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Anti-hyperlipidemic effect of aqueous leaf extract of *Emilia praetermissa* milne-redh (Asteraceae) in rats

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Abstract

This study aims at evaluating the effect of administration of the aqueous leaf extract of *Emilia praetermissa* on total cholesterol (TC) level, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and atherogenetic index of plasma (AIP) in albino rats and to determine its preliminary phytochemical constituents. The phytochemical tests were carried out using standard methods. The control group received distilled water (2ml/kg, orally) only, olive oil (hyperlipidemic) treated group received distilled water and olive oil (5ml/kg, orally) while the other three groups were pre-treated with 100, 200 and 400 mg/kg body weight of aqueous leaf extract of *Emilia praetermissa*, 100mg of the extract plus 15mg/kg atorvastatin and 100mg/kg plus 30mg/kg atorvastatin respectively. Olive oil (5ml/kg) was administered to each of the groups after 30 mins of treatment. After 2 hours following olive oil administration, blood samples were collected through abdominal aortae under chloroform anaesthesia for analysis of plasma TC, TG, HDL, LDL, AIP. Phytochemical screening of the aqueous leaf extract of *Emilia praetermissa* showed the presence of tannins, flavonoids, steroids, cardiac glycosides, carbohydrate, reducing sugar and terpenoids and absence of alkaloids and saponins. The result showed significant decreases in plasma TC, TG, LDL, and AIP levels of the rats and increase in HDL when compared with the hyperlipidemic group. Co-administration of the extract and atorvastatin significantly increased HDL and decreased LDL and AIP. It is then concluded that oral administration of aqueous leaf extract of *Emilia praetermissa* reduces hyperlipidemia and could serve as a good alternative in its treatment.

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Introduction

Hyperlipidemia is an excess of fatty substances called lipids, largely cholesterol and triglycerides, in the blood. It is also called hyperlipoproteinemia because these fatty substances travel in the blood attached to proteins (Chen *et al.*, 2005). This is the only way that these fatty substances can remain dissolved while in circulation.

It has been reported that complications and diseases associated with hyperlipidemia cause almost 12 million deaths each year all over the world (Bakari *et al.*, 2007). The major risk due to hyperlipidemia is related to atherosclerosis and one of the initial events in this process is the accumulation of cells containing excess lipids within the arterial wall (Chisolm and Steinberg, 2000).

Hyperlipidemia is a major contributor to the pathogenesis of cardiovascular diseases which is a leading health problem in the world. Cholesterol is a lipid, waxy steroid found in the cell membrane. It can dissolve and travel in the water-based blood stream at exceedingly small concentrations. Since cholesterol is insoluble in blood, it is transported in the circulatory system within lipoproteins. Abnormal high dietary cholesterol which leads to hypercholesterolemia is strongly associated with cardiovascular diseases because it promotes atherosclerosis (Durrington, 2003). Indeed, It has been demonstrated that the development of CAD is a function of the particle size of LDL-C and HDL-C, with the small particle size exhibiting great atherogenic potential (Nwagha *et al.*, 2010).

Abundant evidence has accumulated relating the concentrations of lipids (total cholesterol and triglycerides) and their associated blood transporting lipoproteins (HDL-C, LDL-C, VLDLC) with the occurrence of atherosclerosis in general and coronary artery disease (CAD) in particular. The strong association between the risk of coronary artery diseases (CAD), high levels of LDL-C and low levels of HDL-C has been well established. (Nwagha *et al.*, 2010).

Hyperlipidemia, along with diabetes, hypertension (high blood pressure), positive family history, and smoking are all major risk factors for coronary heart disease (Chen *et al.,* 2005). Olive oil can induce hyperlipidemia, which in turn can lead to atherosclerosis (Abro et al., 2008).

The first morphological change in the initiation of atherosclerosis is the migration of monocytes through an intact endothelial surface into the intima, these monocytes subsequently take up LDL-C and become lipid filled macrophages called foam cells (Ross, 1986). Progressing atherosclerotic lesion is a continuous extra cellular and intracellular accumulation of lipoproteins (Camejo *et al.*, 1991).

