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Antidiabetic Activity of Some Herbal Plants in Streptozotocin Induced Diabetic Albino Rats

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Abstract: Aqueous extract of leaves of 3 herbs (*Murraya koenigii*, MK; *Psidium guajava*, PG and *Catharanthus roseus*, CR) were used to test their antidiabetic activity in Streptozotocin (STZ) induced diabetic albino rats. MK, PG and CR are given to the STZ induced diabetic rats at the concentration of 500 mg/kg body weight in different groups of 6 diabetic rats each orally once a day for 15 days. Glibenclamide (GBC) is also given to another group to support the results at the concentration of 3 mg/kg body weight orally once a day for 15 days. Diabetic control received vehicle. Body weight showed significant increase (MK and PG: $p < 0.05$, CR and GBC: $p < 0.001$) after 15 days of treatment with herbal extract when compared with the control. Blood glucose level on 15th day of treatment become significantly low ($p < 0.001$). At the termination of the experiment (on 15th day) the urine glucose and ketone were absent in herbal treated group which was present in the diabetic control. Histological study of the pancreas also assesses the results of body weight and blood glucose level. Islets of diabetic control group were damaged, shrunken in size and infiltration of lymphocytes was observed. While islets of herbal extracts treated rats were comparable to normal rats. Many rounds and elongated islets were evenly distributed through out the cytoplasm. No significant histological alteration was found in glomeruli or any other segment of kidney tubule in STZ induced diabetic rats. In herbal extract treated group no difference was found in kidney tubules when compare with their respective diabetic control. Findings of the present study suggest that the aqueous extract of leaves of MK, PG and CR at the dose of 500 mg/kg body weight brings about significant beneficial effects in various physiological/histological parameters altered during diabetic manifestations and these effects are quite comparable with glibenclamide (a standard drug used to treat diabetes mellitus).

Key words: Antidiabetic activity, *Murraya koenigii*, *Psidium guajava*, *Catharanthus roseus*, albino rats

INTRODUCTION

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Several drugs such as biguanides and sulfonylureas are presently available to reduce hyperglycemia in diabetes mellitus. These drugs have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems (Noor *et al.*, 2008). Management of diabetes without any side effect is still a challenge to the medical community. There is continuous search for alternative drugs. Therefore it is prudent to look for options in herbal medicines for diabetes as well. Although, herbal medicines have long been used effectively in treating diseases in Asian communities and through out the world. The mechanism of most of the herbals used has not been defined. Many traditional plants treatments for diabetes are also used. But most of the evidence for their beneficial effects is anecdotal (Bailey and Day, 1989). Traditional antidiabetic plants might provide new oral hypoglycemic compounds, which can counter the high cost and poor availability of the current medicines/ present day drugs for many rural populations in

developing countries. India is well known for its herbal wealth. Medicinal plants like *Trigonella foenum graecum*, *Allium sativum*, *Gymnema slyvestre* and *Syzygium cumini* have been studied (Grover *et al.*, 2002) for treatment of diabetes mellitus. However, detailed studies on the efficacy, mechanism of action and safety of plant extract are needed.

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the world (Burke *et al.*, 2003). Diabetes is one of the leading causes of death in humans and animals. In animals it occurs most frequently in the dog with an incidence of approximately 0.2%. In the indigenous Indian system of medicine good number of plants were mentioned for the cure of diabetes and some of them have been experimentally evaluated and active principle were isolated (Grover *et al.*, 2002). WHO (1980) has also recommended the evaluation of the effective of plants in conditions where there are no safe modern drugs (Upadhaya and Pandey, 1984). The ethnobotanical information reports state that about 800 plants may possess antidiabetic potential (Aguilara *et al.*, 1998). Recently the medicinal values of various plants extracts have been studied by many scientists in the field of diabetic research (Daisy and Eliza, 2007; Noor *et al.*,

2008). Various parts of herbs have been used for medicinal purposes including the treatment of diabetes mellitus.

Streptozotocin (STZ) is a naturally occurring nitrosourea product of *Streptomyces achromogenes*. Usually, the intraperitoneal injection of a single dose (60 mg/kg body weight) of it exerts direct toxicity on β cells resulting in necrosis within 48-72 h and causes a permanent hyperglycemia. STZ breaks nuclear DNA strand of the islet cells (Takasu *et al.*, 1991).

