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## Atherogenic indices demonstrate the significant antilipidaemic and haematinic potentials of aqueous seed extracts from *Syzygium aromaticum*

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**Abstract**

The atherogenic indices of antilipidaemic and haematinic potentials of aqueous seed extract of *Syzygium aromaticum* were studied using Wistar rats. Seventy male rats weighing 70-75 g were grouped into seven of seven rats each. Group 1 (NFC1) served as normal control and were fed with rat-chow and water only. Groups 2-7 (NHFD2, HFD3, HFD4, HFD5, HFD6, HFD7) were fed with a high-fat-diet (HFD) composed of commercial rat-chow, butter, sugar and flour for 28 days to establish hyperlipidemia before treatment. A baseline lipogram test was carried out to evaluate the hyperlipidaemic status of experimental animals. NHFD2 did not receive any treatment and served as negative control. HFD3, HFD4, HFD5, HFD6 and HFD7 were treated with 500, 1000, 1500, 2000, 2500 mgkg<sup>-1</sup> bodyweight aqueous seed extract of *S. aromaticum* respectively for 28 days. Haematology indices, liver function tests and lipid profile were investigated. Also, atherogenic indices were calculated. Baseline results showed an increase in lipid profile except for reduced high-density lipoprotein (HDL). Results from HFD3, HFD4, HFD5, HFD6 and HFD7 showed a significant increase ( $p < 0.05$ ) in HDL ( $89.37 \pm 1.49$  mmol/L), packed cell volume ( $39.00 \pm 1.00\%$ ), haemoglobin ( $12.74 \pm 0.32$  g/L), red blood cell count ( $6.73 \pm 0.55 \times 10^9$ /L) with a significant decrease ( $p > 0.05$ ) in plasma cholesterol ( $123.83 \pm 2.85$  mmol/L), triglyceride ( $135.76 \pm 8.89$  mmol/L), low density lipoprotein ( $19.32 \pm 0.49$  mmol/L), when compared to NHFD2. Atherogenic index of plasma and Castelli Risk Indices (CRI-I and CRI-II) showed that NHFD2 has elevated index while HFD3, HFD4, HFD5, HFD6 and HFD7 groups have significantly reduced indices when compared to NHFD2. This implies that SAE treatment produced significant reductions, especially at 2500 mg/kg ( $p < 0.05$ ), indicating improved lipid balance. In conclusion, the aqueous extract of *S. aromaticum* at 2500 mgkg<sup>-1</sup>, showed ameliorative effect on the lipid profile, haematological indices and the liver enzymes indicating its potential for the management of anaemia, hyperlipidaemia and cardiovascular related ailments.

**Keywords:** High-fat-diet, hyperlipidaemia, Atherogenic index, Castelli risk index, haematology, *Syzygium aromaticum*

**Introduction**

The increasing prevalence of metabolic and haematological disorders across the globe has necessitated the search for effective, safe, and affordable therapeutic agents. Among these disorders, hyperlipidaemia and anaemia stand out as significant contributors to global morbidity and mortality. Hyperlipidaemia, defined as an abnormal elevation of lipids in the blood, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and a concomitant reduction in high-density lipoprotein cholesterol (HDL-C), plays a pivotal role in the pathogenesis of cardiovascular diseases (CVDs) such as atherosclerosis, coronary heart disease, and stroke (Hill & Bordoni, 2023; Netala *et al.*, 2024; Wan *et al.*, 2025) [17, 25, 35]. These are leading causes of morbidity and mortality globally, with dyslipidemia being a primary modifiable risk factor. Dyslipidemia is characterized by abnormal levels of lipids and lipoproteins in the blood, including elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and triglycerides (TG), as well as reduced high-density lipoprotein cholesterol (HDL-c) (Kumar *et al.*, 2020) [20]. These lipid imbalances contribute to the development of atherosclerosis, a process where plaque builds up inside the arteries, leading to narrowed and hardened vessels (Dybiec *et al.*, 2023; Abera *et al.*, 2024) [11, 3].

Atherogenic indices, such as the Castelli's Risk Index-I (CRI-I: TC/HDL-c), Castelli's Risk Index-II (CRI-II: LDL-c/HDL-c), Atherogenic Index of Plasma (AIP:  $\log[\text{TG}/\text{HDL-c}]$ ), and Atherogenic Coefficient (AC:  $[\text{TC}-\text{HDL-c}]/\text{HDL-c}$ ), are valuable tools for assessing an individual's risk of developing atherosclerosis and subsequent CVDs (Dobiášová & Frohlich, 2001; Frohlich & Dobiášová, 2004) [10, 15]. These indices integrate multiple lipid parameters into a single value, providing a more comprehensive prediction of cardiovascular risk than individual lipid markers alone.

On the other hand, anaemia, particularly iron-deficiency anaemia, affects over 1.6 billion people worldwide, impairing oxygen transport, causing fatigue, reducing productivity, and negatively impacting cognitive function, especially in vulnerable populations such as children and pregnant women (World Health Organization, 2021).

