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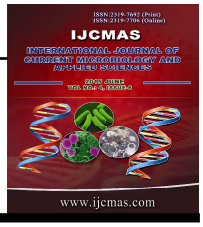

Original Research Article

Evaluation of Free radicals scavenging and glucose uptake by isolated rat hemi-diaphragm study of *Andrographis echioides* an indigenous Medicinal Plant

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Keywords

Hydroalcholic extract, Hyperglycemia, Antioxidant, Nanoparticles, *Andrographis echiodies*

A B S T R A C T

Diabetes is a clinical syndrome characterized by hyperglycemia due to relative deficiency of insulin. There has been an enormous interest in the development of alternative medicine for diabetes specifically screening for phytochemicals with ability to delay or prevent glucose absorption. The synthesis of nanoparticles from plant sources has proved to be an effective and alternate method for the novel production of nanoparticles.The goal of the present study was to provide *in vitro* evidence for potential inhibition of *in vitro* antioxidant and antidiabetic activity of silver nano particles synthesized hydroalcholic extract of *Andrographis echioides*. The extract showed antidiabetic activity was studied using the glucose uptake by isolated rat hemi-diaphragm *in vitro* model. The important outcome of the study will be the development of value added products from medicinal plants of India for biomedical and nanotechnology based industries. Our assay results suggests that hydroalcholic extract of *Andrographis Echiodies* exhibit dose dependent increase in percentage inhibitory activity on *in vitro* antioxidants and antidiabetic activities. The ABTS radical scavenging of Hydroalcholic extract has highest IC_{50} value 14.33µg/ml and other antioxidants of Nitric oxide, DPPH, Ferric ion reducing power, Lipid peroxidation of the extract increased with the increasing amount of the concentration.

Introduction

Reactive oxygen species (ROS) are constantly produced in cells by cellular metabolism and by exogenous agents. They are essential for life because they are involved in cell signaling and are used by phagocytes for bactericidal action (Halliwell, 1999). Recently, increasing evidence highlights that overproduction of ROS and oxygen-derived free radicals may contribute to a variety of pathological effects (e.g. DNA damage, carcinogenesis and cellular degeneration) and induce many

diseases including aging, cancer, atherosclerosis, diabetes and rheumatoid arthritis (Circu and Aw, 2010; Jeong and Liu,2012).

Traditionally, many medicinal plants are currently used in India for the treatment of diabetes and its efficacy has been proved scientifically. *Andrographis echioides* or *Indoneesiella echioides* L. Nees (False Water Willow) is an herb widely distributed in the dry districts of tropical India and Srilanka (Chattopadhyay,1992). In traditional medicine, the leaf juice of this plant is used as a remedy for fevers (Gamble, 1956). The plant from genus *Andrographis* is used in goiter, liver diseases (Kirtikarand Basu,1975), fertility problems, bacterial (Nadkarni and Nadkarni,1976), malarial and fungal disorders. Leaf juice boiled with coconut oil is used to control falling and graying of hair (Qadrie et al., 2009).

Phytochemicals in fruits, vegetable species and traditional herbal medicinal plants have been found to play protective role against many human diseases. Phytocomponents including phenolics, flavonoids and tannins and various plants or herbal extracts have been reported to be radical scavengers and inhibitors to lipid peroxidation (Pandi Kumar, 2007).Due to uniqueness of curing different ailments, the whole plant of *Andrographis echioides* was selected for the study.

The aim of the present study was to examine the in vitro antioxidant and glucose uptake method of rat hemi diaphragm of extracts of the *Andrographis echioides*. Little to no information exists currently in scientific literature on the *in vitro* antioxidant and antidiabetic activities of silver nanoparticle synthesized hydroalcoholic extract of *Andrographis echioides.*

Collection and identification of plant

*Andrographis echioides or Indoneesiella echioides*used in the study was identified in the ABS Botanical Conservation and Training Centre, India-Southern Circle-Salem, Tamil Nadu, India. The reference material was kept under number [No: AUT/MCAS/035]. Fresh plants were collected randomly from the region of ABS Garden, Salem, Tamil Nadu.

Materials and Methods

Animals

Colony bred, healthy Wister albino rats of either sex weighing 200-220 g were taken for the study. The animals were fed on standard laboratory diet with water *ad libitum* and housed at room temperature. The rats were kept of fasting overnight with free access to water during the experiment in the same ambience. The animals were divided into four groups of six animals each. The rats were fasted overnight and killed by cervical dislocation. The diaphragms were dissected out quickly with minimal trauma and divided into two equal halves. Two diaphragms from the same rat were not used for the same set of experiments.

