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The Evaluation of Antihyperlipidemic Activity of Methanol Leaf Extract of *Erythrina senegalensis* on Poloxamer 407-Induced Hyperlipidemic Rat Model

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Abstract:- The evaluation of the antihyperlipidemic activity of methanol leaf extract of *Erythrina senegalensis* (MLEES) on serum lipid profile of poloxamer 407-induced hyperlipidemic rats is the aim of this study. The injection of 1000mg/kg poloxamer 407 intraperitoneally elevated the lipid profile of the experimental animals. Preliminary phytochemical screening of the extract was carried out. The animals were grouped into four (4): Normal control, hyperlipidemic control, standard group (administered atorvastatin at 20mg/Kg body weight), and test group (administered extract of *Erythrina senegalensis* at 200mg/Kg body weight). The administration was done for four (4) days at the above stated doses. Serum lipid profile was estimated by using standard methods. The results were presented as mean ± standard error of mean (SEM).

Keywords:- *Antihyperlipidemic, Erythrina senegalensis, poloxamer 407.*

I. INTRODUCTION

It is no longer news that preference is being given to scientific research recently in the area of traditional /herbal medicine, obtained from medicinal plants. Acute and chronic diseases have been treated with medicinal plants. About 80% of the world's population depend solely on herbal medicine according to WHO report. Thus, the use of traditional herbs for national policies and drug regulatory measures was approved by the WHO, in a bid to promote research and evaluation of the safety and efficacy of herbal products. The discovery of 74% of the enlisted over 100 plant derived drugs according to WHO occurred via chemical studies aimed at isolation of the bioactive components inherent in them [13]. Such biologically active compounds account for their use as traditional medicine. The utilization of medicinal plants is still ongoing within rural locations despite the advent of modern medicines [3]. Medicinal plants continue to gain acceptance in the society given their beneficial roles with little or no side effect. Plants with lipid-lowering activity have been documented to be over 70 in number. As such, medicinal plants are good agents for attenuating hyperlipidemia [2]. *Erythrina senegalensis* like other medicinal plants is a commercial and indigenous tree of African countries including the savannah regions of Sudan [20]. It is widely used in Nigeria and other African countries in the treatment of common ailments [31]. Parasitic diseases like schistosomiasis during immature stages can be treated with *Erythrina senegalensis* when combined with *Prosopis africana* [27]. *Erythrina senegalensis* can serve as ornamental tree as well as hedge plant [10]. An isolate of another variety of this plant, *Erythrina droogmansiana* has been shown to possess anticonvulsant and sedative effect, thus making it useful in the treatment of epilepsy [30].

II. MATERIALS AND METHODS

❖ *Materials*

➢ *Plant Collection and Preparation of Extract*

Mature leaves of *Erythrina senegalensis* were obtained from Ikpa village of Makurdi LGA, Benue state, Nigeria. The botanical identification and authentication were carried out in the Botany unit, Federal University of Agriculture, Makurdi-Nigeria.

The collected leaves of *Erythrina senegalensis* were dried under shade for 14 days. Pestle and mortar were used to pulverize the shade-dried leaves, followed by sieving to have a finer powder. 100g of the sample was macerated in 1000mL of methanol (of analytical grade), allowed to stand for 48 hours, and the supernatant was decanted into another flask. Whatman No.1 filter paper was used to filter the extract. The filtrate was subjected to water bath to dry at 50°C in order to concentrate it. The weight of the crude extract was determined. Air-tight sample bottle was used for storage of the crude extract within a refrigerator for later use.

➢ *Location of the Study*

This study aimed at evaluating the lipid-lowering activity of methanol leaf extract of *Erythrina senegalensis* was carried out in Federal University of Agriculture, Makurdi-Nigeria. The plant extraction, phytochemical screening of the extract, acclimatization of the rats, poloxamer 407-induction of hyperlipidemia, and treatment of the hyperlipidemic rats including the analysis of some biochemical parameters were carried out in the Veterinary pharmacology/Physiology/Biochemistry laboratory of the college of Veterinary medicine.

➢ *Phytochemical Screening of the Extract*

The MLEES was subjected further to screening for phytochemicals using the method described by [29].

➢ *Experimental animals*

Male wistar rats (*Rattus norvegicus*) of weight 130-150g were obtained from National Veterinary and Research Institute (NVRI) Vom, Jos, Plateau State, Nigeria. The rats were housed in plastic cages for two weeks in order to adapt to the new environment. During the 14-day period, the rats had access to light, suitable temperature, and relative humidity. The rats were given pellet feeds and water as often as necessary throughout the experimental period.

➢ *Determination of the Median Lethal Dosage (LD_{50}) of the Plant Extract*

The median lethal dosage of the plant extract was determined to ascertain the safety of the extract at given doses. A limit test is generally performed before the main test during which one animal is given a test dose (2000mg/Kg). The death of an animal leads to the conduct of the main test to find out the LD_{50}. However, a sequential dose of 4 animals is carried out upon the survival of the animal. The limit test is terminated and main test is performed upon the death of 3 animals. LD_{50} is said to be less than 2000mg/kg upon the death of 3 or more animals. Main test is carried out if death of a third animal is recorded. But the survival of 3 or more animals shows that the LD_{50} is greater than 2000mg/kg.