Current pharmacological interventions for hyperlipidaemia, including statins, fibrates, and bile acid sequestrants, while effective, are often associated with adverse side effects such as hepatotoxicity, muscle pain, gastrointestinal disturbances, and high cost, making long-term compliance difficult (Abbasi *et al.*, 2024; Feng-Huang *et al.*, 2025) [1, 13]. Similarly, conventional haematinics, including iron salts and erythropoiesis-stimulating agents, can induce gastrointestinal discomfort, constipation, and oxidative stress (Koury & Ponka, 2004) [19]. These limitations underscore the urgent need to explore alternative therapies derived from medicinal plants, which are widely perceived to be more accessible, cost-effective, and less toxic (Asafo-Agyei *et al.*, 2023; Frimpong *et al.*, 2024) [5, 14].

Medicinal plants have historically played a vital role in traditional medicine and continue to contribute significantly to modern drug development. The search for effective and safer antilipidaemic agents has led to increased interest in medicinal plants. *Syzygium aromaticum*, commonly known as cloves, is a spice derived from the flower buds of the clove tree, native to the Maluku Islands of Indonesia and widely cultivated in tropical and subtropical regions. It has been used for centuries in traditional medicine for its various therapeutic properties, including anti-inflammatory, antioxidant properties, antidiabetic, and antimicrobial effects (Cortés-Rojas *et al.*, 2014; Pandey *et al.*, 2024; Abdelmuhsin *et al.*, 2025; Valarezo *et al.*, 2025) [7, 29, 2, 34].

Previous studies have demonstrated the potential of *S. aromaticum* extracts in modulating lipid profiles. For instance, some research has shown that clove extracts can lower TC and TG levels while increasing HDL-c (Cortés-Rojas *et al.*, 2014) [7]. However, the specific effects of aqueous seed extracts of *S. aromaticum* on atherogenic indices and their potential to improve hematological parameters (haematinic potential) are not extensively documented. Anaemia, a condition characterized by a deficiency of red blood cells or hemoglobin, is often associated with chronic diseases, including dyslipidemia, and its correction is crucial for overall health (Weiss & Goodnough, 2005) [36].

Phytochemical investigations of *S. aromaticum* have revealed a rich composition of bioactive compounds, including eugenol (its principal constituent), flavonoids, tannins, alkaloids, saponins, phenolic acids, sterols, and essential oils (Rabizadeh *et al.*, 2022; Mary *et al.*, 2025) [31, 24]. These constituents are known to exhibit a wide range of biological activities. Eugenol, for example, has been

extensively studied for its hypolipidaemic, antioxidant, and anti-inflammatory effects (Damasceno *et al.*, 2024; Silva *et al.*, 2024) [9, 33]. Flavonoids and phenolic compounds, on the other hand, contribute to free radical scavenging, modulation of enzyme activity, and enhancement of haematopoiesis (Chaieb *et al.*, 2007; Marvilliers *et al.*, 2020; Javed, 2023) [6, 23, 18].

While most pharmacological studies on clove have focused on its flower buds and essential oils, there is a notable paucity of information regarding the pharmacological efficacy of its seeds. The seeds, although less studied, may contain unique or complementary phytochemicals capable of modulating lipid profiles and enhancing haematological parameters. This study presents a novel area of investigation, particularly in understanding the antilipidaemic (lipid-lowering) and haematinic (blood-forming) potentials of the aqueous seed extract of *S. aromaticum*.

Given the dual burden of hyperlipidaemia and anaemia in many populations, especially in low-resource settings where reliance on plant-based remedies is high, the current study aims to investigate the antilipidaemic and haematinic potentials of aqueous seed extracts of *S. aromaticum* using appropriate *in vivo* experimental models. This research not only seeks to validate the ethnopharmacological claims associated with clove seeds but also to contribute scientific evidence to support their potential integration into complementary or alternative therapeutic strategies. Therefore, this study aims to investigate the antilipidaemic and haematinic potentials of aqueous seed extracts from *S. aromaticum*. By analyzing changes in key atherogenic indices and hematological parameters, this research will provide a more detailed understanding of the therapeutic efficacy of *S. aromaticum* seeds in managing dyslipidemia and related cardiovascular risks. The findings could pave the way for the development of a natural, plant-based therapeutic agent for the prevention and treatment of cardiovascular diseases.

## Materials and Methods

### Preparation of the Extracts

Dried seeds of *S. aromaticum*, purchased from Oil Mill market in Port Harcourt, Rivers State, Nigeria, were properly washed with distilled water, air dried under room temperature for 3 weeks in the absence of moist and dust. They were ground and pulverized to coarse powder using an electric blender. A 100 ml of Distilled water was added to each 10g portion of the blended seed. The Extract was allowed to ferment for 3 days before it was vacuum-filtered through Whatman No 1 filter paper and concentrated using a vacuum rotary evaporator (Eyla N-1000, Japan). The extract was stored in an air-tight container.