The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history (Barnes *et al.*, 2007). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and food plants. *Emilia praetermissa* milne-Redh (Asteraceae) was originally described from Sierra leone and Nigeria (Milne- Redhead, 1950) and was subsequently found in other West African countries, including Cameroun, Cote d' Ivoire, Ghana, Guinea and Liberia (*Hepper*, 1963; *Nicholson*, 1980; *Lisowski*, 1997). It has similar uses as *Emilia lisowskiana* (Jeffery, 1997), these are as follows; in West Africa and DR Congo, the leaves are occasionally eaten as a vegetable, either fresh in salads or cooked. In Nigeria, Cameroon and Gabon, the leaves are used to treat eye disorders, and also filariasis (*Lisowski*, 1997). In Gabon, the macerated leaves are used to treat heart problems and crushed leaves mixed with copper filings are used to dress ulcers. In Nigeria, a leaf decoction is used as a febrifuge. In Congo, the leaf sap is used to treat all kinds of skin troubles (breast abscesses, ulcers caused by yaws, leprous affections), as well as against mange, lice and

ringworm. Hernia, backache, syphilis, gonorrhea, sore throat, convulsions, enlarged spleen, vertigo, epilepsy and menstrual problems are all recorded as being treated traditionally with *E. Lisowskiana*. Laxative and antiabortificient properties are also attributed to *E. Lisowskiana*. The plants serve as fodder for rabbits and guinea pigs in Gabon. (Jeffrey, 1997). As a vegetable *Emilia Lisowskiana* is likely to remain only locally important. In view of the local medicinal uses and interesting properties of its close relatives, pharmacological research is desirable (Jeffrey, 1997).

There is no known scientific report whatsoever on the antihyperlipidemic activity of *Emilia praetermissa*. We therefore designed this work to determine the preliminary phytochemical constituents of the aqueous leaf extract of *Emillia praetermissa* and to evaluate its effect in-vivo in lowering lipids and sugar levels in albino rats.

Materials and method

Plant material and extraction

The plant was collected from Ifite-Oraifite in Ekwusigo Local Government Area of Anambra State in the month of September 2010, for identification. It was authenticated by the Forest Research Institute of Nigeria, Ibadan where the herbarium sample with voucher number FHI- 108836 was deposited.

Fresh leaves of *E. praetermissa* (100 g) were rinsed by dipping in clean water to remove debris that may be present. The leaves were cut to small pieces into a porcelain mortar and ground with a pestle. About 50 ml of distilled water was added to the mortar and used to macerate the leaves while crushing with the pestle for about 30 min. The extract was decanted and another 50 ml of the distilled water was added. The process was repeated until a total of 200 ml of distilled water was used. The decanted extracts were combined and then centrifuged at 1000 revolutions per minute for 5 min in order to remove suspended particulate matter within the extract solution. The supernant fraction, representing the aqueous extract of *E. praetermissa* was then dried in an oven at 40^{o} C for 3 days (yield = 5.67 % w/w). The dried extract was preserved in a bottle and stored in a refrigerator at 4^{o} C until required for use.

Experimental animals

Experiments were performed using adult male albino rats of both sexes (180 – 200 g) bred locally in the animal house of the Department of Pharmacology & Toxicology, University of Benin, Benin City, Nigeria. The animals fasted for 12-14 hours before experimentation but they were allowed free access to water and feed (Bendel Feeds and Flour Mill Ltd, Ewu, Nigeria). Animals were exposed to natural room temperature and lighting conditions and were handled according to standard protocols for the use of laboratory animals (National Institute of Health USA: Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002).

Phytochemical screening

Qualitative chemical tests were performed to assess the presence of various phytoconstituents of the aqueous extracts of *Emilia praetermissa*. All the phytochemical tests were carried out according to the methods of Evans, (1989).

