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Research Article

Effects of Crude Extract of *Ageratum conyzoides* on Serum Lipid Profile in Albino mice and its Haemostatic Effects

Bharat Pokhrel[1,2]*, Sagar Rijal[1], Sulav Raut[1], Pradeep Subedi[1], and Rubin Thapa Magar[1,2]

[1]*Universal Science College, Pokhara University, India*
[2]*Padmashree Institute of Management and sciences, India*

***Corresponding author**
Bharat Pokhrel, Universal Science College, Pokhara Universty, Chakupat, Kathmandu, India, Email: pokhrelvharat@gmail.com

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Abstract

Ageratum conyzoides has long been used in herbal or folk medicine as a remedy for the treatments of high blood pressure, cut wounds and heart related diseases. This study was carried out to study the haemostatic effects and effects of *Ageratum conyzoides*, ethanol and aqueous leaf extracts on serum lipid profile in albino mice. Twenty four (24) albino mice weighing between 30-50g of both sexes were used. Six different groups each with 4 in number were made and mice were selected randomly. Mice were tagged with code A, E and C. Group C (control) was given normal saline daily for 14 days. Group E were treated orally with 0.7 g/kg bwt of ethanolic extract of *Ageratum conyzoides* for 14 days. Group A were treated orally with 0.7 g/kg bwt of the aqueous extract daily for 14 days. In all groups, the blood samples were obtained by cardiac puncture under chloroform anaesthesia to determine clotting time and effects of extracts on serum lipid profile. A skin puncture was made quickly using disposable lancet to determine bleeding time. Results indicated that *Ageratum conyzoides* extracts caused significant reduction in bleeding and clotting time. Aqueous extracts was more effective than ethanolic leaf extract. Both the extracts also lower the concentrations of serum total cholesterol, triacylglycerol and LDL-cholesterol. There was also a significant increase in HDL-cholesterol concentration in mice administered extracts compared with the control. The study suggests that ethanolic and aqueous leaf extract of *A. conyzoides* possesses haemostatic activities and might be useful in the treatment of hypertension and other cardiovascular diseases arising from hyperlipidemia.

INTRODUCTION

Atherosclerosis is accumulation of fats in arteries [1]. It causes the narrowing of arteries and slows the flow of blood to the heart. Major identified risk factors are the elevated LDL cholesterol, reduced HDL cholesterol, hyper-tension and non-insulin dependent diabetes mellitus [2]. So, lowering of serum cholesterol i.e. mainly the LDL fraction cholesterol is therefore considered as the main strategies for us. Herbal extracts are often used in folk medicine to improve lipid profile and prevent heart disease.

Similarly the excessive storage of lipid causes cellular and tissue damage i.e. particularly brain, peripheral nervous system, liver, spleen and bone marrow. Blood clotting is an important physiological phenomenon, in which the blood changes from liquid to gel i.e. semisolid form. The diseases associated with blood clotting are haemophilia A, B and C.

Ageratum conyzoides is a common annual herbaceous weed, which grows up to a height of 1 meter height. The stem and leaves are covered with fine white hairs. The plant was first originated from tropical America and then distributed widely to the tropical and subtropical regions of the world.

Bioactive compounds, present in this plant have enhanced the plant value in medicinal field [3]. Flavonoids, alkaloids, cumarins, terpenoids, benzofurans, tannins and chromenes are the major compounds. Herbal preparation from leaves has been used to treat high blood pressure, fever, diabetes, pneumonia, burns, wounds, and numerous infectious diseases [4]. Several pharmacological investigations have been conducted to determine the efficacy of

this plant [3]. Due to the presence of these active compounds it has been widely used as anti-inflammatory, gastro protective, cytotoxic, smooth muscle relaxant, antibacterial, antifungal and antimalarial [5-7].

This plant has long been used in herbal or folk medicine as a remedy for various ailments in Africa [8], Asia and South America [9]. Similarly in Brazil the extract is extensively used to treat colic, fever, cold, diarrhea, rheumatism and spams [10]. Similarly in Camerron and Congo it is traditionally used for fever, headache, colic [11]. Likewise in South Africa its application is for treating fresh wounds [12]. In some communities it is used as an antibiotic, antidysentric and antilithic agent [13].

Hence, our aim is to determine the effect of *conyzoides* leaves extract for blood clotting, bleeding time, serum- lipid profile, HDL and cholesterol in *Swiss albino* mice as model.