### Procurement of Experimental Animals

Seventy (70) male Wistar rats 70-75 g were purchased from the animal house of the Department of Biochemistry, University of Port Harcourt Choba, Rivers State. The rats were weighed and divided into seven (7) groups of ten (10) each and were housed differently in standard cages. The wistar rats were housed according to the laboratory conditions (12 hours light; dark cycle,  $28 \pm 2$  °C) and acclimatized for 2 weeks with free access to standard rat chow and water *ad libitum* before the study began. All experiments were carried out in strict compliance with

internationally accepted principles for laboratory animal use and care in accordance with the Canadian Council on Animal Care Guidelines and Protocol Reviews.

Experimental protocol

After the period of acclimatization during which all the rats were fed with normal rat chow and water *ad libitum* via oral gavage on a daily basis, they were grouped into seven of ten rats each (n=10). Group 1 (NFC1) served as normal control and were fed with rat-chow and water only. Groups 2-7 (NHFD2, HFD3, HFD4, HFD5, HFD6, HFD7) were fed with a high-fat-diet composed of commercial rat-chow, butter, sugar and flour (20% sucrose + 20% butter + 60% grower mash) for four weeks to establish hyperlipidemia before treatment. A baseline lipogram test was carried out to evaluate the hyperlipidaemic status of experimental animals. NHFD2 did not receive any treatment and served as negative control. HFD3, HFD4, HFD5, HFD6 and HFD7 were treated with 500, 1000, 1500, 2000, 2500 mgkg<sup>-1</sup> bodyweight of the aqueous seed extract of *S. aromaticum* (SAE) respectively for 28 days.

Experimental design

The Seventy Wistar rats were divided according to their body weight into seven (7) groups of ten (10) rats each as follows:

Table 1: Experimental design for haematinic and anti-lipidaemic screening

Experimental Groups (n=10)	Treatment Plan
NFC1	Normal feed + water
NHFD2	High-fat-diet (HFD)
HFD3	HFD + 500 mgkg <sup>-1</sup> body weight SAE
HFD4	HFD + 1000 mgkg <sup>-1</sup> body weight SAE
HFD5	HFD + 1500 mgkg <sup>-1</sup> body weight SAE
HFD6	HFD + 2000 mgkg <sup>-1</sup> body weight SAE
HFD7	HFD + 2500 mgkg <sup>-1</sup> body weight SAE

Determination of haematology and blood biochemical indices

The animals from all the groups were sacrificed at the end of the treatment. They were anaesthetized with chloroform. The animals were sacrificed by severing the jugular vein with a surgical blade. Blood was allowed to flow freely and

was collected and put into ethylene diamine tetraacetic acid (EDTA) bottles for haematological studies and Lithium Heparin bottles for the lipid profile analysis. Component of haematological parameter such packed cell volume (PCV), red blood cell count (RBC), was determined using haematocrit method; white blood cell count (WBC) was estimated using haemocytometer. Differential white cell count was determined using the method previously described by Osim *et al.*, 2004 [28].

Based on the results of the lipid profile, the following Atherogenic Indices and Castelli's risk index were calculated: Atherogenic Index of Plasma (AIP) is calculated as the logarithmic transformation of the triglyceride to HDL-cholesterol ratio (log10 [TG/HDL-C]), while Atherogenic Index (AI), also known as the atherogenic coefficient is the ratio of non-HDL cholesterol to HDL-cholesterol (non-HDL-C/HDL-C) (Sastre-Alzamora *et al.*, 2024) [32]. Castelli's risk index I (CRI-I) is the ratio of the total cholesterol to HDL cholesterol while Castelli's risk index II (CRI-II) is the ratio of the LDL cholesterol to HDL cholesterol (Raaj *et al.*, 2024) [30].

Statistical Analysis of Results

Data obtained from this study were analyzed using Statistical Product and Service Solution (SPSS) for window version 23.0 USA. Descriptive statistics was done by one way analysis of variance (ANOVA) and multiple comparisons of the mean values were done using Turkey Post hoc at 95% (p<0.05) confidence level. All data obtained from this study are represented as means ± standard deviation (M±SD).

Results

Effect of the aqueous extracts of the seeds of *S. aromaticum* on average weekly weight gains and total weight gains of Wistar rats

The effect of SAE on average weekly weight gains and total weight gains of wistar rats are presented in Figure 1. The result revealed a weekly and total weight gain across all groups in the study. However, HFD7 group which was administered 2500 mgkg<sup>-1</sup> body weight of SAE were observed to have the highest average weight gain (129.16±6.68 g) when compared to the other experimental groups while NFC1 had the least average weight gain (71.68±20.84 g) at the end of the study.