Murraya koenigii (MK) is widely distributed in India and commonly known as 'curry patta', belongs to the family Rutaceae. *Psidium guajava* (PG) is known as 'amrud' in Hindi and belongs to the family Myrtaceae. And *Catharanthus roseus* (CR) is commonly known as 'sadabahar' and belongs to the family Apocynaceae. Leaves of above 3 herbal plants were used in the present study to clarify their effect in the treatment of STZ induced diabetes, on blood glucose, body weight and possible effects on pancreatic and kidney tissue in rat model. A comparison was made with the Glibenclamide (GBC), a standard drug used in treating diabetes mellitus.

MATERIALS AND METHODS

Animals: Adult albino rats weighing about 100-150 g were used in the present investigation. All the rats were given a period of acclimatization for 15 days before starting the experiment. They were fed *ad libitum* everyday with standard chow diet and were given free access to water. Animals described as fasting were deprived of food for at least 16 h but were allowed free access to drinking water.

Induction of diabetes: Streptozotocin (Batch No.T1829656) was purchased from Sisco Research laboratories Pvt. Ltd. Mumbai, India and was freshly dissolved in 0.1 M citrate buffer (pH = 4.5) at the dose of 50 mg/kg body weight and injected intraperitoneally within 15 min of dissolution in a vehicle volume of 0.4 mL with 1 mL of tuberculin syringe fitted with 24 gauge needle, whereas normal control group was given citrate buffer only (0.4 mL). Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin.

Plant extracts: Leaf extract of *Murraya koenigii* (Batch No.08MK/5002), *Psidium guajava* (Batch No.TAPG/5005) and *Catharanthus roseus* (Batch No. 05VR/5002) were obtained as a sample from Tulsi amrit Pvt. Ltd. Indore, India.

Glibenclamide: Single dose of glibenclamide provokes a brisk release of insulin from pancreas. It acts on β -cell membrane leading to enhance calcium flux across it, hence degranulation. After chronic administration the

insulinemic action of glibenclamide declines, but improvement in glucose tolerance is maintained. Thus it is an oral antidiabetic preparation with an efficient hypoglycemic action. Daonil (glibenclamide) manufactured by Aventis Pharma Ltd. Goa, India was collected from market and preserved at room temperature.

Experiments: Rats were divided into the following groups.

Group I: Consisted of 6 rats which served as normal control and were given only distilled water daily.

Group II: Consisted of 6 STZ induced diabetic rats and served as diabetic control and were given distilled water only.

Group III: Consisted of 6 STZ induced diabetic rats and were treated orally with aqueous extract of *Murraya koenigii* leaves at the dose of 500 mg/kg body weight daily for 15 days, once a day.

Group IV: Consisted of 6 STZ induced diabetic rats and were treated orally with aqueous extract of *Psidium guajava* leaves at the dose of 500 mg/kg body weight daily for 15 days, once a day.

Group V: Consisted of 6 STZ induced diabetic rats and were treated orally with aqueous extract of *Catharanthus roseus* leaves at the dose of 500 mg/kg body weight daily for 15 days, once a day.

Group VI: Consisted of 6 STZ induced diabetic rats and were given Glibenclamide (GBC) at the dose of 3 mg/kg body weight daily for 15 days, once a day.

After 15 days of herbal treatment experiments were terminated and observations were made. Body weight was taken before and after experiment with the help of single pan balance. Blood glucose level was estimated on 0 day and 15th day of experiment with the help of glucometer using strip method and blood was taken from tip of the tail. Fresh urine was collected by slightly pressing the tail and back of the rat. Glucose and ketone in urine was checked using keto-diastrix strips on 0 and 15th day of experiment. After termination of experiment the rats from all the groups were anesthetized and dissected out. Pancreas and kidney were fixed in bouin's fluid and histological preparations were made. 5 μ thick sections were cut and stained with haematoxyline and eosin.

Statistical analyses were done with the help of student's 't' test (Bruning and Kintz, 1977). Animal housing, care and application of experimental procedures were in accordance with institutional animal ethic guidelines.