➢ *Design of Study*

Four (4) groups, each containing four (4) animals were formed as follow:
Group 1: Normal control
Group 2: Hyperlipidemic control
Group 3: Standard group
Group 4: Test group

➢ *Collection and Preparation of Sera samples for Biochemical Analysis*

The experimental animals were anaesthesized with chloroform at the end of treatment period. Cardiac puncture was used to bleed the anaesthesized animals. Anticoagulant tubes were used to collect the blood samples. Centrifugation of the blood samples at a speed of 3000 rpm for ¼ hour yielded sera. Plain sample bottles were used to collect the sera for further biochemical examination.

➢ *Statistical analysis*

The data collected at the end of the experiment were presented as mean ± standard error of mean (SEM), followed by analysis using the one-way analysis of variance (ANOVA) with the aid of Graph Pad Prism version 3.0. Duncan's Post Hoc was used to compare the difference between the extracts and animal groups. Probability level of less than 0.05 was taken to be significant (p<0.05).

III. RESULTS

Results of Phytochemical Screening

Table 1: Phytochemical Composition of Methanol Leaf Extract of *E. senegalensis*.

Bioactive compounds	Results
Alkaloids	+
Tannins	+
Flavonoids	+
Saponnins	+
Steroids	+
Phlobatannins	+
Cardiac glycosides	+

Key+ = present, - = absent.

Result of LD_{50}

The LD_{50} of the MLEES was estimated to be approximately ≥ 2000mg/Kg having shown no mortality rate from 100 to 2000 doses tested, and the extract was assumed to be safe. The experimental doses used (100mg/Kg, 200mg/Kg and 400mg/Kg) were relatively safe.

Serum Lipid Profile

Following the last day of treatment, blood samples of all the animals were collected. The concentration of each serum lipid biomarker was determined.

Table 2: The table below is the mean ± SEM of serum biochemical changes of hyperlipidemic and non-hyperlipidemic rats treated with atorvastatin and methanol leaf extract of *Erythrina senegalensis* for four (4) days.

Groups	TC (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
NC	169.1 ± 2.86^{a}	111.9± 8.72^{a}	28.9 ±3.34^{b}	195.6 ±5.36^{a}
HyperCp407	427.2±11.50^{d}	332.2±17.66^{d}	12.2±11.03^{a}	390.9 ±3.79^{d}
Hyper+ATV	303.9±11.25^{b}	129.8 ±1.55^{c}	64.5±1.77^{d}	290.2±10.40^{c}
Hyper+MLEES	315.2± 1.73^{c}	122.0±29.25^{b}	51.5±11.11^{c}	242.3±9.33^{b}

Statistically significant values are those with different superscripts down the column.

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Key
NC: Normal Control, **HyperCp407**: Hyperlipidemic rats control, induced by poloxamer 407, **Hyper + ATV**: Hyperlipidemic rats + atorvastatin (20mg/Kg), and **Hyper + MLEES**: Hyperlipidemic rats + methanol leaf extract of *Erythrina senegalensis* (200mg/Kg).

TC: total cholesterol, TGL: triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein.

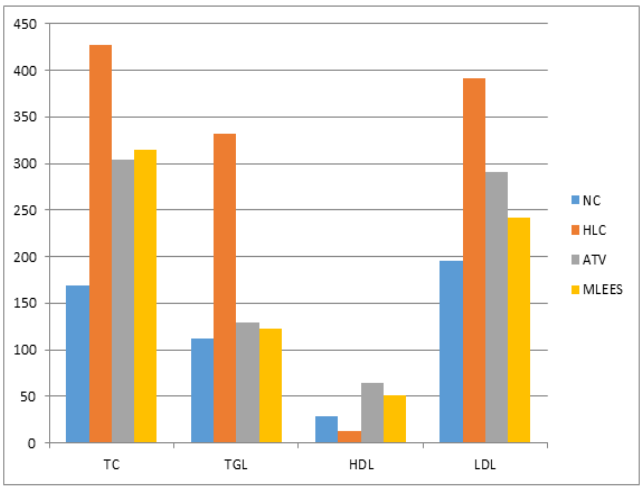

Chart 1: Representing data of the mean of Serum biochemical changes of Hyperlipidemic and non-Hyperlipidemic rats treated with atorvastatin and methanol leaf extract of *Erythrina senegalensis*.