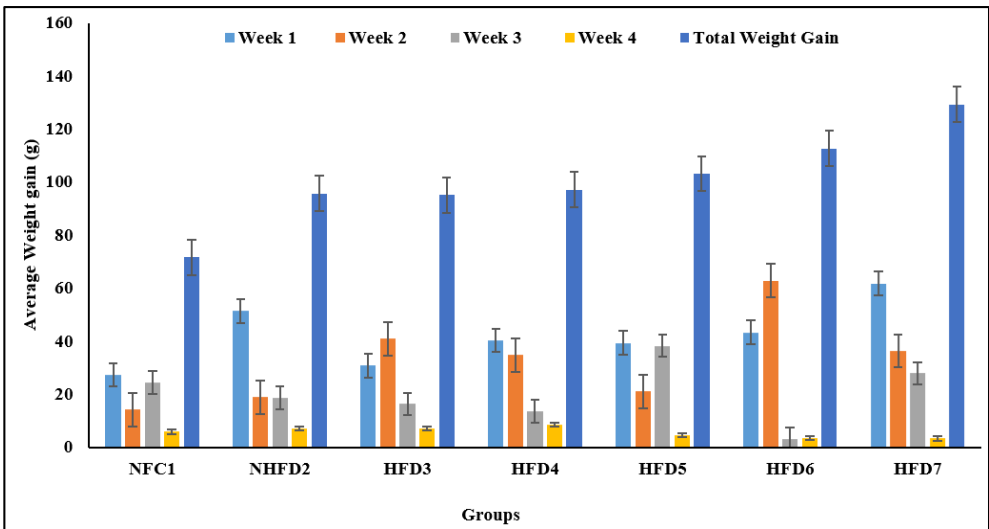


Fig 1: Effect of the aqueous extracts of the seeds of *S. aromaticum* (clove) on weekly weight gains and overall weight gains of wistar rats

### Effect of the aqueous extracts of the seeds of *S. aromaticum* on liver enzymes of wistar rats

Serum levels of ALT, AST, and ALP were significantly ( $p < 0.05$ ) elevated in the NHFD2 group relative to NFC1,

reflecting hepatic stress and injury. SAE administration produced a dose-dependent reduction in these enzyme levels, with the highest dose restoring values nearly to control levels (Figure 2).

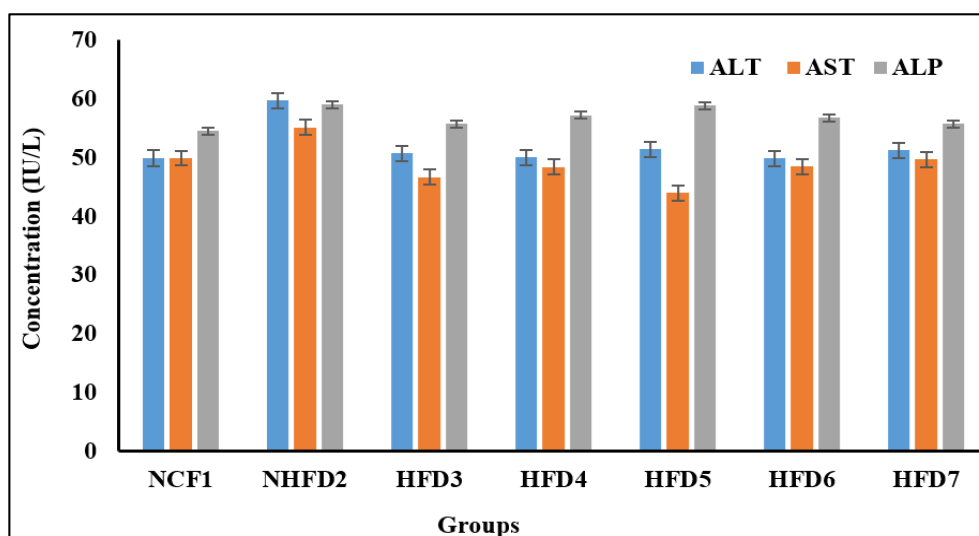


Fig 2: effect of the aqueous extracts of the seeds of *S. aromaticum* (clove) on hepatic enzymes of wistar rats

### Effect of aqueous extracts of the seeds of *S. aromaticum* on lipid profile of wistar rats.

The effect of SAE on lipid profile levels of wistar rats are presented in Figure 3. The result revealed a significant decrease ( $p < 0.05$ ) in the total cholesterol, triglyceride and low-density lipoprotein levels of NFC1, HFD3, HFD4,

HFD5, HFD6 and HFD7 groups when compared to that of NHFD2. Conversely, the high-density lipoprotein levels of NFC1, HFD3, HFD4, HFD5, HFD6 and HFD7 groups were significantly increased ( $p > 0.05$ ) when compared to that of NHFD2.

