non-standard interactions: A status report. *SciPost Physics Proceedings*, (2), 001.
- Elmahdi B, Khalil MM, Abulgasim AI. 2008. The Effect of Fresh Crushed Garlic Bulbs (*Allium sativum*) on Plasma Lipids in Hypercholesterolemic Rats. *J Animal Vet Sci*;3:15–19.
- Joshi, H., Parle, M., 2007. Pharmacological evidences for anti-amnesic potentials of *Phyllanthus amarus* in mice. *African Journal of Biomedical Research* 10, 165–173.
- Lu Y, He Z, Shen X, Xu X, Fan J, Wu S, Zhang D. 2012; Cholesterol-lowering effect of allicin on hypercholesterolemic ICR mice. *Oxidative Med Cellular Longevity*;2012:1–6.
- Mukthamba P, Srinivasan K. 2016. Protective effect of dietary fenugreek (*Trigonella foenum-graecum*) seeds and garlic (*Allium sativum*) on induced oxidation of low-density lipoprotein in rats. *J Basic Clin Physiol Pharmacol*;27(1):39–47.
- Nazir I. Chauhan RS. 2019. Qualitative phytochemical analysis of *Allium sativum* (Garlic) and *Curcuma longa* (Turmeric). *J Entomol Zoology Studies*;7(1):545–547.
- Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK. *Phyllanthus amarus*: ethnomedicinal uses, phytochemistry and pharmacology: a review. *J Ethnopharmacol*. 2011 Nov 18;138(2):286-313. doi: 10.1016/j.jep.2011.09.040. Epub 2011 Sep 29. PMID: 21982793.
- Patel, vandana D. Modi, bhaswat S. Chakraborty, neeta mathuria*, paresh dadhaniya, prasad A. Borade and Pravin Morankar (2010) Effect of a novel anti-inflammatory polyherbal preparation (sudarshanam oil) on hematological parameters in wistar rats. *Drug Research*, Vol. 67 No. 3 pp. 277–281, ISSN 0001-6837
- Sil S, Roy UK, Biswas S, Mandal P, Pal K. 2020. A Study To Compare Hypolipidemic Effects Of *Allium Sativum* (Garlic) alone and in combination with Atorvastatin Or Ezetimibe in experimental model. *Ser J Exp Clin Res*;22(1):11–19.
- Singh , Vandana and Kumar, Ramesh. 2017; Study of Phytochemical Analysis and Antioxidant Activity of *Allium sativum* of Bundelkhand Region. *Int J Life Sci Scienti Res*;3(6):1451–1458.
- Singh, A., & Avupati, V. R. (2017). Development and validation of UV-spectrophotometric method for the estimation of curcumin in standardised polyherbal formulations. *Journal of Young Pharmacists*, 9(4), 491.
- Talekar, S., Patti, A. F., Vijayraghavan, R., & Arora, A. (2018). An integrated green biorefinery approach towards simultaneous recovery of pectin and polyphenols coupled with bioethanol production from waste pomegranate peels. *Bioresource technology*, 266, 322-334.
- Udom G.J., Ise U.P., Aturamu A, Omoirri M.A., Ogbonnaya M, Umana I.K., Okokon J.E. (2023). Toxicity assessment of a polyherbal drug on haematological parameters,

- brain and spleen histoarchitecture in exposed Wistar rats. *Physiology and Pharmacology*; 27: 261-270. <http://dx.doi.org/10.61186/phypha.27.3.261>
- Umaru, I. J., Fasihuddin, B. A., Otitoju, O. O., & Hauwa, A. U. (2018). Phytochemical evaluation and antioxidant properties of three medicinal plants extracts. *Medicinal & Analytical Chemistry International Journal*, 2(2), 1-8.
- Yakubu, O. E., Abu, M. S., Akighir, J., Onuche, J. I., & Arabi, A. (2021). Comparative determination of total antioxidant effects of ethanol extract of *Phyllanthus amarus* leaves. *Asian Journal of Natural Product Biochemistry*, 19(2).
- Yakubu, O. E., Ezeonu, C. S., Agyo, D. A., Jonah, T., & Ale, E. M. (2024). Methanolic stem-bark extracts of *Adansonia digitata* modulates haematological and antioxidant parameters in streptozotocin-induced diabetic rats. *Scientific African*, 26, e02419.