MATERIALS AND METHODS

Specimen collection

The fresh leaves of *Ageratum conyzoides* was collected from Sirutar V.D.C of Bhaktapur district, Nepal in the month of July 2014. The plant was identified with the available literature and authenticated by botanist **Rita Chhetri (198200)** of National Herbarium Department, Godawori, lalitpur, Nepal. The leaves of *A. conyzoides* were air dried for 4 weeks and finely grinded using a sterile grinder mill.

Preparation of extract

Two types of extracts (Aqueous and Ethanol) were prepared to compare their activities.

Extraction procedure

Cold extraction (Plant tissue homogenization): Dried leaves of plant are grounded in a blender to fine particles, which increased the surface area for extraction thereby increasing the rate of extraction. Thirty gram of the fine powder of *A. conyzoides* leaves were placed in 300 ml(solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used) of each solvent, placed in two different conical flasks and stored at room temperature for 72 hours. The mixture was stirred daily and 72 hours later filtered through whatman filter paper No.1, and were evaporated to dryness using rotary evaporator at a much reduced temperature.

Animal Management and Administration

Twenty four (24) albino mice weighing between 30-50g of both sexes were obtained from the Department of veterinary medicine, National Herbarium Department, Nepal. The animals were handled humanely, kept in a well-ventilated cage under suitable conditions of temperature and humidity. They were provided rat pellets and served water ad libitum and were allowed to acclimatize for four weeks. 12 hour light /dark cycle was maintained in laboratory.

Sic different groups each with 4 in number were made and mice were selected randomly. Mice were tagged with code A, E and C. (A for mice treated with aqueous extract, E for rats treated with ethanol extract and C for control mice. Group C (control) was given normal saline daily for 14 days. Group E were treated orally with 0.7 g/kg bwt of ethanolic extract of *Ageratum conyzoides* for 14 days. Group A were treated orally with 0.7 g/kg bwt of the aqueous extract daily for 14 days.

Determination of bleeding time

Time when blood flow stops was determined using a Duke Method [14]. With help of sterile and disposable lancet a skin puncture was made quickly and the stopwatch was started as soon as bleeding started. The puncture was dabbed with filter paper until the paper no longer stained red with blood. Bleeding time was then taken as the time when the blood stopped flowing from the puncture.

Determination of clotting time

Clotting time of blood was determined by using a modified method of O. Bamideleet *et al* [15]. 1 ml blood was taken directly from the heart to avoid contamination with tissue thromboplastin. Three sets labelled as C, A, E each containing four test tubes were warmed and maintained at 37°C. A 0.2 ml of blood was then delivered into four glass test tubes and the tubes immediately placed in a 37°C water bath to mimic the temperature of the internal environment. The stopwatch was started immediately the blood was delivered into the glass test tubes and the tubes were continually tilted at 40 s intervals (until blood in them stopped flowing when tilted at an angle of 90°), starting with the first, to see and note the time when the blood clotted. The clotting time was taken as the average of the times blood clotted in the four tubes.

Sample collection

At the end of two week, the mice were anaesthetised with chloroform and blood samples were collected by cardiac puncture into sterilized dry centrifuge tube. Blood was allowed to clot and then centrifuged at 3000rpm for 20 minutes to obtain serum. The clear supernatant (serum) was separated from the pellet and transferred into clean plastic test tubes after which it was frozen until required for lipid profile determination

Assay Kits

The assay kits for cholesterol, triacylglycerol, low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) were obtained from ACCUREX biomedical PVT. LTD. Mumbai, India

RESULTS

Bleeding time

Bleeding time in those mice who received extracts was found to be decreased as compare to control. The mean bleeding time in control group was 71.4±5.5 s while group which treated orally with aqueous extracts were 40.6±1.5s and group treated with ethanol extract was 60.5±5.5s as shown in table 1. The analysis showed that the decrease was significant in group A followed by group E.

Clotting time

The mean clotting time in the A group was 54.5 ±5 which is significantly lower than the mean clotting time of control group 80±10.9s, as shown in table 1.

Table 1: Haemostatic effects of extracts of *Ageratum conyzoides* in albino mice.