RESULTS

Body weight: Diabetes is characterized by weight loss and it was also seen in this study. Streptozotocin administration brought about marked reduction in body weight of rats. This reduction was found to be statistically significant ($p < 0.05$) when compared with normal control group. These reduced body weights were found to be increased when compared to their respective diabetic control group and this increase was found to be statistically significant in rats treated with *Murraya koenigii* leaf extract ($p < 0.05$), *Psidium guajava* leaf extract ($p < 0.001$) and Glibenclamide ($p < 0.001$) (Fig. 1). Percent increase in body weight was 6.85% for *Murraya koenigii*, 4.36% for *Psidium guajava*, 7.96% for *Catharanthus roseus* and 5.34 for glibenclamide.

As for as the relative efficacy in increasing or maintaining body weight is concerned, *Catharanthus roseus* seems to be the most promising than *Murraya koenigii* but these two herbs were even more effective than glibenclamide in maintaining body weight. *Psidium guajava* treated group showed minimum increase in percentage body weight and it was also less than glibenclamide treated group.

Blood glucose: Streptozotocin causes selective destruction of β cells of islets of pancreas and brings an increase in blood glucose levels. It is evident from the present investigation that streptozotocin administration at the dose of 50 mg/kg body weight causes significant diabetogenic response in albino rats. Blood glucose levels in diabetic control rats treated with *Murraya koenigii*, *Psidium guajava*, *Catharanthus roseus* and glibenclamide were raised nearly to 3.5 fold as compared to their respective normal control group rats on 15th day after treatment. Interestingly, the increase in glucose levels in diabetic control groups was found to be highly statistically significant ($p < 0.001$) when compared to their respective normal control groups. These raised levels of blood glucose in diabetic rats were declined sharply after oral feeding of aqueous extract of leaves of *Murraya koenigii*, *Psidium guajava*, *Catharanthus roseus* and Glibenclamide. When comparisons were made between diabetic and drug treated animals, blood glucose levels were found to be declined sharply from 290.33-173.00 mg/dL on 15th day after oral feeding of *Murraya koenigii*, from 287.50-162.16 mg/dL on 15th day after oral feeding of *Psidium guajava* and from 292.00-156.33 mg/dL on 15th day after oral feeding of *Catharanthus roseus* aqueous extract of leaves. Glibenclamide treatment also decreased blood glucose levels on 15th day (from 291.16-152.16 mg/dL). This decline in blood glucose levels of drug treated groups when compared with their respective diabetic control group was found to be highly statistically significant ($p < 0.001$) (Fig. 2).

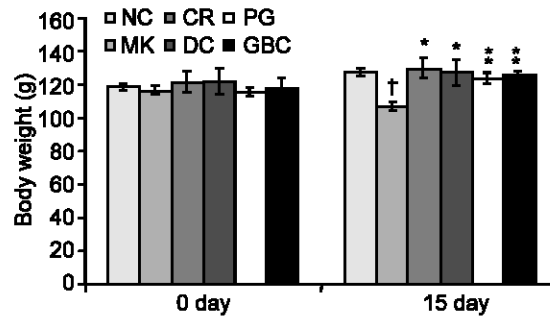


Fig. 1: Histogram showing changes in body weight following treatments of MK, PG, CR and GBC on 15th day in streptozotocin induced diabetic rats. Significance of difference from Diabetic control (* $p < 0.05$, ** $p < 0.001$; Student's 't' test). Significance of difference from Normal control ($\dagger p < 0.001$; Student's 't' test)

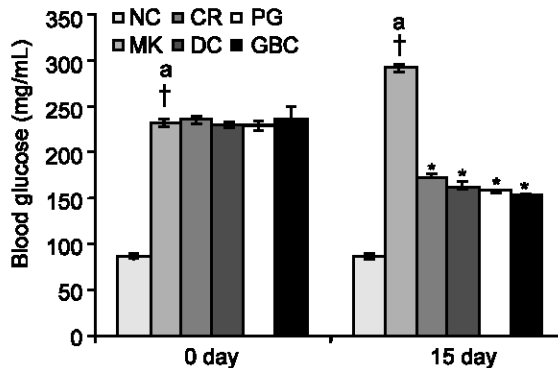


Fig. 2: Histogram showing changes in blood glucose level following treatments of MK, PG, CR and GBC on 15th day in streptozotocin induced diabetic rats. Significance of difference from Diabetic control (* $p < 0.001$; Student's 't' test). Significance of difference from Normal control ($\dagger p < 0.001$; Student's 't' test). ^aExperimental groups differ significantly ($p < 0.001$; ANOVA)