Measurement of biochemical parameters

Fasted rats were divided into 8 groups of 5 rats each. Group I served as the control and received distilled water at the dose of 2 ml/kg. Group II served as hyperlipidemic group and received distilled water (2ml/kg) and olive oil (5ml/kg), administered after 30mins. Animals in group III received atorvastatin at the oral dose of 50 mglkg, olive oil was administered 30 minutes after treatment. Group IV, V and VI were treated with the aqueous leaf extract at the oral doses of 100mg/kg, 200mg/kg, 400 m/kg respectively and olive oil administered 30min latter. Group VII was treated with 15mg/kg of atorvastatin, alongside 100 mg/kg of the aqueous extract and olive oil given after 30 mins while animals in group VIII were treated with 30mg/kg atorvastatin along side 100mg/kg of the aqueous extract and oil given after 30mins. The dose of olive oil received by the rats in all the groups was 5ml/kg oral dose (Iyer and Patil, 2011).

Blood samples were collected via the abdominal aorta, 2 hours after olive oil treatment under chloroform anaesthesia. The blood samples were transferred immediately into lithium heparin tubes and then centrifuged for 10mins at 4000 rpm. The supernatant clear plasma thus obtained was transferred carefully with the aid of a micropipette into small plain test tubes for estimation. The clear supernatant from the lithium heparin was used for the estimation of total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL). The samples were analyzed by measuring absorbance by UV Spectrophotometer (Spectrum Lab 22 CP-), using diagnostic kits (Randox Laboratories Ltd, United Kingdom) according to manufacturer's protocol.

Total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C) and arterogenic index of plasma (AIP) were determined according to the methodology described by Abell *et al.,* 1952, Allain *et al.,* 1982 and Roeschlaw *et al.,* 1974; Jacobs and Vandemark, 1960, Tietz, 1990; Friedewald, 1972; Friedewald, 1972; Dobiasova, 2006, Nwagha *et al.*, 2010 and Tietz, 1990 respectively.

Results

Phytochemical screening

Phytochemical screening of the aqueous leaf extract of *Emilia praetermissa* showed the presence of tannins, flavonoids, steroids, cardiac glycosides, carbohydrate, reducing sugar and terpenoids and absence of alkaloids and saponins as shown in Table 1.

Effect of extract on TC

The aqueous extract significantly ($p < 0.01$) reduced (47.80 ± 4.75 mg/dl to 37.22 ± 2.18 mg/dl) in the TC level at the dose of 200 mg/kg of the extract after 2 hours. When 100 mg/kg of the extract was co-administered with 30 mg/kg atorvastatin, TC was also reduced but not significantly. This is as shown in Table 2.

Table 1. The preliminary phytochemical screening of the aqueous leaf extract of *Emilia praetermissa*.

Constituents	Observation
Carbohydrate	Present
Saponins	Absent
Tannins	Present
Alkaloids	Absent
Cardiac Glycosides	Present
Flavonoids	Present
Terpenoids	Present
Reducing Sugar	Present
Steroids	Present

Table 2. Effect of Aqueous Extract of the Leaves of *Emilia praetermissa* on the Total cholesterol (TC) level in rats.

Groups (mg/kg)	TC after 2 hrs (mg/dl)
Distilled water (2ml/kg)	40.09 ± 3.23
Olive oil (5 ml/kg)	47.96 ± 4.57
ATV	40.80 ± 2.61**
EP (100)	48.20 ± 2.03
EP (200)	37.22 ± 2.18*
EP (400)	54.50 ± 7.43*
EP (100 +15 ATV)	45.02 ± 3.82
EP (100+ 30ATV)	47.63 ± 3.80

Values are expressed as Mean ± SEM, n=5 per group. *$p < 0.01$, ** $p < 0.001$ significantly lower when compared to olive oil (hyperlipidemic) group, ATV: Atorvastatin, EP: *Emilia praetermissa*.

