Hypolipidemic effect of aqueous and ethanol extracts of *Alafia barteri* (leaf and root) in formaldehyde-induced arthritis in male mice

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Abstract

Alafia barteri is valued for its effectiveness in the traditional medicine system in Nigeria and other African countries. This study aims at evaluating the hypolipidemic effect of the aqueous and ethanol leaf and root extracts of *Alafia barteri* in formaldehyde-induced arthritic mice. 77 healthy Swiss albino male mice (30-35 g) were randomized into eleven groups of five mice each and extracts were administered for 10 days at 200 mg/kg and 400 mg/kg. Arthritis was induced by sub-plantar injection of 0.1 mL of 2 % formaldehyde. Serum lipid profile was evaluated after blood sample collection. Results of this study revealed that the aqueous and ethanol leaf extracts caused significant reduction($p<0.05$) in triglyceride and total cholesterol levels, but increased HDL-cholesterol levels compared to the arthritic control which is suggestive of their hypo-lipidemic effects in arthritic mice.

***Keywords*:** *Alafia barteri, A*rthritic, Sub-plantar, Hypolipidemic

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune and systemic inflammatory disease that causes irreversible damage to the joint resulting in significant disability, painful joints and destructive bone erosion (Almutairi *et al.*,2021). It affects over 21 million people worldwide and is one of the leading causes of chronic morbidity in the developed world, mostly affecting the work force population throughout the world. Many studies have shown that there is a correlation between abnormal lipid metabolism and rheumatoid arthritis. Arthritic patients have been found to have decreased levels of serum total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein- cholesterol compared to non-arthritic healthy individuals. This has also been observed in the serum of arthritic rats. Increasing evidence reveals that treatments used to manage rheumatoid arthritis may modify the nature and impact of cardiovascular risk factors (Sattar *et al.*, 2003) and that the risk of dying from a first cardiovascular event is increased in arthritic individuals (Goodson *et al.,* 2005). Medicinal plant extracts are rich in phytoconstituents used by man as prophylactic or therapeutic agents in the treatment of several inflammatory-related disorders (Anyasor, 2015). The undesirable side effects of some modern anti-inflammatory drugs have prompted a switch to the search for alternative sources(herbs) due to their easy accessibility, cost, and lesser side effects.

Alafia barteri is a medicinal plant belonging to the family *Apocynaceae* and also known as Agbari etu (immediate fever treatment) (Yoruba), Loko or Mende (Sierra Leone), Anyi (Ivory Coast), Akan-asante or Fante (Ghana), Ota nza (Igbo) (Leeuwenberg 1997). Previous literature evidences reveal that the leaf extracts possess antibacterial and antifungal activities (Adekunle and Okoli, 2002; Hamid and Aiyelaagbe, 2011). The root and leaf decoctions are used for toothache and eye infections (Odugbemi, 2008); fiber from the stem of the plant serves as tying material for roofs; aqueous leaf extract displayed potent antiplasmodial activity (Lasisi *et al*., 2012). The root extract showed analgesic effect (Ishola *et al*., 2015) while the stem extract of the plant has also been reported to show anti-proliferative activity (Hamid and Aiyelaagbe, 2017). Furthermore, the leaf extracts reduced the blood glucose level of the diabetic animals and maintained histo-renal architecture (Atilade, *et al*., 2018); its effect on spermatogenesis and steroidogenesis has been studied by Adelakun *et al*., 2018. The ethanol leaf and root extracts of *Alafia barteri* have also been shown to possess anti-inflammatory activity using the carrageenan induced paw edema model (Sofidiya and Akindele, 2014; Ishola *et al.*, 2015). The aim of this study is to evaluate the hypolipidemic potential of the aqueous and ethanol leaf and root extracts of *Alafia barteri* in formaldehyde-induced arthritic mice.

2. Methodology

2.1 Chemicals

Ethanol (70%), distilled water, formaldehyde, phosphate buffer saline, **Randox Kits**, Diclofenac sodium

2.2 Collection and authentication of the plant material

The whole plant material of *Alafia barteri* was collected from Osun State, Nigeria, January 2021. Botanical identification and authentication was done by Dr. Nodza George of the Department of Botany, University of Lagos, where a voucher specimen LUH 8789 was deposited.