Hence if decline in blood glucose levels is to be the only indices, then treatment with aqueous extract of leaves of *Murraya koenigii*, *Psidium guajava*, *Catharanthus roseus* and Glibenclamide has proved highly effective in causing significant antihyperglycemic response in this strain of rats. A comparative account of the antihyperglycemic activity of the 3 chosen plants and glibenclamide is well displayed in the present study. The reduction of sugar after administering *Murraya koenigii* was 48.82%, *Psidium guajava* was 43.59%, *Catharanthus roseus* was 46.46% and glibenclamide was 47.74%. It is evident from these results that reduction in blood glucose levels brought by 3 herbal extracts is quite comparable with reduction brought about by glibenclamide.

Table 1: Effect of aqueous extract of leaves of MK, PG, CR and GBC on urine glucose and ketone in streptozotocin induced diabetic rats

Groups	Glucose and Ketone level in urine			
	0 day		15 day	
	Glucose	Ketone	Glucose	Ketone
Normal control	-	-	-	-
Diabetic control	+++	Trace	+++	Trace
<i>Murraya koenigii</i>	+++	Trace	-	-
<i>Psidium guajava</i>	+++	Trace	-	-
<i>Catharanthus roseus</i>	+++	Trace	-	-
Glibenclamide	+++	Trace	-	-

Glucose: - = absence of glucose, +++ = 1 g/dL. Ketone: - = absence of ketone, Trace = 5 mg/dL

As far as the relative efficacy is concerned, *Murraya koenigii* has been proven the highest antihyperglycemic activity among the 4 drugs given in this study. *Murraya koenigii* was found even more anti hyperglycemic than glibenclamide, whereas, *Psidium guajava* and *Catharanthus roseus* exhibited little less hyperglycemic activity than glibenclamide in this study.

Urine glucose and ketone: Urine analysis on 0 day showed the presence of glucose and traces of ketone in the entire group except normal control. But on 15th day glucose and ketone traces were absent in herbal (MK, PG and CR) and glibenclamide treated groups while they were present in diabetic control (Table 1).

Pancreas histology: Histologically, the islets of Langerhans of normal control group I were unevenly scattered in the pancreatic tissue and they were often quite abundantly distributed and were of varying sizes in the same lobule of pancreas. Each islet was separated from the acini by a reticular membrane and islet was arranged in anastomosing cellular plates or cords. In the control group, well delineated islets were completely enmeshed in the surrounding acinar cells of the exocrine pancreas. The islet cells were compactly arranged, with negligible intercellular space and no inflammatory cells were observed (Fig. 3a). Pancreatic islets of diabetic control group II rats revealed significant architectural disarray which also extended into surrounding exocrine tissue. Further, streptozotocin treatment seems too brought about an increase in intercellular space compared to control rats. Another significant change following streptozotocin treatment was the occurrence of peripheral widening between pancreatic acinar (exocrine tissue) and islet cells (Fig. 3b). Pancreatic islets of group III rats (treated with *Murraya koenigii*) (Fig. 3c), group IV (treated with *Psidium guajava*) (Fig. 3d), group V (treated with *Catharanthus roseus*) (Fig. 3e) and group VI (treated with glibenclamide) (Fig. 3f) also showed architectural disarray but to a lesser extent as compared to streptozotocin induced diabetic rats (group II). Even

though the peripheral widening between acinar and islet cells were existed but it was not as prominent as in the streptozotocin induced diabetic animals. All the changes brought about by streptozotocin induction in diabetic rats like architectural disarray and peripheral widening between acinar and islet cells were although present in *Murraya koenigii*, *Psidium guajava*, *Catharanthus roseus* and glibenclamide treated groups but were much less pronounced and at certain places, peripheral widening between acinar and islet cells was quite reduced. Both acinar and islet component of pancreas seems near to each other showing as if islet is returning back to its normal structure and it correlates well with the significant decrease in blood glucose and increase in body weight in all 3 herbal extract and glibenclamide treated groups.