Effect of extract on TG

Table 3 shows the result of the effect of aqueous leaf extract of *Emilia praetermissa* on TG after 2 hours. The extract at 100 mg/kg and 200 mg/kg significantly ($p < 0.03$) reduced TG. That is 40.01% and 38.34 % reduction respectively. Co-administered of 100 mg/kg of the extract with 15 mg/kg and 30 mg/kg atorvastatin did not cause any further reduction in the TC. 50 mg/kg atovastatin significantly ($p < 0.01$) reduced the TG.

Table 3. Effect of Aqueous Extract of the Leaves of *Emilia praetermissa* on the Triglyceride (TG) level in Rats.

Groups (mg/kg)	TG after 2 hours (mg/kg)
Distilled water (2ml/kg)	54.43 ± 2.64
Olive oil (5ml/kg)	91.68 ± 12.10
ATV	57.60 ± 9.52
EP (100)	55.00 ± 8.82
EP (200)	56.53 ±5.47
EP (400)	139.32 ± 16.29
EP (100 +15 ATV)	111.55 ± 3.70
EP (100+ 30AVT)	98.39 ± 6.83

Values are expressed as Mean ± SEM, n=5 rats per group. * p < 0.03, p < 0.01 significantly lower when compared to the hyperlipidemic group, ATV : Atorvastatin, EP: *Emilia praetermissa*

Effect of extract on HDL

Table 4 shows that HDL was significantly (p < 0.004) increased (48.44 %) by 400 mg/kg of the extract. Concomitant administration of 100 mg/kg of the extract with 15 mg/kg and 30 mg/kg atorvastatin respectively resulted in significant (p < 0.007 and p < 0.002 respectively) increase in HDL. The percentage reductions are (54.36 %) and (58.43 %) respectively. This was dependent on the dose of the atorvastatin since the dose of the extract remained constant in the two groups. Atorvastatin (50 mg/kg) alone also significantly (p < 0.004) increased the HDL level. There appears to be a synergistic effect observed when 100 mg/kg of the extract was co-administered with 30 mg/kg atorvastatin.

Effect of extract on LDL

Table 5 shows that there were significant (p < 0.005 and p < 0.002 respectively) reductions in LDL level by the aqueous extract of *Emilia praetermissa* (100 mg/kg) when it was administered concomitantly with 15 mg/kg and 30 mg/kg atorvastatin respectively. Atorvastatin (50 mg/kg) also significantly (p < 0.004) reduced the LDL level.

Table 4. Effect of aqueous Extract of the Leaves of *Emilia praetermissa* on the high density lipoprotein (HDL) level in rats.

Groups (mg/kg)	HDL after 2 hours (mg/dl)
Distilled water (2 mg/ml)	40.87 ± 3.74
Olive oil(5 mg/ml)	21.52 ± 1.82
ATV	26.74 ± 1.03
EP (100)	25.65 ± 1.25
EP (200)	35.01 ± 4.21
EP (400)	41.74 ± 3.33
EP(100+15 ATV)	47.15 ± 5.36*
EP(100+ 30ATV)	51.77 ± 5.44

Values are expressed as Mean ± SEM, n=5 rats per group. * p< 0.007 significantly lower than distilled water group, p< 0.004, p < 0.002 significantly higher than hyperlipidemic group. ATV: Atorvastatin, EP: *Emilia praetermissa*.

Table 5. Effect of Aqueous Extract of the Leaves of *Emilia praetermissa* on the Low-density lipoprotein (LDL) level in rats.

Groups (mg/kg)	LDL after 2hours (mg/dl)
Distilled water (2 mg/ml)	-3.77 ± 4.20
Olive oil (5 mg/ml)	0.86 ± 1.41
ATV	-7.02 ± 2.87**
EP (100)	6.75 ± 1.77
EP (200)	-14.01 ± 9.15
EP (400)	-15.11 ± 8.28*
EP (100 +15 ATV)	-26.14 ± 4.63
EP (100 + 30ATV)	-27.64 ± 6.16***

Values are expressed as Mean ± SEM, n=5 rats per group. * p< 0.005, **p< 0.004, ***p < 0.002 significantly lower than hyperlipidemic group. ATV: Atorvastatin, EP: *Emilia praetermissa*. < 0.02). ATV: Atorvastatin, EP: *Emilia praetermissa*.