2.3 Preparation of plant extracts

The leaves and root barks of *Alafia barteri* plant were washed, dried in the laboratory oven at 50ºC. The leaves were pulverized while the bark was chopped to pieces. 60 g of the powdered leaves and chopped root bark were weighed respectively into separate glass wares containing 300 mls distilled water and 70% ethanol respectively. The mixtures were macerated at room temperature for 72 hours with intermittent vigorous shaking. The mixtures were filtered using Whatman's filter paper no 1 Filtrates were collected separately in clean beakers and subjected to evaporation using a laboratory

oven at 45ºC and also the rotary evaporator to obtain dark viscous extracts which were preserved in a refrigerator for use throughout the period of the study.

2.4 Experimental animals.

Seventy seven (77) Swiss albino mice weighing between 30-35 g were used for the experiments and were acclimatized for a week at room temperature and relative humidity with a 12 hr. light/dark cycle. They were fed with crushed feed and water ad libitum. The animals were randomly allocated to eleven(11) treatment groups in plastic cages with paddy husk sawdust as bedding after acclimatization as follows:

Groups	Treatment
Group I	Normal control
Group II	Arthritic control
Group III	200mg/kg *Alafia barter*i aqueous leaf extract +0.1ml of 2% formaldehyde
Group IV	400mg/kg *Alafia barteri* aqueous leaf extract +0.1ml of 2% formaldehyde
Group V	200mg/kg *Alafia barteri* ethanol leaf extract +0.1ml of 2% formaldehyde
Group VI	400mg/kg *Alafia barteri* ethanol leaf extract +0.1ml of 2% formaldehyde
Group VII	200mg/kg *Alafia barteri* aqueous root extract +0.1ml of 2% formaldehyde
Group VIII	400mg/kg *Alafia barteri* aqueous root extract +0.1ml of 2% formaldehyde.
Group IX	200mg/kg *Alafia barteri* ethanol root extract +0.1ml of 2% formaldehyde
Group X	400mg/kg *Alafia barteri* ethanol root extract +0.1ml of 2% formaldehyde
Group XI	Standard drug, diclofenac +0.1ml of 2% formaldehyde

2.5 Induction of arthritis.

Arthritis was induced by sub plantar administration of 0.1ml formaldehyde (2% v/v) into the left hind paw in all groups except normal control on days 1 and 3 respectively. Oral administration of the aqueous and ethanol leaf and root extracts at 200mg/kg bwt and 400 mg/kg body weight was done for 10 days.

2.6 Measurement of Biochemical Parameters.

At the end of the treatment protocol, blood samples were obtained from the mice through cardiac puncture under chloroform anesthesia. Centrifugation was done at 5000g for 15mins and serum samples were analyzed for their lipid profile using RANDOX diagnostic kits. Parameters analyzed include: Total Cholesterol (TC), Triglycerides (TG), and High-density lipoprotein-cholesterol (HDL-

C). Low density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C) concentrations were estimated according to the formula of Friedwald *et al.,*1972 as reported in the commercial kit's instructions.

2.7 Statistical analysis

Data obtained from the study were statistically analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's Multiple comparisons (post-hoc) using GraphPad prism 9.0 for Windows (GraphPad Software, San Diego, California, USA). Data were expressed as mean ± standard deviation (SD). Values of $p< 0.05$ were regarded as statistically significant.

3. Results and Discussion

Rheumatoid arthritis is the most common autoimmune arthritis associated with increased cardiovascular mortality. (Shah *et al.,* 2017). While common manifestations include synovial damage and inflammation, the systemic effects majorly cardiovascular disorders are also life threatening. Such effects have been shown by an altered lipid profile in RA patients which have been found to correlate with cardiovascular risks. (Behl *et al.,* 2020).

RA patients are marked with high levels of inflammatory molecules and cytokines (IL-6 and TNF-alpha) which promote dysfunction of endothelial cells, structural vessel deformities and induction of other cardiovascular risk (CV) risk factors.(Steiner *et al* 2009; Schultz *et al.*,2010). Figures 1 -10 show the effect of aqueous and ethanol leaf extracts of *Alafia barteri* on the different biochemical parameters assessed in formaldehyde-induced arthritic rats. Active Rheumatoid arthritis is associated with reduced levels of low-density lipoprotein (LDL) cholesterol and total cholesterol (Ridker *et al.,* 2009).