Preparation of hydroalcoholic extract

The shade dried coarsely powdered leaves of *Andrographis echioides*(200g) was extracted with 500 ml of 80% aqueous ethanol by maceration at room temperature for 72 hours. After extraction, the extract was filtered, concentrated to dryness in rotavapour under reduced pressure and controlled temperature (40-500C). Dark yellowish brown colour residue was obtained and it was coded as *Andrographis echioides.* The residue was then stored in desiccators. The extractive value of hydro-

alcohol extract of *Andrographis echioides* was found to be 2-5g.

Silver nano particles synthesis using

3 mM solution of silver nitrate was prepared. 20 ml of the plant extract was mixed with 80 ml of 3 mM of silver nitrate solution. The colour changed from yellow to reddish brown colour indicating the formation of silver nanoparticles. The AgNps thus obtained was purified by repeated centrifugation at 7000 rpm for 10 min. The pellet was collected and dried. The Chemical tests were carried out in AgNPs for Antioxidant and Antidiabetic properties . The pH of the solution was also determined.

Radical scavenging activity

The efficacy of the hydroalcoholic extract of *Andrographis echioides* was studied under *in vitro* conditions. The free radical scavenging activity of plant of the *Andrographis echioides* against 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Molyneux, 2004), 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) [Re,1999], hydrogen peroxide (Ruch,1989), super oxide (Liu et al., 1997), hydroxyl (Halliwell,1992) and nitric oxide radical (Govindarajan et al., 2003) and total reducing potential(Oyaizu, 1986) and lipid peroxidation (Ohkawa, 1979) assay were also studied.

Statistical analysis

The biochemical parameters studied were subjected to statistical analysis using Sigma Stat statistical package (Version3.1). The experimental results were expressed as mean ± SD.

Experimental design

Five sets of six graduated test tubes each, were grouped as follows:

Group 1: 2 mL Tyrode solution with 2 g% glucose (Normal control);
Group 2: 2 mL Tyrode solution with 2 g% glucose +insulin (Nova Nordisk) 0.62 mL of 0.4 U/mL solution (insulin-treated group)
Group 3: 2 mL of Tyrode solution with 2 g% glucose + extract (1000 µg/mL)
Group 4: 2 mL of Tyrode solution with 2 g% glucose + extract (500, µg/mL)
Group 5: 2 mL of Tyrode solution with 2 g% glucose +insulin (Nova Nordisk) 0.62 mL of 0.4 U/mL solution + chrysin (1000 µg/mL).

The volumes of all graduated test tubes were made up to 4 mL with distilled water. The hemi-diaphragms were placed in test tubes and incubated for 30 min at 37°C bubbled with oxygen with continuous shaking. Glucose uptake per g of tissue was calculated as the difference between the initial and final glucose content in the incubated medium.

Results and Discussion

Several concentrations ranging from 75-500 µg/ml of the hydroalcohol extract of plant of *Andrographis echioides* were tested for their antioxidant activity indifferent *in vitro* models. The percentage of inhibition was observed and found that free radicals were scavenged by the test compounds in a concentration dependent manner upto the given concentration in all the models.

DPPH radical scavenging activity

The plant extract demonstrated H-donor activity. The DPPH radical scavenging activity was detected and compared with standard Rutin (Table 1). The IC50 values for *A.echioides* plant extract and ascorbic acid are 457.33µg/ml. DPPH assay is one of the most widely used methods for screening

antioxidant activity of plant extracts. DPPH radical was used as a stable free radical to determine antioxidant activity of natural compounds (Ozturk et al.,2007). The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals (Stoilova et al 2007) Thus, the purple colour of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) will reduce to 2, 2-diphenyl-1-picrylhydrazine (yellow coloured) (Akowuah,2005). Scavenging of the stable radical (DPPH) is considered a valid and easy assay to evaluate scavenging activity of antioxidants. Figure1 Radical scavenging activity of *Andrographis echioides* and Rutin against DPPH radical increase at increasing concentration.

Radical scavenging activity

A.echioides plant extract effectively reduced the generation of nitric oxide from sodium nitroprusside. Inhibition increased with increasing concentration ofthe extract. It showed good NO scavenging activity with IC50 of 453.33µg/ml compared with Rutin which served as positive control(Table 1). Nitric oxide is a free radicals product in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases [Sun,2005]. In the present study the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 250C was reduced by the hydroalcoholic extract of *A.echioides*. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. Figure2 Radical scavenging activity of *Andrographis echioides* and Rutin against NO radical increase at increasing concentration.