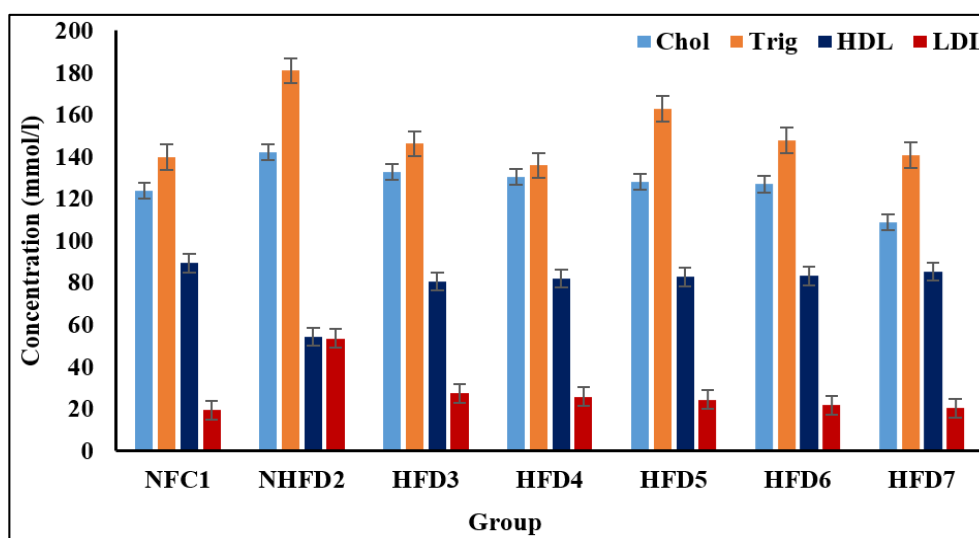


Figure 3: Effect of aqueous extracts of the seeds of *S. aromaticum* on lipid profile of wistar rats.

### Effect of aqueous extracts of the seeds of *S. aromaticum* on haematological indices of wistar rats

The effect of SAE on hematological indices of wistar rats are presented in Table 2. The result showed that there was a significant increase ( $p > 0.05$ ) in packed cell volume, hemoglobin concentration and red blood cell count of HFD3, HFD4, HFD5, HFD6 and HFD7 groups when compared to that of NHFD2. Conversely, there was a significant decrease ( $p < 0.05$ ) in white blood cell count, platelet levels and mean cell hemoglobin count of HFD3, HFD4, HFD5, HFD6 and HFD7 groups when compared to that of NHFD2. Also, results on blood differential counts presented in Table 3 revealed that SAE produced a

significant reduction ( $p < 0.05$ ) in the blood differential count (neutrophils, leukocytes, eosinophil and monocytes) in HFD3, HFD4, HFD5, HFD6 and HFD7 groups when compared to that of NHFD2.

### Atherogenic Index of Plasma, Castelli Risk Index I and II calculated

Atherogenic index of plasma calculated showed that NFC1 and NHFD2 groups have an AIP of 0.194 and 0.524 respectively while HFD3, HFD4, HFD5, HFD6 and HFD7 groups have values which range from 0.218 to 0.295 and is significantly lower ( $p < 0.05$ ) when compared to that of NHFD2. The HFD group displayed significantly higher



levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), alongside a reduction in high-density lipoprotein cholesterol (HDL-C) compared with NFC. SAE treatment significantly reversed these changes, reducing TC, TG, and LDL-C, while improving HDL-C levels, particularly at 2500 mg/kg ( $p<0.05$ ) (Figure 4). CRI-I was markedly elevated in the

NHFD2 group compared with NFC1. SAE significantly ( $p<0.05$ ) reduced CRI-I across all treated groups (Figure 5). CRI-II followed a similar pattern, showing significant elevation in NHFD2 rats compared to NFC1. SAE treatment produced significant reductions, especially at 2500 mg/kg ( $p<0.05$ ), indicating improved lipid balance (Figure 6).