Numerous studies have shown that anti-inflammatory therapies raise lipid levels especially in rheumatoid arthritis conditions. This may be explained by the fact that the overproduction of acute phase reactants (APR) under an elevated inflammatory burden leads to an impairment in cholesterol trafficking in the liver (Choy *et al.,* 2014). HDL possess anti-inflammatory property thus aiding the reverse cholesterol transport from circulation to the liver blocking LDL oxidation (Berrougui *et al.,* 2012).

The expression of LDL-C is due to the activation of pro-inflammatory cytokines like IL-6 and TNF-alpha which causes elevated liver uptake of LDL and biliary secretion of cholesterol. (Calin *et al.*, 2015). Anti-inflammatory therapies decrease inflammation, modifies lipoprotein spectrum and has been associated with decrease in cardiovascular risk in rheumatoid arthritis.

According to research, atherogenic index (AI) may also be an appropriate tool used to assess the relative contribution of lipids to CV risk in rheumatoid arthritis. In addition, HDL levels and AI are more suitable parameters of lipid profile as CVD determinants in RA conditions. (Carlin et *al.,* 2012) The observed reduction in HDL cholesterol and triglyceride (TG) levels on induction agrees with the findings of Choy *et al.*,2014 where a drop in the level of lipids, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides in the blood was observed in active RA. HDL levels were significantly increased upon administration of all extracts however a significant effect was seen with the ethanol leaf and root extracts at both doses administered. Triglyceride levels were significantly decreased upon administration of the aqueous and ethanol leaf extracts at all doses suggesting its hypolipidemic effect. A consistent pattern of decreased HDL-cholesterol levels has been observed in RA patients Agents that can decrease serum TC,LDL-C,VLDL-C and raise HDL-C levels may confer significant protection against inflammatory related diseases(Dursunoglu *et al.,* 2005).

4. Conclusion

Based on the results of these findings, the leaf extracts of *A. barteri* possess hypo-lipidemic potential in formaldehyde induced arthritic mice and thus may be considered safe during arthritis treatment.

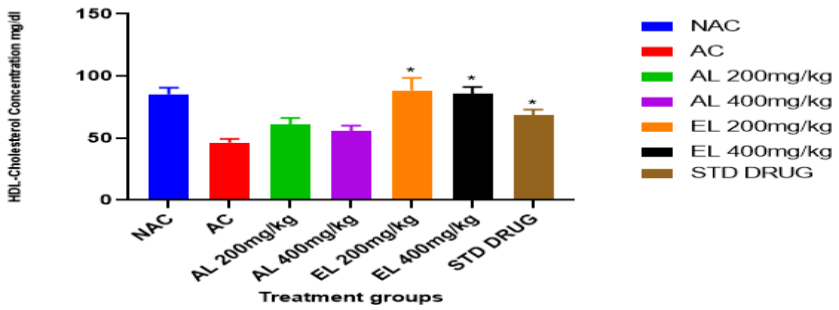

Figure 1: Effect of aqueous and ethanol leaf extracts of *Alafia barteri* on HDL-C concentration of formaldehyde-induced arthritic rats (values are expressed as mean ±standard deviation; $p<0.05$)

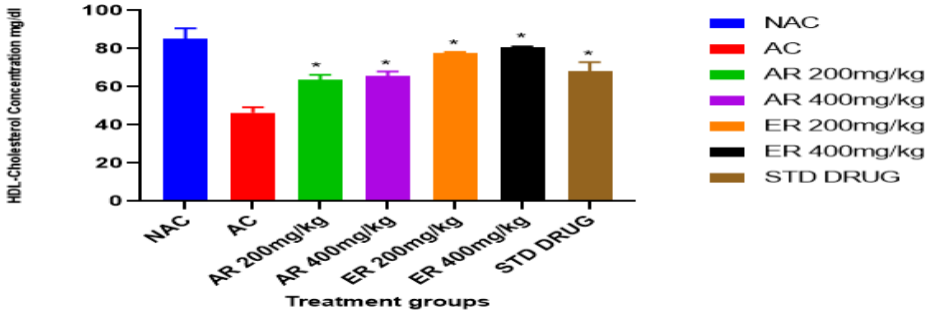

Figure 2: Effect of aqueous and ethanol root extracts of *Alafia barteri* on HDL-C levels of formaldehyde-induced arthritic rats (values are expressed as mean ±standard deviation; $p<0.05$)