Kidney histology: The histological preparations of kidney from buffer injected control rats showed that the various segments of kidney tubules were well preserved. Abundant glomeruli, portion of nephron segment with interspersed blood capillaries were also clearly seen (Fig. 4a)

In the group II (diabetic control) there was no significant histological alteration in the glomeruli or any other segment of kidney tubule following streptozotocin treatment under the present experimental protocol. All the constituent structures of kidney tubule in streptozotocin treated group appear to be well maintained with no indication of hyaline deposition or increase in messangial thickening (Fig. 4b).

No difference was found in structure of glomerulus of group III, IV, V and VI rats treated with MK, PG and CR extract and GBC respectively for 15 days when compared with their respective diabetic control (group II) rats. Various regions of kidney tubules appeared to be normal without any change in messangial thickening (Fig. 4c-f).

Toxicity evaluation: The water extract of MK, PG and CR tested for in acute toxicity (if any) in rats. To determine acute toxicity of a single oral administration of the herbal drug different doses of the drug (0.5, 1.0, 1.5 and 2 g/kg

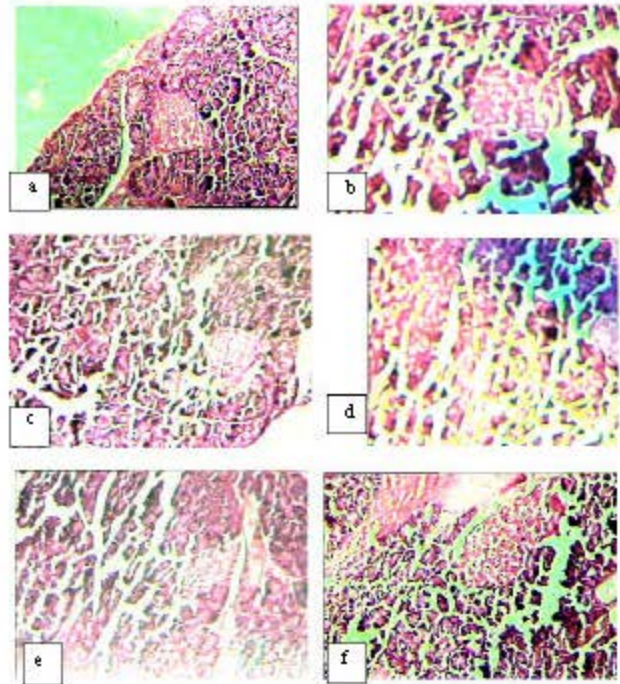


Fig. 3: Photomicrograph of Pancreatic islet of rats stained with Haematoxyline and eosin $\times 10$. a-Buffer injected control group. b-Streptozotocin induced diabetic rat. c-Diabetic rats treated with MK. d-Diabetic rats treated with PG. e-Diabetic rats treated with CR. f-Diabetic rats treated with GBC

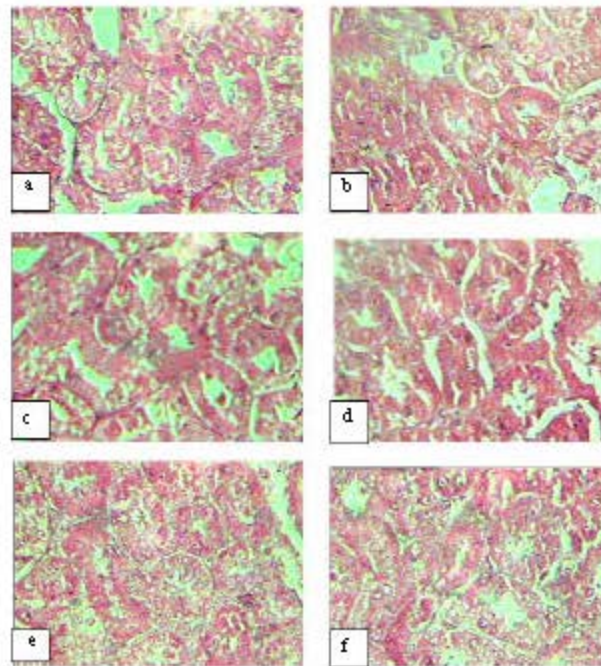


Fig. 4: Photomicrograph of Kidney of rats stained with Haematoxyline and eosin $\times 40$. a-Buffer injected control group, b-Streptozotocin induced diabetic rat, c-Diabetic rats treated with MK, d-diabetic rats treated with PG, e-Diabetic rats treated with CR, f-Diabetic rats treated with GBC

body weight were administered to different groups of rats (2 rats were used for each group); control rat received distilled water only. Mortality and general behavior of the animals were observed continuously for initial 4 h and intermittently for the next 6 h and then again at 24 and 48 h following drug administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion.