ABTS radical scavenging activity

Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals (Halliwell, and Gutteridge, 1993).The decolorization of ABTS•+ cation radical is an unambiguous way to measure the antioxidant activity of phenolic compounds (Gulcin et al., 2005). Recently, Awika*et al*, (RajuIlavarasan et al., 2005) found positive correlations between phenolic content and antioxidant activity tested using the Oxygen Radical Absorbance Capacity (ORAC), ABTS and the 2, 2- iphenyl-1-Picrylhydrazyl (DPPH) assays. Thus the ability of a compound to scavenge ABTS•+ radical can demonstrate oxygen radical absorbance capacity. Hydro-alcoholic extract of AE plant extract showed potent ABTS radical scavenging activity (IC50 14.3µg/ml) which is comparable to Rutin (Table 1). Figure2 Radical scavenging activity of *A.echioides* and ascorbic acid against NO radical increase at increasing concentration. Figure3 Radical scavenging activity of *A.echioides* and Rutin against ABTS radical increase at increasing concentration.

Total reducing potential assay

Figure 4 shows the total reducing power of the extract. *A.echioides* plant extract exhibited maximum reducing power. The reducing power of the extract increased with increase in extract concentration and exhibited moderate reducing power that was comparable with that of vitamin C**.** The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom (Raju Ilavarasan et al.,

2005). The presence of reductants (i.e.antioxidants) in *A.echioides* plant extract causes the reduction of the Fe3+/ ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

Assay of lipid peroxidation

The lipid peroxidation inhibitory activity of the extract was detected and compared with standard ascorbic acid. The IC50 values for *A.echioides* plant extract and standard BHA273.33 µg/ml respectively. In addition to free radical scavenging activity, the extract was evaluated for its ability to protect bio membrane from oxidative damage. Initiation ofthe lipid peroxidation by ferrous sulphate takes place either through ferryl-perferryl complex or through $^{\cdot}OH$ radical by Fenton's reaction. The inhibition could be caused by the absence of ferryl-perferryl complex or by scavenging the **.**OH radical or the superoxide radicals or by changing the$Fe^{3+/}Fe^{2+}$ or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself (Aruoma, 1999). The result shows that *A.echioides* plant extract has the capacity to prevent oxidative deterioration of mitochondrial membrane lipids. The beneficial effect of *A.echioides* plant extract on lipid peroxidation is attributed to its phenolic content.

Table.1 In vitro antioxidant properties of hydro alcoholic extract of *Andrographis echioides*

Samples	**DPPH**	**Nitric oxide**	**ABTS**	**Lipid peroxidation assay**
Hydro alcoholic extract of *Andrographis echioides*	457.33±3.06	453.33±5.77	14.33±0.58	273.33±5.77
Standard	Rutin	Rutin	Rutin	BHA
	3.16±0.08	58.63±0.47	15.77 ±0.12	123.27±4.25

Table.2 Glucose uptake in rat hemidiaphargm of AE extract

Test	**Glucose uptake (mg/dl/30 min)**
Normal	3.325±1.00
Insulin	16.85±2.89***
High dose (1000µg/ml)	11.14±1.94*
Low dose (500µg/ml)	7.125±1.06
Insulin + High dose	12.23±1.88**

Figure.1 DPPH Radical Scavenging Activity. Values are expressed as mean ± standard deviation

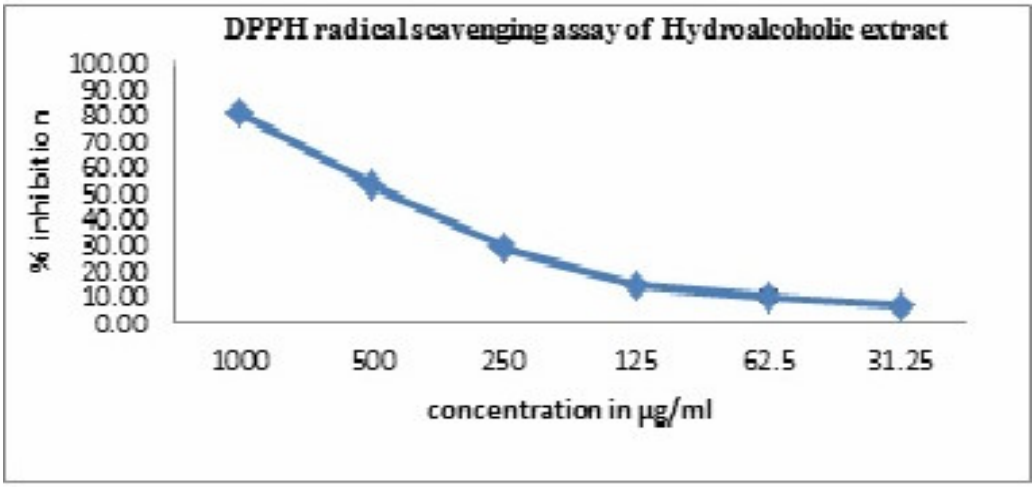


Figure.2 Radical Scavenging Activity. Values are expressed as mean ± standard deviation