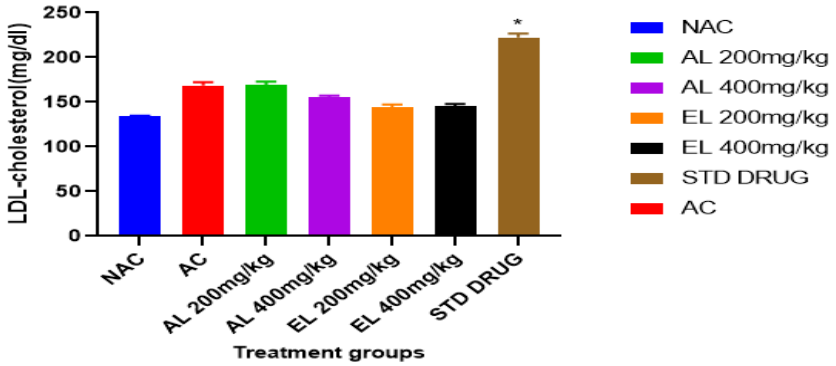

Figure 3: Effect of aqueous and ethanol leaf extracts of *Alafia barteri* on LDL-C levels of formaldehyde-induced arthritic rats (values are expressed as mean ±standard deviation; $p<0.05$)

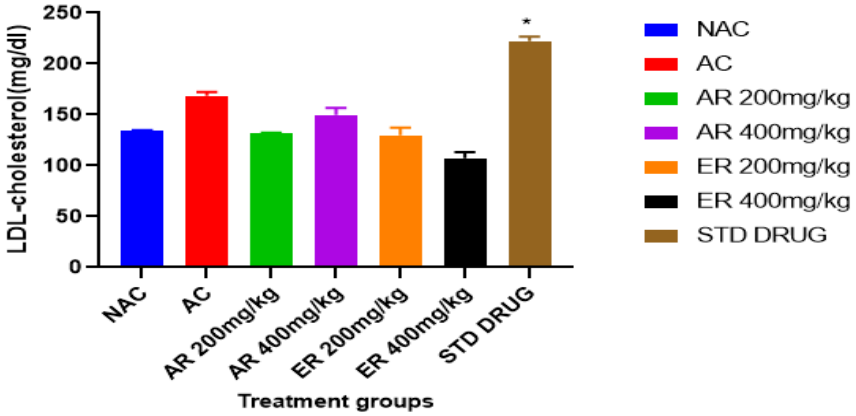

Figure 4: Effect of aqueous and ethanol leaf extracts of *Alafia barteri* on LDL-C levels of formaldehyde-induced arthritic rats (values are expressed as mean ±standard deviation; $p<0.05$)

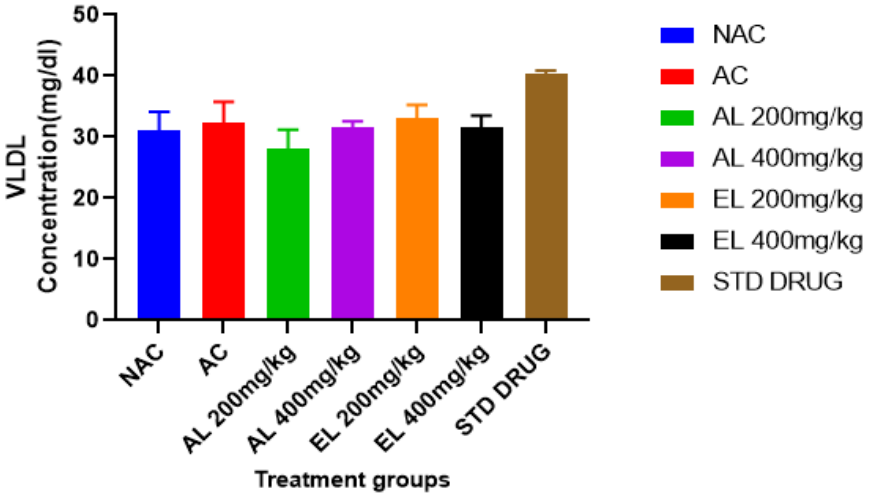

Figure 5: Effect of aqueous and ethanol leaf extract of *Alafia barteri* on VLDL levels of formaldehyde-induced arthritic rats (values are expressed as mean ±standard deviation; $p<0.05$)

