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Effects of *Andrographis paniculata* Crude Extract in Normal and Alloxan Induced Hyperglycaemic Rats

Asmah Rahmat, Bibi Rohaniza Baharudin and Mohd Fadzelly Abu Bakar
Department of Nutrition and Health Science, Faculty of Medicine and Health Science,
University Putra Malaysia, 43400, Serdang, Selangor, Malaysia

Abstract: The antidiabetic effect of crude extract of *Andrographis paniculata* leaves were studied on normal and alloxan induced hyperglycaemic rats. Oral administration of *Andrographis paniculata* leaf extract (10 mg kg⁻¹ body weight) for 8 weeks resulted in significant reduction in glucose level as well as increased in body weight in alloxan induced hyperglycaemic rats but not in normal rats, which clearly shows the antidiabetic properties of *Andrographis paniculata* crude extract. The effect of the extract at dose 10 mg kg⁻¹ body weights was more effective than tested hypoglycaemic agent (glibenclamide) at dose 10 mg kg⁻¹ body weights in restoring the values of these parameters.

Key words: *Andrographis paniculata*, alloxan hyperglycaemic, glucose, total cholesterol, protein

INTRODUCTION

Prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and to rise 5.4% by the year 2025. It is higher in developed than in developing countries. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in years 2025. By the year 2025, more than 75% of people with diabetes will reside in developing countries, as compared with 62% in 1995^[1].

The use of natural products with therapeutic properties is as ancient as human civilisation and for a long time, mineral, plant and animal products were the main sources of drugs^[2]. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants^[3]. However, the potential use of plants as a source of new drugs is still poorly explored. Of the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties^[4].

Until present, there is no treatment that can cure completely diabetes mellitus. Nowadays, we use insulin for treatment in diabetes mellitus type 1 and use of oral hypoglycaemic agents (biguanide and sulfonylurea) for treatment in diabetes mellitus type 2. However, insulin and hypoglycaemic agent can cause side effects. Current insulin regimens (in type 1 diabetes mellitus) are

problematic in maintaining a physiological blood glucose profile^[5]. Hypoglycaemic agent such as glibenclamide can cause acidosis and impair of cardiac function^[6] and cannot be efficient in long term treatment^[7].

That is why efficient research have been done to search for new hypoglycaemic agent from plants and herbs that can be use to cure or control diabetes mellitus traditionally by certain community^[8].

The selection of a suitable plant for a pharmacological study and drug development is a very important and decisive step. There are several ways in which this can be done, including traditional use, chemical content, toxicity, randomised selection or a combination of several criteria.

Andrographis paniculata or locally known as Hempeđu bumi is widely known for its pharmacotherapeutic values. This plant has been proven scientifically effective as anti-malarial, anti-microbial^[9] and high in flavonoid contents^[10]. This plant also has hepatoprotective compounds^[11]. This plant has been used traditionally in Malaysia especially in Malay community to treat diabetes and related disorder. Due to the pharmacotherapeutics potential of this plant in diabetic prevention, we report here, the potential antidiabetic activities of *Andrographis paniculata*.

MATERIALS AND METHODS

Plant material: The leaves of *Andrographis paniculata* were grown and collected from the herb garden of

Faculty of Medicine and Health Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Extraction of *Andrographis paniculata* aqueous extract:

Crude extract of *Andrographis paniculata* was prepared from the modification according to Wang *et al.*^[12]. The leaves of *Andrographis paniculata* were blended to obtain crude extract. The dosage that was used is 10 mg kg⁻¹ bodyweight that were diluted in distilled water at 50 mL/rats. The mixture was stirred using magnetic stirrer for 30 min. Later, the mixture was filtered and the filtrate were supplemented *ad libitum* (approximately 50 mL/rat).

Experimental rats: Albino white rats from Sprague dawley species, weight 100-1500 g were used in this study. The rats were supplied by Institute for Medical Research, Kuala Lumpur, Malaysia. They were housed individually in animal cage at animal house in Faculty of Medicines and Health Sciences, UPM, Malaysia. Upon arrival, the animals were allowed to acclimatize for at least 7 days before starting the experimental studies. They were maintained on regular commercial laboratory diet (Gold coin Sdn. Bhd.) and tap water *ad libitum*.

Induction of hyperglycaemic in rats: Rats were made hyperglycaemic by a single intraperitoneal injection of alloxan monohydrate (Sigma, 45 mg kg⁻¹ body weight) to induce a mild hyperglycaemic. Alloxan was weight and diluted with normal saline prior to injection to fasting rats. To prevent hypoglycaemic shock, the rats were fed 1% glucose for 24 h after alloxan injection. Three days after alloxan injection, rats with glucose level more than 10 mmol mL⁻¹ were included in the study for mild hyperglycaemia model.

Study design: In this experiment, a total of 30 rats (15 normal and 15 alloxan hyperglycaemic surviving rats) were used. The rats were divided into 6 groups with 5 rats in each group.

