Full Length Research Paper

Pharmacognostic and hypoglycemic studies of *Achyranthus aspera* L.

Ishtiaq Ahmad¹, Muhammad Ibrar¹, Barkatullah¹, Naveed Muhammad^{2*}, Zahir Muhammad¹
and Niaz Ali¹

¹Department of Botany, University of Peshawar, Peshawar, KPK, Pakistan.

²Department of Pharmacy, Hazara University, Havelain Campus, Abbottabad, KPK, Pakistan.

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In the present study, the ethanolic extract of *Achyranthus aspera* L. Leaves (EEA) were investigated for their pharmacognostic characteristics and hypoglycemic effects. The pharmacognosy of the plant was carried out using microscopic and macroscopic tools. Macroscopic studies revealed that the leaves were cauline, ramel, opposite, exstipulate, simple, sub-sessile, ovate, entire, acute, unicostate, reticulate, rough, coriaceous and hairy. The stem was erect, herbaceous, quadrangular, branched, solid, green and pubescent, while various internal parts were observed using powder drug for microscopic study. The hypoglycemic effect of the plant was studied in healthy normoglycemic rabbits. The EEA was tested in three doses (100, 150 and 200 mg/kg), and a dose dependent hypoglycemic effect was observed. The most significant hypoglycemic effect was observed against higher dose (200 mg/kg) which remained for 5 h while the hypoglycemic effect remained significant for 3 h against the lower dose. It was concluded that EEA has hypoglycemic properties and it is recommended for the treatment of diabetes mellitus II.

Key words: Pharmacognostic, hypoglycemic, *Achyranthus aspera* L.

INTRODUCTION

Achyranthus aspera L. is a common perennial herb, growing as a wild plant, in natural and cultivated habitats. *A. aspera* L. has a branched tap root system. The stem is herbaceous, quadrangular, branched, solid, green and pubescent. Leaves are simple and exstipulate. A spike type of racemose inflorescence occurs in this specimen. Almost all parts (seed, root, stem and leaf) of *A. aspera* L. possess medicinal value (Lans, 2007) but leaves have greater importance in the traditional (unani) system of health and herbal medicine formulation. Alcoholic decoctions of *A. aspera* L. are mostly used as anti-inflammatory (Girach Aminuddin and Khan, 1992) and infertility inducing agents (Chakraborty et al., 2002; Shibeshi et al., 2006). Furthermore, the plant possesses laticidal (Bagavan et al., 2008), antioxidant (Edwin et al., 2008),

anticancer (Chakraborty et al., 2002), anti-diarrheal and antidiysentric (Boily and Van Puyvelde, 1986; Girach Aminuddin and Khan, 1992), antimicrobial (Boily and Van Puyvelde, 1986) properties. The hypoglycemic effect of methanolic and aqueous extract of the whole plant of *A. aspera* has been reported (Akhtar and Iqbal, 1991). The pharmacognostic profile and hypoglycemic effect of the leaves of this plant was studied in order to validate traditional uses.

MATERIALS AND METHODS

Plant

The study was conducted in the Department of Botany, University

*Corresponding author. E-mail: dnaveedrph@gmail.com

of Peshawar. Plants of *A. aspera* was collected from different localities in the district of Charsadda, KPK, Pakistan. Plants were identified by Dr. Muhammad Ibrar, Department of Botany, and specimen was deposited there in the herbarium under voucher number 7455/Bot. The whole plant was powdered for microscopic study.

Preparation of extract

The ethanolic extract of the leaves was prepared using our previous reported method (Barkatullah et al., 2011; Muhammad and Saeed, 2011; Muhammad et al., 2012b, c). Powder of leaves was macerated in ethanol for 10 to 15 days. The extract was filtered and then evaporated. The dry extract was stored in refrigerator for use.

Animals

Domesticated rabbits weighing 1 kg obtained from the district Charsadda District, Pakistan were used. The animals were kept at standard laboratory conditions. The animals were shifted from animal house to the laboratory one hour prior to start the experiment.