Effect of extract on AIP

Table 3.6 shows that the AIP level was significantly ($p < 0.002$) decreased by the aqueous leaf extract of *Emilia praetermissa* (100 mg/kg) when it was co-administered with 30 mg/kg atorvastatin. It was also reduced at 200 mg/kg and 400 mg/kg, although not significantly.

Table 6. Effect of Aqueous Extract of the Leaves of *Emilia praetermissa* on the atherogenic Index (AIP) of olive oil induced hyperlipidemia in rats.

Groups (mg/kg)	AIP after 2hours (mg/dl)
Distilled water(2 ml/kg)	0.39 ± 0.03
Olive oil (5 ml/kg)	0.53± 0.08
ATV	0.48 ± 0.13
EP (100)	0.42 ± 0.13
EP (200)	0.34 ± 0.06
EP (400)	0.49 ± 0.09
EP (100 +15 ATV)	0.49 ± 0.08
EP (100+ 30ATV)	0.30 ± 0.01*

Values are expressed as Mean ± SEM, n=5 per group. * p< 0.002 significantly lowered when compared to hyperlipidemic group ($P < 0.02$). ATV: Atorvastatin, , EP: *Emilia praetermissa*.

Discussion

Emilia praetermissa significantly caused decrease in plasma TC, TG, LDL, and AIP levels of the rats and also increased HDL significantly when compared with the hyperlipidemic group. Co-administration of the extract and atorvastatin significantly increased HDL and decreased LDL and AIP. Phytochemical screening of the aqueous leaf extract showed the presence of tannins, flavonoids, steroids, cardiac glycosides, carbohydrate, reducing sugar and terpenoids and absence of alkaloids and saponins.

The cholesterol lowering effect of the extract may have been due to the tannins, terpenoids and flavonoids content (Soetan, 2008; European Food and Safety Authority, 2009). Tannins significantly reverse the increased blood glucose, total cholesterol, triglycerides, low density lipoprotein and also significantly restore the insulin and high density lipoprotein in the serum. In addition tannins significantly restore the activity of antioxidant enzymes such as superoxide dismutase, catalase and decreased the, glutathione peroxidase, and glutathione, thereby restoring the antioxidant status of the organs to almost normal levels. (Velayutham *et al.*, 2012).

Flavonoids have been reported to reduce the levels of TG, TC and LDL-C remarkably. They therefore possess lipid-favourable activity. (da Silva *et al.*, 2001; Farboodniay *et al.*, 1993). Some terpenoids, which are intermediates in cholesterol synthesis, regulate the activity of 3-hydroxy-3-methylglutaryl-coemzyme A (HMG-CoA) reductase, a key enzyme in cholesterol synthesis by controlling the degradation of the enzyme (Bradfute and Simoni, 1994; Lehmann *et al.*, 1995). Such functions of dietary terpenoids are significant for the trials to manage disease conditions such as cancers or cardiovascular diseases using food factors. Daily intake of dietary terpenoids, which activate PPARs as we described above, may be valuable for the control of carbohydrate and lipid disorders. (Tsuyoshi *et al.* 2010).

Raised plasma total cholesterol level is a recognized and well-established risk factor for developing atherosclerosis and other cardiovascular diseases (Ademuyiwa *et al.*, 2005) and is often found in hypertension (Zicha *et al.*, 1999). Therefore, a reduction in plasma total cholesterol level reduces the risk of cardiovascular diseases. Thus, the significantly lower plasma total cholesterol levels produced by the extract, connotes the ability of the extract to protect against cardiovascular diseases.