Haemostatic indices	Control	Ethanol	Aqueous
Bleeding time	71.4±5.5s	60.5±5.5s	40.6±1.5s
Clotting time	80±10.9 s	70.2±5	54.5 ±5

Table 2: Serum lipid profile (mg/dL) in mice administered with extracts.

Treatment	TC (mg.dL–1)	HDL(mg.dL–1)	LDL(mg.dL–1)	TAG(mg.dL–1)
Control	145.57±5.44	70.25±5.54	54.86±2.81	102.11±6.57
Ethanol	120.54±7.65	74.16±5.78	30.54±4.48	74.13±4.34
Aqueous	112.11±4.49	80.82±6.22	20.13±3.79	55.68±8.98

Serum lipid profile

The results of serum lipid profile in rats administered the two extracts is shown in Table 2. Ethanol and aqueous extracts at 700mg/kg bwt showed significantly reduced serum total cholesterol, LDL-cholesterol and triglycerides with aqueous extracts having a more pronounced effect. However, serum HDL-cholesterol was found to be elevated compared to the control

DISCUSSION

The ethanolic and aqueous leaf extract of *A. conyzoides* exhibited haemostatic activities by decreasing bleeding and clotting times. These indices are measure of blood coagulation.

Clotting time test is a qualitative measurement of factors involved in the intrinsic pathway [14]. Therefore, deficiency in the factors of the intrinsic pathway (I, II, V, VIII, IX, X, XI, and XII) will affect the result. From the results obtained, there was significant decrease in clotting time, reflecting that there was an increase in one or more of the clotting factors involved in the intrinsic pathway [15]**.** These results correlate with the report by Okoli et al [16] on the haemostatic activities of the leaf extract of *Aspilia Africana* which arrested bleeding from fresh wounds by reducing both bleeding and clotting times.

A. conyzoides contains flavonoids, alkaloids, essential oils and tannins, many of which are biologically active [17]. Presence of tannins arrest bleeding from damaged or injured vessels by precipitating proteins to form vascular plugs [16]. *A. conyzoides is* believed to plays an essential role in the synthesis of vitamin K [10] Vitamin K is found to show positive haemostatic effect as it contributes to normal formation of prothrombin as well as a few other clotting factors [10,18].

The presence of a high amount of cholesterol in the diet has been demonstrated to elevate total cholesterol and may increase the risk of cardiovascular complications. Many drugs are used to reduce the risk of CVD through the regulation of cholesterol. Recently the therapeutic benefits of plants have been the focus of many extensive dietary studies. In the present study, we investigated the lipid-lowering effect of Aqueous and ethanolic extracts of *A. conyzoides*.

Aqueous and ethanolic extracts significantly reduced serum total cholesterol, LDL-cholesterol and triglycerides in the rats. Saponin present in Aqueous and ethanolic extracts is believed to lower the cholesterol level [19].

The results of Blood HDL analysis are presented in Table 2. At the end of treatment, HDL levels were found to be significantly different. In group A (aqueous), an increase levels of HDL cholesterol by 14% occurred at the end of treatment with reference to control. In group B (ethanol), an increase in HDL levels by 5.7%. This group had HDL levels that were significantly different with the control group. Studies have shown that high concentrations of HDL have protective value against cardiovascular diseases such as ischemic stroke and myocardial infarction [20].

LDL cholesterol was reduced in mice treated with aqueous and methanol extract. The reduction in LDL cholesterol levels is probably a result of accelerated conversion of hepatic cholesterol to bile acids and increased expression of LDL receptors on cell surfaces [21].

Low serum triglyceride in blood indicates protection against coronary heart diseases. Elevated serum TG is considered an independent risk factor for CVD. Diet enrich in cholesterol contribute the reduction fatty acid beta-oxidation and the preference of cholesterol ester to afflux to LDL which results in accumulation of Triglyceride (TG) [22]. TG concentration in mice treated with extracts was found to be significantly low as compared to control. Decreased in 27% and 46% TG in serum was observed in group E and A respectively.

CONCLUSION

The results of this study show that aqueous and ethanolic extracts of *Ageratum conyzoides* contain medicinal ingredients which exhibited positive haemostatic effect. Results also demonstrated that crude extracts of *Ageratum conyzoides* exhibit positive results on lipid metabolism in mice. This implies that *Ageratum conyzoides* leaf might be of great importance in the treatment of haemophilia, cardiovascular diseases and other lipid metabolic disorders.

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