Pharmacognostic study

The pharmacognostic profile of the plant was reported using our previous reported method (Muhammad et al., 2012; Ismail et al., 2011). Vein islet number, vein termination number, palisade ratio, stomatal number and stomatal indices were calculated from 1 mm² pieces of leaf of *A. aspera* boiled in concentrated chloral hydrate solution under high power compound microscope.

Macroscopical features

The following macroscopic characters of plant were noted: size, shape, margins, apex, surface, color, odour, taste, nature, texture of leaves, stem and root were also studied (Wallis, 1985; Evans and Trease, 2002).

Powder drug Study

Powdered drug was studied using chloral hydrate and iodine solution under compound microscope (Compound microscope). Organoleptic evaluation (color, odour, taste, fracture and touch) of the powdered drug was done using five senses (Pharmacopoeia, 1986).

Phytochemical Study

Qualitative chemical identification tests

Various phytochemical tests were performed for detection of various constituents preliminary, using well established procedures (Trease and Evans, 1989; Muhammad and Saeed, 2011).

Hypoglycemic effects

The healthy rabbits were divided into five groups of six. Group I was treated with normal saline (10 ml/kg) and acted as a control, Group II was treated with the standard hypoglycemic drug, glibenclimide,

Group III, IV and V were treated with the ethanolic extract of *Achyranthus aspera* (EEA) at doses of 100, 150 and 200 mg/kg, respectively. Blood glucose level was noted at the start of the experiment and then after 1, 2, 3, 4 and 5 h, respectively. Glibenclimide was used as a standard hypoglycemic drug. Swab soaked in methylated spirit was used as a disinfectant during collection of blood. Blood was obtained by puncturing the veins in the pinna of each animal by using a sharp lancet. A swab soaked in methylated spirit was used to disinfect the lancet after each use.

RESULTS

Macroscopical features

Leaves of *A. aspera* L. are cauline, ramel, opposite, exstipulate, simple, sub-sessile, ovate, entire, acute, unicostate, reticulate, rough, coriaceous and hairy. The stem is erect, herbaceous, quadrangular, branched, solid, green and pubescent. A tap and branched root system is present.

Microscopical features

Vein islet No., vein termination No., palisade ratio, stomatal No., and stomatal index are given as in Table 1.

Powder drug study

Trichomes of various types that is, multicellular, shagay, unicinate, uniseriate etc (a-d), glandular hairs (e-f), tracheids (l), parenchyma cells (j), mesophyll cells (i), fragments of small veins (h), starch granules (g), fragments of epidermis and stomata (k) were found in the powder drug. Organoleptic evaluation of the powder drug is as seen in Figure 1.

Phytochemical screening tests

Alkaloid, saponin, lignin, ca-oxalate, starch, protein, mucilage, tannin were identified in the plant extract. (Table 2).

Hypoglycemic effect

The hypoglycemic effects of ethanolic extracts of *A. aspera* L. was compared with standard (glibenclamide). Both standard and ethanolic plant extracts reduced the blood glucose level of rabbits significantly ($P < 0.05$). The result showed that ethanolic plant extracts of *A. aspera* L. possess strong hypoglycemic effects. Higher doses of plant extract is more effective in decreasing blood glucose level when compared to low doses. The efficacy of low doses last for 2 to 3 h while high doses last for more than 3 h. (Table 3).

Figure 1. Powder drug study of the *A. aspera*. Multicellular (a), shaggy (b), uniciliate (c), uniseriate (d), glandular hairs (e-f), starch granules (g), fragments of small veins (h), mesophyll cells (i), parenchyma cells (j), fragments of epidermis and stomata (k) and tracheids (l).

Table 1. Organoleptic evaluation of *A. aspera* leaves.

S/No	Parameter	Observation
1	Color	Dark green
2	Odour	Pungent
3	Taste	Slightly disagreeable
4	Fracture	Fibrous
5	Touch	Soapy

DISCUSSION

The pharmacognostic profile is essential for crude drugs as it helps in the identification of specific drugs within the same genus. There are several plants within the same genus with similar structure and with different microscopic characteristics, however the medicinal value of these morphological similar plants vary from each other. Pharmacognostic studies help in the identification of specific plants possessing organoleptic and microscopic characteristics (Ismail et al., 2011).