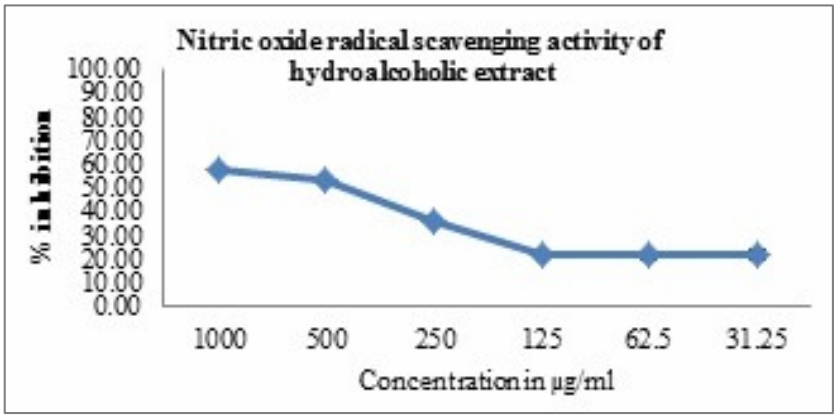


Figure.3 ABTS Radical Scavenging Activity.Valuesare expressed as mean ± standard deviation

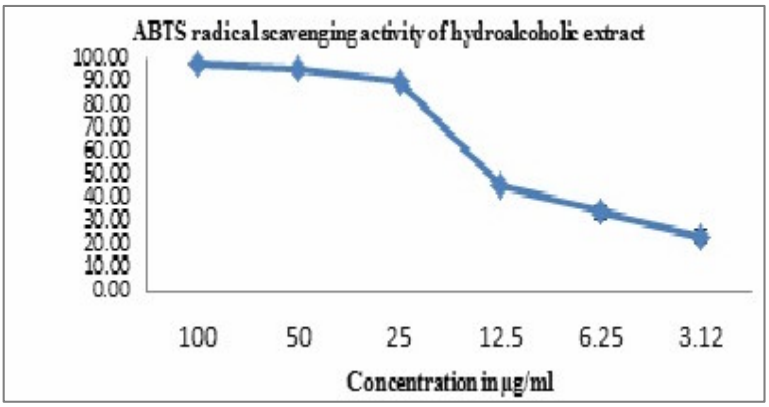
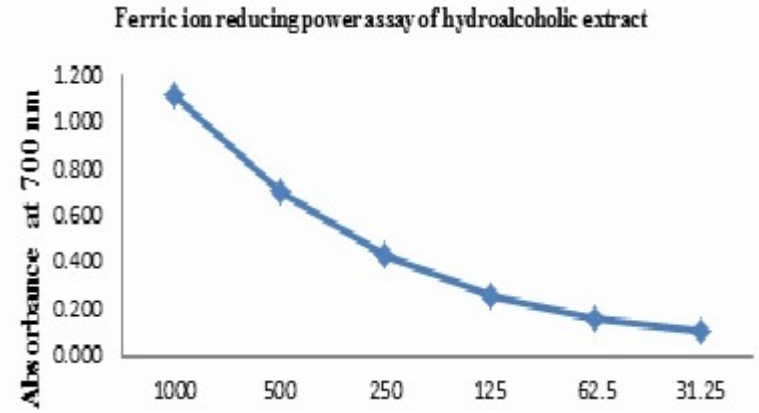


Figure.4 Total Reducing Potential Assay. Values are expressed as mean ± standard deviation



In Vitro antidiabetic activity

In vitro α-glucosidase inhibition of the hydro alcholoic extract of *Andrographis echioides* treated rats showed a significant inhibitory action of alpha glucosidase enzyme (Table 2). There was a proportionate increase in the percentage of α- glucosidase inhibition in a concentration dependent manner. Ascorbic acid and Rutin was used as a reference standard for the evaluation of α- glucosidase inhibitory action. Alpha glucosidase are the enzymes involved in the metabolism of carbohydrates. Alpha amylase degrades complex dietary carbohydrates to oligosaccharides and disaccharides, which are ultimately converted into monosaccharide by alpha glucosidase. Liberated glucose is then absorbed by the gut and results in postprandial hyperglycemia. Inhibition of alpha glucosidase limits postprandial glucose levels by delaying the process of carbohydrate hydrolysis and absorption. The plant based alpha glucosidase inhibitor offers a prospective therapeutic approach for the management of post-prandial hyperglycemia (Sunil *et al*., 2009). In the present study, exhibited appreciable alpha glucosidase inhibitory effects when compared with standard drug acarbose.

In conclusions, this study may provide pharmacological evidence for folklore medicinal uses of *Andrographis echioides* against the treatment of *in vitro* antidiabetic and antioxidant activities. Further studies are under progress in the laboratory to understand precise pharmacological properties of silver nano particles syntheseis of hydroalcholoic extract of *Andrographis echioides*.

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