IV. DISCUSSION

The medical term used to describe the elevation of serum total cholesterol above 240mg/dl, and/or low density lipoprotein above 160mg/dl with total cholesterol to high density lipoprotein ratio greater than 5.7, and total triglyceride above 150mg/dl in adult is known as hyperlipidemia [4]. This arises when there is an alteration in serum lipid and lipoprotein profile due to increase in the concentration of total cholesterol, low density lipoprotein, very low density lipoprotein and triglycerides with a concurrent reduction in the concentration of high density lipoprotein in the blood [17]. Although hyperlipidemia is asymptomatic, it is a risk factor of cardiovascular disease (CVD). In fact, an elevated cholesterol level has been associated with type 1 and type 2 diabetes mellitus [28]. Death has been associated with type 2 diabetes due to complications from hypertension, another risk factor of cardiovascular disease [8]. Sedentary lifestyle, tobacco smoking are also linked to excessive CVD risk among adolescents and adults including mood disorder [15]. Excessive alcohol intake like the factors mentioned above can also lead to abnormal cholesterol level in the blood [19]. The reality of the asymptomatic nature of hyperlipidemia necessitates the need for individuals to monitor their serum lipid profile via regular medical check-ups [18].

Globally, cardiovascular diseases rank second in terms of mortality [1]. Incidence rate of over 400 million in CVDs with over 17 million deaths was recorded in 2015 [26]. It has been well established that elevated levels of cholesterol, triglyceride, and low density lipoprotein place one at risk of developing CVD. On the other hand, increased level of high density lipoprotein protects against CVD.

Bioactive compounds such as saponins, steroids, terpenoids, tannins, alkaloids and others were found to be present in MLEES based on the result (table 1). The result is in concordance with earlier studies [16].

The LD_{50} of MLEES is above 2000mg/Kg because neither death nor any sign of acute toxicity (hair erection, shivering, heat-seeking behaviour, and so on) was observed during the observation period. Although, 4000mg/kg was estimated as the LD_{50} of its aqueous extract from earlier studies [22] and confirmed the safety of its oral administration.

The intra-peritoneal administration of poloxamer 407 (P407) to rats resulted in an enormous elevation of serum total cholesterol (TC), triglyceride (TGL), and low-density lipoprotein (LDL), but lowered high-density lipoprotein (HDL) at the same time. P407-induced hyperlipidemia is artificial even though it provides a rapid means of evaluating the hypolipidemic activity of plants.

The administration of methanol leaf extract of *Erythrina senegalensis* (MLEES) at the stated dose (200mg/Kg) via oral route lowered the serum TC, TGL and LDL while it raised the level of serum HDL. Thus, the MLEES could be said to possess an anti-atherogenic potential by inhibiting the biosynthesis of cholesterol, triglyceride and LDL, while stimulating an increase in the biosynthesis of HDL. The intensive conversion of LDL into HDL and clearance of circulating lipids are indicated by increase HDL levels. Based on the result of this study, there is a competition between elevated level of HDL and LDL receptor sites on arterial smooth muscle cells, which limits the uptake of low density lipoprotein. Elevated high density lipoprotein in the blood hampers the oxidation of low density lipoprotein. These must have occurred because of biologically active compounds inherent in *Erythrina senegalensis*. Such compounds have the potential to decrease lipid peroxidation to about normal while upregulating the levels of glutathione –S transferase (GST), superoxide dismutase (SOD) and catalase [5, 6, 7]. The integrity and function of membrane can become disrupted upon lipid peroxidation, which results in the formation of plagues and subsequently generates carcinogenic derivatives [14]. Mechanisms of action of the bioactive components of plants are the upregulation of the activity of antioxidant enzyme system, and subsequent attenuation of free radicals via scavenging [25]. Studies have reported an increment in the level of glutathione after treatment with *E. senegalensis* due to its phytochemicals [5, 6, 7]. *E. senegalensis* spares glutathione by reducing the level of oxidative stress. The arrangement as well as structures of the polyphenols (flavonoids and tannins) in *E. senegalensis* contributes to its ability to mop up reactive oxygen species [9]. This further illustrates the antioxidant properties of the plant [24]. Hypolipidemic activity of *Erythrina senegalensis* may be

due to presence of polyphenols such as flavonoids, tannins and other allied phytochemicals [12]. The MLEES decreased TC, TG and LDL by 37.49%, 57.54% and 34.58% respectively while it increased serum HDL by 32.50%. ATV decreased TC, TG, and LDL by 35.72%, 55.50% and 10.38% respectively while it increased serum HDL by 28.96%. These results suggest that methanol leaf extract of *Erythrina senegalensis* possesses antihyperlipidemic activity.

However, earlier studies revealed that aqueous leaf extract of *Erythrina senegalensis* has no significant effect on lipid profile when administered orally at doses of 50, 150 and 300mg/Kg [22]. This agrees with the hepatoprotective effect of *Erythrina senegalensis* as demonstrated in previous research [11]. This is a possibility because significant difference exists between the hyperlipidemic control, the test group treated with MLEES, and the standard group administered atorvastatin. Dyslipidemia, hyperglecemia, among others have been attenuated with aqueous extract of *E. senegalensis* [8]. The activity of the enzyme, diacylglycerol acyltransferase can be inhibited by the flavones inherent in *E. senegalensis* [23]. And this could be responsible for the cardioprotective effect of the plant [21].

V. CONCLUSION

In the light of this research and other similar studies earlier carried out, it can be inferred that coral tree (*Erythrina senegalensis*) has lipid-lowering ability, thus making it a very useful regimen for the management of hyperlipidemia, a prominent risk factor for cardiovascular disease.

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