Glibenclamide is a sulfonylurea derivative and commonly used in the management of diabetes mellitus type II; it causes hypoglycemia by stimulating beta cells of pancreas and increasing release of insulin and inhibiting glucagon secretion. As these effects require a

functional pancreas, it can lower blood sugar levels in non-diabetic subjects (Soon and Tan, 2002) and a similarity was obtained in our tested ethanolic extract. It is very interesting to note that the ethanolic extract of the leaves of our selected plant is used in the tradition system of medicines in the management of various ailments. Our results show that the extract can decrease the serum glucose level in normoglycemic individuals. However, an adverse effect is that the plant can decrease the normal glucose level, just like glibenclamide, therefore, it is recommended that in the use of this plant as an anti-inflammatory or even for diabetic management, the glucose level should be monitored periodically. It is also worth mentioning that the plant should not be used in high doses for the treatment of ailments other than diabetes because it lowers the normal serum glucose level in normal patients.

In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and a sharp reduction of anti-oxidant defenses (Oberley, 1988; Brownlee, 2001; Muhammad et al., 2012a). Hence, compounds with both hypoglycemic and anti-oxidative properties would be useful anti-diabetic agents (Brownlee, 2001). It has been reported that the methanolic extract of *A. aspera* is a good antidiabetic and antioxidant (Akhtar and Iqbal, 1991; Edwin et al., 2008) agent. The phytochemical study revealed the presence of

Table 2. Phytochemical screening of the EEA.

Phytochemicals	Result	Remarks
Alkaloid	Reddish brown	++
Saponin	Frothing	++
Lignin	Pink coloration	++
Ca-oxalate	Dissolve in HCl without effervescence	++
Starch	Deep blue coloration	++
Protein	Yellow ppt.	++
Mucilage	Gummy suspension	++
Tannin	Greenish coloration	++

Table 3. Effect EEA on blood glucose level of normoglycemic rabbits.

Treatments (mg/kg)	0 h	1 h	2 h	3 h	4 h	5 h
Control	153±2	151±1.5	152±2	149±2	147.7±2.5	142.3±1.15
Glb (0.2)	153.7±1.5	111.3±2.1**	113.6±1.5**	117.3±2.5**	120.3±1.5**	122.7±1.5**
EEA (100)	149.7±1.5	114.3±2.1**	118±2**	123.6±2.5**	133.7±3	139±2.6
EEA (150)	144±2	109.7±2.5**	127.7±2.1**	131.3±2.1**	133±2	135.3±2.1
EEA (200)	150.7±2.5	90.6±2.1**	101.0±1.5**	111.3±2.5**	116.7±1.5**	124.7±1.5**

Glb= Glibenclamide, Data presented as mean ± SEM (n = 6). *P < 0.05, **P < 0.01.

starch which produce hyperglycemia but the presence of alkaloids, saponin and tannin in our results as hypoglycemic. Besides *A. aspera* L, many other plants like *Averrhoa bilimbi* (Pushparaj et al., 2000) and *Indigofera pulchra* (Tanko et al., 2008) have hypoglycemic effects on blood glucose level of normoglycemic rats. While plants like *Nauclea latifolia* (Gidado et al., 2005), *Rhinacanthus nasutus* (Rao and Naidu, 2010) and *Veranonia amygdalina* (Michael et al., 2010) show hypoglycemic effects in alloxan and streptozocin induced diabetic rats but have no pronounced effects on blood glucose in normoglycemic rats.

Conclusion

The ethanolic extract of the leaves of *A. aspera* validates its use as a hypoglycemic drug. It can be used safely for the treatment of diabetes type two, however in certain cases, use of the drug by non-diabetic patient in high doses will lower the normal glucose level. It is therefore recommended that the plant should not be used in large doses for the treatment of non-diabetic conditions.

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