**Table 2:** Effect of aqueous extracts of the seeds of *S. aromaticum* on hematological indices of wistar rats

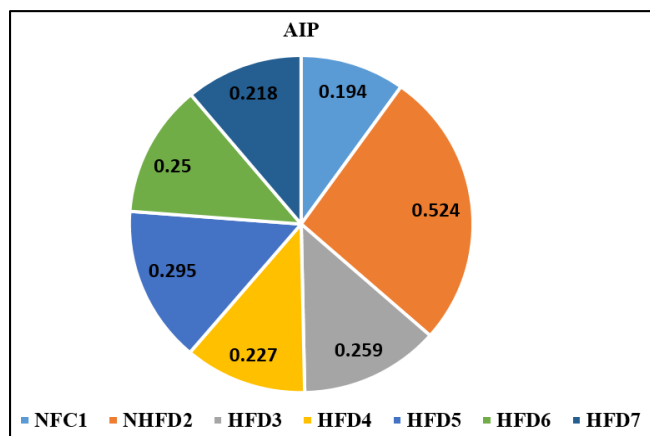
Experimental groups	PCV (%)	HB (g/L)	RBC ( $\times 10^9/L$ )	WBC ( $\times 10^9/L$ )	PLT ( $\times 10^9/L$ )	MCHC (g/dl)	MCH (pg)	MCV (fL)
NFC1	39.00 $\pm$ 1.00 <sup>b</sup>	12.74 $\pm$ 0.32 <sup>b</sup>	6.62 $\pm$ 0.17 <sup>b</sup>	3.23 $\pm$ 0.21 <sup>a</sup>	462.00 $\pm$ 14.11 <sup>b</sup>	32.03 $\pm$ 0.81 <sup>a</sup>	9.37 $\pm$ 0.50 <sup>a</sup>	57.80 $\pm$ 1.51 <sup>a</sup>
NHFD2	27.00 $\pm$ 1.00 <sup>a</sup>	4.70 $\pm$ 1.56 <sup>a</sup>	1.07 $\pm$ 1.04 <sup>a</sup>	16.37 $\pm$ 3.43 <sup>c</sup>	746.67 $\pm$ 6.43 <sup>c</sup>	30.37 $\pm$ 2.37 <sup>a</sup>	17.73 $\pm$ 1.58 <sup>b</sup>	50.00 $\pm$ 7.46 <sup>a</sup>
HFD3	33.33 $\pm$ 7.31 <sup>b</sup>	9.13 $\pm$ 0.70 <sup>b</sup>	6.67 $\pm$ 0.47 <sup>b</sup>	6.43 $\pm$ 1.86 <sup>b</sup>	466.33 $\pm$ 9.09 <sup>b</sup>	33.93 $\pm$ 1.36 <sup>a</sup>	9.63 $\pm$ 0.91 <sup>a</sup>	58.70 $\pm$ 3.35 <sup>a</sup>
HFD4	32.00 $\pm$ 1.73 <sup>b</sup>	10.50 $\pm$ 0.56 <sup>b</sup>	6.07 $\pm$ 0.85 <sup>b</sup>	8.87 $\pm$ 2.95 <sup>b</sup>	488.00 $\pm$ 15.54 <sup>b</sup>	33.03 $\pm$ 3.05 <sup>a</sup>	7.50 $\pm$ 3.46 <sup>a</sup>	52.33 $\pm$ 7.15 <sup>a</sup>
HFD5	33.67 $\pm$ 9.87 <sup>b</sup>	11.43 $\pm$ 5.08 <sup>b</sup>	5.77 $\pm$ 1.89 <sup>b</sup>	7.17 $\pm$ 2.65 <sup>b</sup>	387.00 $\pm$ 9.70 <sup>a</sup>	32.23 $\pm$ 0.95 <sup>a</sup>	8.10 $\pm$ 1.35 <sup>a</sup>	58.37 $\pm$ 3.15 <sup>a</sup>
HFD6	38.33 $\pm$ 1.15 <sup>b</sup>	9.83 $\pm$ 1.00 <sup>b</sup>	6.73 $\pm$ 0.55 <sup>b</sup>	6.47 $\pm$ 2.29 <sup>b</sup>	414.67 $\pm$ 9.96 <sup>b</sup>	33.23 $\pm$ 2.06 <sup>a</sup>	10.70 $\pm$ 0.36 <sup>a</sup>	52.73 $\pm$ 4.93 <sup>a</sup>
HFD7	36.33 $\pm$ 3.79 <sup>b</sup>	9.77 $\pm$ 0.91 <sup>b</sup>	5.17 $\pm$ 0.38 <sup>b</sup>	4.07 $\pm$ 1.68 <sup>a</sup>	391.67 $\pm$ 6.46 <sup>a</sup>	32.13 $\pm$ 1.21 <sup>a</sup>	9.40 $\pm$ 2.24 <sup>a</sup>	52.27 $\pm$ 2.77 <sup>a</sup>

Values are expressed as Mean  $\pm$  SD. (n= 10 rats)

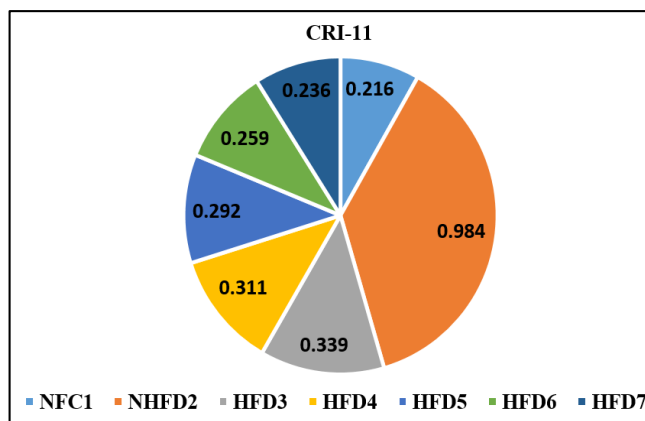
**Table 3:** Effect of aqueous extracts of the seeds of *S. aromaticum* on blood differential counts of wistar rats