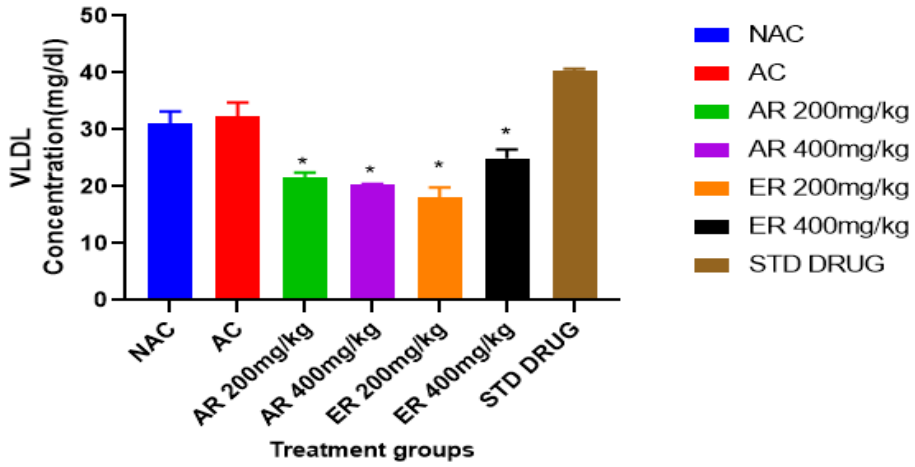

Figure 6: Effect of aqueous and ethanol root extracts of *Alafia barteri* on VLDL concentration of formaldehyde-induced arthritic rats (values are expressed as mean ±standard deviation; $p<0.05$)

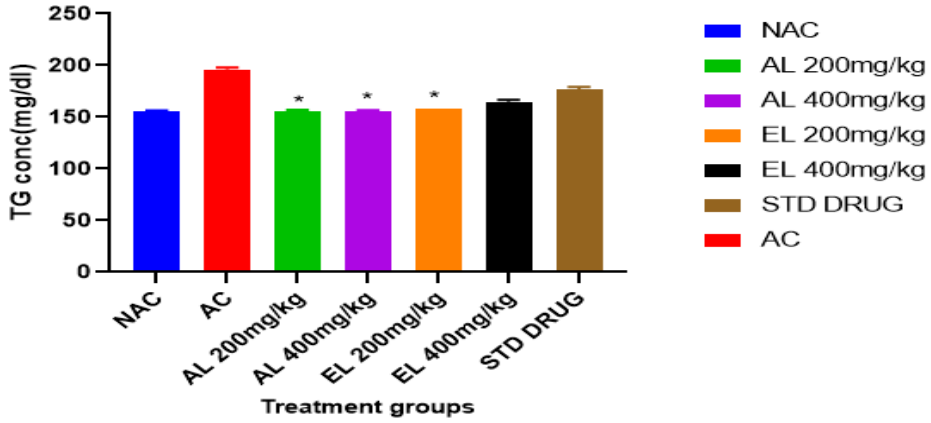

Figure 7: Effect of aqueous and ethanol leaf extracts of *Alafia barteri* on triglyceride levels in formaldehyde-induced arthritic rats (values are expressed as mean ±standard deviation; $p<0.05$)

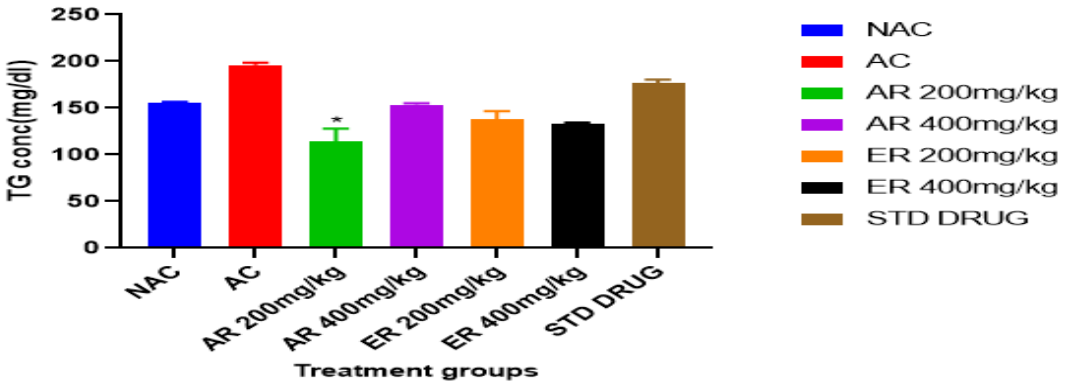

Figure 8: Effect of aqueous and ethanol root extracts of *Alafia barteri* on TG concentration of formaldehyde-induced arthritic rats (values are expressed as mean ±standard deviation; $p<0.05$)