The extract significantly reduced plasma levels of triglycerides. Elevated plasma triglyceride levels is both an independent and synergistic risk factor for cardiovascular diseases (Martirosyan *et al.*, 2007; McBride, 2007; Brunzell *et al.*, 2008) and is often associated with hypertension (Shepherd, 1998; Zicha *et al.*, 1999), abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus (Shepherd, 1998; Krauss *et al.*, 2006; McBride, 2007; Brunzell *et al.*, 2008). There is an inverse

association between plasma TG concentration and HDLC levels and therefore, since there is reduction in the TG concentration by the aqueous leaf extract of *Emilia praetermissa,* one should expect an increase in the plasma concentration of HDLC.

The result of the study showed a significantly lower plasma LDL levels in the treated animals. Decreases in plasma LDL cholesterol have been considered to reduce risk of coronary heart disease (Rang *et al.*, 2005). High plasma levels of LDL and VLDL cholesterol is a risk factor for cardiovascular disease (Ademuyiwa *et al.*, 2005; Lichtennstein *et al.*, 2006) and often accompanies diabetes mellitus (Rang *et al.*, 2005; Brunzell *et al.*, 2008), obesity (Krauss *et al.*, 2006) and hypertension (Shepherd, 1998; Zicha *et al.*, 1999).

In this study, the extract increased plasma HDL cholesterol levels. Decreased plasma HDL cholesterol is a risk factor for cardiovascular diseases (Lewis and Rader, 2005; Rang *et al.*, 2005; Lichtennstein *et al.*, 2006; Martirosyan *et al.*, 2007) and is often found in hypertension (Shepherd, 1998; Zicha *et al.*, 1999), obesity (Krauss *et al.*, 2006) and diabetes mellitus (Shepherd, 1998; Rang *et al.*, 2005; Brunzell *et al.*, 2008). Clinical data show that increase in plasma HDL cholesterol concentration decreases cardiovascular risk (Assmann and Gotto, 2004; Rang *et al.*, 2005). Increases in plasma HDL cholesterol have been considered to reduce risk in coronary heart disease (Assmann and Gotto, 2004; Rang *et al.*, 2005). High HDL exerts a protective effect by decreasing the rate of entry of cholesterol into the cell via LDL and increasing the rate of cholesterol release from the cell (Marcel *et al* ., 1980) i.e. by enhancing reverse cholesterol transport by scavenging excess cholesterol from peripheral tissues followed by esterification through lecithin: cholesterol acyltransferase and delivering it to the liver and steroidogenic organs for subsequent synthesis of bile acids and lipoproteins and eventual elimination from the body (Assmann and Gotto, 2004; Ademuyiwa *et al.*, 2005); and inhibiting the oxidation of LDL as well as the atherogenic effects of oxidized LDL by virtue of its antioxidant (Assmann and Gotto, 2004; Ademuyiwa *et al.*, 2005; Brunzell *et al.*, 2008) and anti-inflammatory property (Ademuyiwa *et al.*, 2005). Significant increase observed in this study could be an advantage in reducing cardiovascular risk.

In this study, we observed that the extract produced significantly lower atherogenic index, especially when it was used in combination with artovastatin. Atherogenic indices are powerful indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease and vice versa (Brehm *et al.*, 2004; Dobiásová, 2004; Usoro *et al.*, 2006; Martirosyan *et al.*, 2007). It has been suggested that AIP values of -0.3 to 0.1are associated with low, 0.1 to 0.24 with medium and above 0.24 with high CV risk. (Dobiasova, 2006).
Low atherogenic indices are protective against coronary heart disease (Usoro *et al.*, 2006).

All of these results indicate a possible protective mechanism of the extract against the development of atherosclerosis and coronary heart disease, as well as the dyslipidemic conditions that characterize obesity, hypertension and diabetes mellitus.

Conclusion

The results suggest a possible protective role of the extract against the development of atherosclerosis and coronary heart disease, as well as the dyslipidemic conditions that characterize diabetes mellitus, hypertension, metabolic syndrome and obesity. It can therefore be concluded that the aqueous extract of *Emilia praetermissa* could be used as an effective supplement in hyperlipidemic patients.

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