Experimental groups	NEUT ( $\times 10^9/L$ )	LEU ( $\times 10^9/L$ )	ESO ( $\times 10^9/L$ )	Mono ( $\times 10^9/L$ )
NFC1	92.00 $\pm$ 1.00 <sup>b</sup>	6.47 $\pm$ 0.58 <sup>a</sup>	1.00 $\pm$ 0.00 <sup>a</sup>	7.33 $\pm$ 0.58 <sup>a</sup>
NHFD2	103.33 $\pm$ 3.21 <sup>c</sup>	17.67 $\pm$ 1.53 <sup>b</sup>	10.33 $\pm$ 0.58 <sup>b</sup>	11.67 $\pm$ 1.53 <sup>b</sup>
HFD3	82.00 $\pm$ 4.36 <sup>a</sup>	8.67 $\pm$ 0.13 <sup>a</sup>	1.33 $\pm$ 0.58 <sup>a</sup>	6.00 $\pm$ 2.00 <sup>a</sup>
HFD4	86.67 $\pm$ 3.21 <sup>a</sup>	14.33 $\pm$ 4.04 <sup>b</sup>	1.33 $\pm$ 0.58 <sup>a</sup>	5.67 $\pm$ 2.52 <sup>a</sup>
HFD5	82.67 $\pm$ 5.69 <sup>a</sup>	9.67 $\pm$ 3.06 <sup>a,b</sup>	1.33 $\pm$ 0.58 <sup>a</sup>	6.00 $\pm$ 2.65 <sup>a</sup>
HFD6	84.67 $\pm$ 7.51 <sup>b</sup>	16.33 $\pm$ 6.11 <sup>b</sup>	1.33 $\pm$ 0.58 <sup>a</sup>	7.33 $\pm$ 2.08 <sup>a</sup>
HFD7	89.67 $\pm$ 2.89 <sup>b</sup>	6.33 $\pm$ 1.15 <sup>a</sup>	1.67 $\pm$ 0.58 <sup>a</sup>	6.67 $\pm$ 0.58 <sup>a</sup>

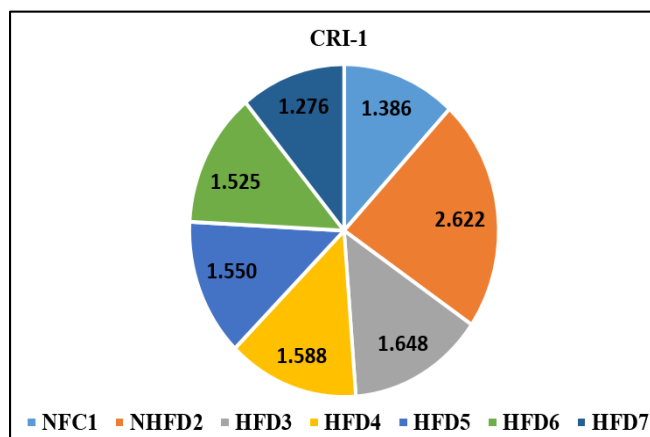
Values are expressed as Mean  $\pm$  SD. (n= 10 rats).



**Fig 4:** Atherogenic index of plasma of aqueous extracts of the seeds of *S. aromaticum*



**Fig 6:** Castelli Risk Index II of plasma of aqueous extracts of the seeds of *S. aromaticum*



**Fig 5:** Castelli Risk Index I of plasma of aqueous extracts of the seeds of *S. aromaticum*

## Discussion

The present study demonstrates that aqueous seed extract of *Syzygium aromaticum* exerts hypolipidemic and haematonic effects in high-fat-diet-induced Wistar rats. Treatment with the extract at various doses significantly reduced total cholesterol, triglycerides and LDL-C while increasing HDL-C. These changes were reflected in favorable shifts in atherogenic indices (AIP, Castelli indices), suggesting reduced cardiovascular risk in treated animals.

The observed increase in overall weight gain across groups NHFD2 to HFD7 compared to the normal control (NFC1) suggests that the HFD, enriched with butter, sugar, and flour, successfully induced a hyperlipidaemic state. However, the aqueous extract of *S. aromaticum* seeds mitigated some of these effects. Notably, there was no significant difference ( $p>0.05$ ) in alkaline phosphatase (ALP) levels across treated groups compared to NHFD2,

indicating that the extract did not adversely affect liver function in this aspect. However, a significant reduction ( $p < 0.05$ ) in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in treated groups (HFD3-HFD7) compared to NHFD2 highlights the extract's potential to alleviate liver and plasma membrane dysfunction caused by the HFD. This aligns with previous findings by Wild *et al.* (2004) [37], Oboh *et al.* (2017) [27], and Emejuru *et al.* (2023) [12], suggesting that flavonoids in the extract may regulate liver enzyme activities, thereby protecting hepatic health. These hepatoprotective effects evidenced by normalization of ALT and AST in extract-treated groups point toward improved hepatic function and reduced steatosis-related damage. Since dyslipidemia and hepatic dysfunction are mutually reinforcing, amelioration of liver enzymes is an important contributor to improved lipid profiles. Also, Eugenol and other phenolic constituents in clove possess potent antioxidant properties that are likely to mediate much of the observed pharmacological activity. Antioxidants reduce lipid peroxidation of lipoproteins, preserve hepatocellular integrity, and can modulate lipid metabolic enzymes; these mechanisms being supported by previous work on clove and related phytochemicals (Cortés-Rojas *et al.*, 2014; Dahiru *et al.*, 2022) [7, 8].