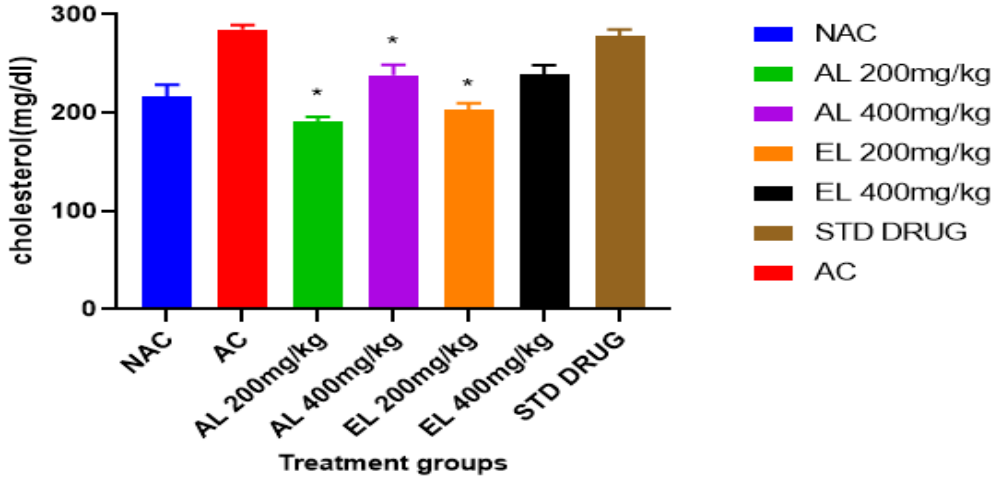

Figure 9: Effect of aqueous and ethanol leaf extracts of *Alafia barteri* on cholesterol levels of formaldehyde-induced arthritic rats (values are expressed as mean ±standard deviation; $p<0.05$)

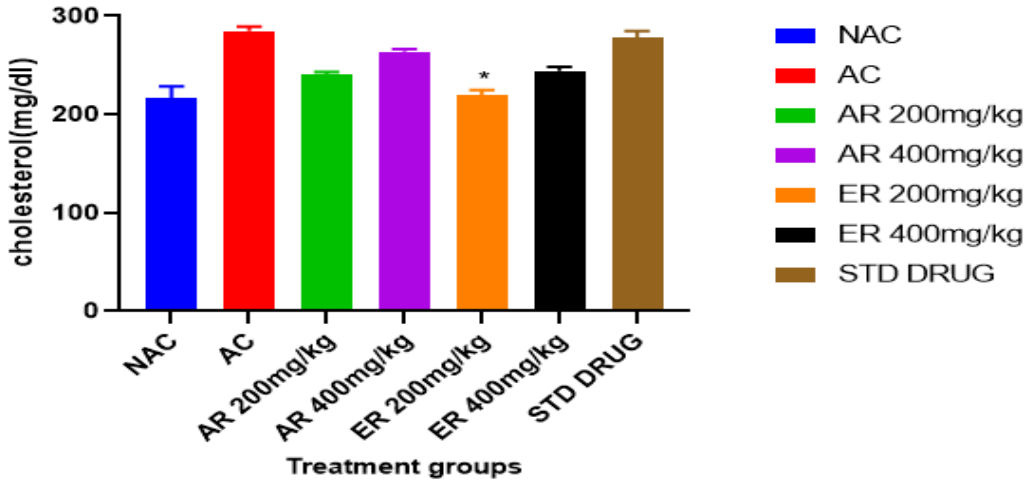

Figure 10: Effect of aqueous and ethanol root extracts of *Alafia barteri* on cholesterol levels of formaldehyde-induced arthritic rats (values are expressed as mean ±standard deviation; $p<0.05$)

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