- Group 1: Normal untreated rats
- Group 2: Normal treated with *Andrographis paniculata* (10 mg kg⁻¹ body weight)
- Group 3: Normal treated with glibenclamide (10 mg kg⁻¹ body weight)
- Group 4: Hyperglycaemic untreated rats
- Group 5: Hyperglycaemic treated with *Andrographis paniculata* (10 mg kg⁻¹ body weight)
- Group 6: Hyperglycaemic treated with glibenclamide (10 mg kg⁻¹ body weight)

After 8 weeks of treatment, all rats were sacrificed after overnight fasting. Blood was collected in lithium heparin tube for biochemical test. Plasma was separated using refrigerated centrifuged at 3000 rpm for 10 min for estimation of glucose, total cholesterol and protein. Whole blood was also used to estimate glucose level.

Estimation of glucose and total cholesterol: Fasting plasma glucose and total cholesterol as well as blood glucose were estimated based on enzymatic colorimetric test automatically using reflotron and the kits were provided by Boehringer Mannheim, Germany.

Estimation of protein: Protein was estimated using spectrophotometry method describe by Pinnell and Northam^[13].

Statistical analysis: All the values of body weight and biochemical estimations were expressed as mean±SD and analysed using Statistical Package for Social Sciences (SPSS) version 10.0 using ANOVA. Differences between groups were considered significant at p<0.05 levels.

RESULTS

There was a significant increased of body weight in all groups (p<0.05). But, there were no significant difference in all groups based on statistical analysis (Table 1).

For blood glucose level, there were a significant difference between normal and hyperglycaemic group (Table 2). But, there were no significant difference between normal and normal treated *Andrographis paniculata* and normal treated with glibenclamide. This result show that *Andrographis paniculata* extract had no effect in normal rats. In the other hand, there was a significant reduction of blood glucose in *Andrographis paniculata* and glibenclamide treated in hyperglycaemic rats compared to hyperglycaemic rats without treatment. Interestingly, the reduction of blood glucose showed a better trend compared to tested drug (glibenclamide) (Table 2).

Table 1: Body weight changes after 8 weeks of treatment

Groups	Body weight changes (g)
1	64.6±7.1
2	52.0±6.0 ^a
3	63.0±6.3 ^a
4	85.6±29.8 ^a
5	109.8±23.3 ^{ab}
6	99.0±8.3 ^{ab}

Each value is the mean±SD of five rats in each group. Values not sharing a common superscript(s) letter differ significantly at p<0.05 (DMRT)

Table 2: Level of blood glucose, total cholesterol and plasma protein after 8 weeks of treatment

Groups	Blood glucose (mmol L ⁻¹)	Plasma protein (g dL ⁻¹)	Plasma cholesterol (mg dL ⁻¹)
1	7.51±0.71	5.14±0.5	111.8±5.9
2	5.82±0.30 ^a	3.62±1.3	103.4±1.5 ^a
3	7.22±1.10 ^a	3.54±0.8 ^a	111.4±4.2 ^a
4	9.42±0.30 ^a	2.82±0.6 ^a	108.3±3.0 ^a
5	5.34±0.50 ^{ab}	2.42±0.6 ^a	103.0±1.5 ^a
6	6.46±0.62 ^{ab}	2.10±0.7 ^a	104.6±2.1 ^a

Each value is the mean±SD of five rats in each group. Values not sharing a common superscript(s) letter differ significantly at p<0.05.

There were similar trend was observed in plasma glucose level as shown in Table 2. The result show that *Andrographis paniculata* extract gave a similar regimen as hypoglycaemic agent (glibenclamide). There were no significant difference between group for plasma total cholesterol and plasma protein level between group.

DISCUSSION

In light of the result, present study strongly indicates that *Andrographis paniculata* leaf extracts have good antidiabetic activities, in which this case lowering the blood glucose of alloxan induced mild hyperglycaemic rats. According to Asmawi *et al.*^[14], *Andrographis paniculata* was able to act as hypoglycaemic agent by lower the absorption of glucose in intestine based on study that was conducted in normal rats. In addition, *Andrographis paniculata* crude extracts have increased the body weight of hyperglycaemic rats which also a good phenomenon because hyperglycaemic or diabetic patients tend to loss body weight due to unutilization of glucose in body and tend to utilize protein from muscle. The possible components from *Andrographis paniculata* that may act as hypoglycaemic agent are diterpenoids (andrographolide, neoandrographolide and 14-Deoxy-11, 12-didehydroandrographolide) that was isolated by Jain *et al.*^[11] as well as flavonoids (secondary metabolites of plants under polyphenol group that possess antioxidant activity) that were identified by Gupta *et al.*^[15]. For examples, green tea polyphenol and quercetin (one of flavonoids) have been proven to possess antidiabetic activities in diabetic rats^[16,17].

There were trend of reduction for both total cholesterol and plasma protein content in *Andrographis paniculata* treated hyperglycaemic rats and glibenclamide treated hyperglycaemic rats, however the reduction was not statistically significant.

According to Bhandari *et al.*^[18], there was reduction of protein synthesis in streptozotocin-induced diabetic rats. This phenomenon was caused by proteolysis pathway in degradation of muscle protein. If the extract can increase the level of protein in plasma, it shows that

there is a pathological reverse reaction after secondary complication of diabetes mellitus. In this study, the consistence of plasma protein level showed there was no pathological disturbance occurred or no secondary complication of diabetes mellitus in group treated with *Andrographis paniculata* crude extract.

In conclusion, the present investigation clearly showed that *Andrographis paniculata* crude extract had a very great potential as drug alternative in diabetic patients and related disorder.

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