DISCUSSION

Historical literatures reveal that knowledge regarding diabetes existed since Brahmic period as this was mentioned in Ayurvedic text books-Sushruta samhita-written in fourth and fifth centuries B.C (Dhanukar and Thatte, 1989). In this ancient text, 2 forms of diabetes were described: one genetically based and the other as a result of dietary indiscretion (Dhanukar and Thatte, 1989). Even the treatment in the Indian ancient pharmacopoeia mentioned specific treatments for the two types including dietary modifications, medicinal plant remedies and minerals. Moreover, the researches conducted over last several decades has shown plant and plant based therapies have a potential to control and treat diabetes (Oliver and Zahnd, 1979; Bailey and Day, 1989; Ivorra *et al.*, 1989; Marles and Farnsworth, 1995) and its complications (Grover *et al.*, 2001). Role of Indian medicinal plants as antidiabetic has also been reviewed by Grover *et al.* (2002). For testing antidiabetic potential of plants, STZ induced hyperglycemia in rodents is considered to be a good preliminary screening model (Ivorra *et al.*, 1989) and is widely used. Diabetes is probably the fastest growing metabolic disease in the world and as knowledge of the heterogeneous nature of disease increase so does the need for the more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of the diabetes (Akhtar and Ali, 1984) but only a few have been scientifically evaluated. STZ is well known for its selective pancreatic islet cell toxicity and has been extensively used in induced diabetes mellitus in animals. STZ is taken up by the β cells via the glucose transporter GLUTZ and causes alkylation of DNA (Delancy *et al.*, 1995) and reduction of ATP and NAD⁺ content (Heller *et al.*, 1994). STZ induces severe and irreversible hyperglycemia in experimental animals. STZ was used to induced diabetes rather than alloxan. Since with STZ there is no incidence of spontaneous revision and greater of islets resulting in more than 90% of rats becoming diabetic (Mitra *et al.*, 1995).

Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an over exertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing

deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycemia. This presents a moving therapeutic target that requires a range of different agents to address the different features of the disease at different stages of its natural history. Although biomedical science has unraveled substantially the pathologically processes involved in causing/fostering diabetes and has designed therapeutic agents with a range of action to fight hyperglycemia, the efficacy of these therapeutic agents is compromised in several ways. Individual agents act only on part of the pathogenic process and only to a partial extent. This may be the reason that even after so much advancement in understanding the disease process and availability of a wide range of therapeutic agents, the disease is still progressing.

The most significant findings of the present study is that the aqueous leaves extract of MK, PG and CR at the dose of 500 mg/kg body weight for 15 days have shown beneficial effect not only on blood glucose but also on body weight, glucose and ketone levels of urine and tissue of pancreas in streptozotocin induced diabetic rats. Results obtained from the present study are very much promising and comparable with glibenclamide, a standard drug used to treat diabetes mellitus. Similar to our observations, Noor *et al.* (2008) reported the antidiabetic activity of *Aloe vera* in streptozotocin induced diabetic rats. Noor *et al.* (2008) have also mentioned that there are two possible explanations for this finding. First, *A. vera* may exert its effect by preventing the death of β cells and/or second, it may permit recovery of partially destroyed β cells. Burcelain *et al.* (1995) reported that the hypoglycemic action of the extract of herbal plants in diabetic rats may be possible through the insulinomimetic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles.

The antidiabetic activity of the 3 herbs (MK, PG and CR) used in the present investigation may be possible through the mechanism as reported by Noor *et al.* (2008) and Burcelain *et al.* (1995). This is an interesting finding and suggests that these herbs may have antioxidant or free radical scavenger properties in preventing these changes.

Conclusion: In conclusion, we suggest that MK, PG and CR have antidiabetic properties in animal model. Although the exact chemical compound (s) responsible for the antidiabetic activity of MK, PG and CR extract still remain speculative. More detailed studies on MK, PG and CR using different doses and covering longer period of observation are needed before reaching a clear-cut conclusion. Further research to refine the extraction of procedure of MK, PG and CR could lead to improved pharmaceutical products.

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