The lipid profile analysis revealed a dose-dependent reduction in total cholesterol, triglycerides, and low-density lipoprotein (LDL) levels, alongside a significant increase ( $p < 0.05$ ) in high-density lipoprotein (HDL) levels in treated groups compared to NHFD2. This indicates the extract's antilipidaemic potential, supporting earlier studies by Luka & Tijani (2013) [22] and Ajiboye *et al.* (2017) [4], which highlighted plant extracts' role in managing atherosclerosis by lowering serum lipids. The ameliorative effect was most pronounced at the highest dose (2500 mg/kg), suggesting a dose-response relationship.

Haematological indices further underscore the extract's haematinic properties. Treated groups (HFD3-HFD7) showed significant increases ( $p < 0.05$ ) in packed cell volume (PCV), haemoglobin (HB), and red blood cell (RBC) counts compared to NHFD2, indicating enhanced erythropoiesis. Conversely, significant ( $p < 0.05$ ) decreases in white blood cell (WBC), platelet (PLT), mean cell haemoglobin (MCH), neutrophils, leukocytes, eosinophils, and monocytes were observed, suggesting a regulatory effect on immune and inflammatory responses. These findings are consistent with previous research by Longe *et al.* (2015) [21], Gabriel *et al.* (2022) [16], and Nwaogwugwu *et al.* (2022) [26], which demonstrated plant extracts' positive impact on the erythropoietic system. These improvements in haematological parameters (PCV, Hb, RBC) further suggest haematinic potential, possibly via reduced oxidative damage to erythrocytes and improved iron utilization. However, decreases in WBC and certain differential counts should be interpreted cautiously; while they may reflect an anti-inflammatory effect, marked reductions could indicate immune modulation that warrants further immunotoxicity assessment.

We evaluated composite lipid risk ratios to quantify cardiovascular risk: Castelli Risk Index-I (CRI-I = Total Cholesterol/HDL-C), Castelli Risk Index-II (CRI-II = LDL-C/HDL-C), and the Atherogenic Index of Plasma (AIP =  $\log_{10}[\text{TG}/\text{HDL-C}]$ ). These atherogenic indices, including the Atherogenic Index of Plasma (AIP), Castelli Risk Index I (CRI-I), and Castelli Risk Index II (CRI-II), provide a

comprehensive assessment of cardiovascular risk. As shown in Figure 4, SAE treatment significantly reduced the Atherogenic Index of Plasma (AIP) compared with the HFD control, suggesting a cardioprotective effect consistent with previous reports (Dobiášová & Frohlich, 2001) [10]. The AIP values decreased significantly in treated groups (ranging from 0.218 to 0.295) compared to NHFD2 (0.524), indicating reduced atherogenic risk. Similarly, CRI-I and CRI-II values were lower in treated groups, with the highest dose (HFD7) showing the most favourable profile (CRI-I: 1.276, CRI-II: 0.236), suggesting a protective effect against atherosclerosis. The reduction in Castelli Risk Index I (CRI-I) observed in SAE-treated groups (Figure 5) further supports the lipid-lowering and anti-atherogenic properties of the extract, particularly at higher doses. Similarly, SAE significantly lowered Castelli Risk Index II (CRI-II) values relative to HFD (Figure 6), reflecting an improved LDL-C/HDL-C balance which reduces cardiovascular risk. These indices integrate multiple lipid parameters, offering a more robust predictor of cardiovascular risk than individual markers alone (Dobiášová & Frohlich, 2001; Frohlich & Dobiášová, 2004; Raaj *et al.*, 2024) [10, 15, 30].

## Conclusion

The aqueous seed extract of *Syzygium aromaticum* exhibits significant antilipidaemic and haematinic potentials, as evidenced by its ability to lower cholesterol and triglyceride levels, improve atherogenic indices (AIP, CRI-I, and CRI-II), while boosting blood parameters such as PCV, HB, and RBC counts in Wistar rats. The extract's efficacy in reducing atherogenic indices and liver enzyme levels further supports its potential as a natural therapeutic agent for managing hyperlipidaemia, anaemia, and cardiovascular-related ailments. These findings validate ethnopharmacological claims and pave the way for the development of novel plant-based drugs, offering a cost-effective and safer alternative to conventional treatments.

## Conflict of Interest

The authors declare no conflict of interest in connection with this article.

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