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Effect of Methanolic extract of *Musa sapientum* leaves on Gastrointestinal Transit time in Normal and Alloxan induced Diabetic rats: Possible Mechanism of Action

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Summary: Disorders of gastrointestinal motility have been associated with Diabetes mellitus. Hyperglycaemia particularly has been reported to inhibit gastrointestinal transit time while glibenclamide, a sulphonylurea and insulin, both increased transit time. *Musa sapientum* has also been reported as an antidiabetic agent but there is dearth of information on the effect of this plant on gastrointestinal motility. This study was therefore carried out to investigate the effect of methanolic extract of *Musa sapientum* leaves (MEMSL) on Gastrointestinal Transit time (GITT) in male albino rats with and without hyperglycaemia and to elucidate possible mechanism by which this extract functions. Fifty five albino rats were divided into 11 groups of five animals each. All animals were fasted for 24hrs before the begining of the experiment. Group 1 served as control; while the remaining groups (2 – 11) were treated with 250mg/kg; 500mg/kg MEMSL; diabetic control; diabetic treated with 250mg/kg; 500mg/kg MEMSL; diabetic treated with glibenclamide (5mg/kg); normal rats treated with Nifedipine (50mg/kg); normal rats treated with calcium chloride ($CaCl_2$) only (10mg/kg); groups 10 and 11 were both pretreated with $CaCl_2$ and subsequently treated with 250mg/kg and 500mg/kg MEMSL respectively. All plant extracts used for treatments were dissolved in normal saline and administered orally using orogastric tube. Charcoal meal was used as marker in the estimation of GITT. The study showed significant decrease in GITT in the normal rats treated with 250mg/kg and 500mg/kg of extract. However, in the diabetic rats treated with 500mg/kg MEMSL, there was significant increase in GITT and this is comparable with the gut response to glibenclamide (5mg/kg). Musa sapientum extract produced significant decrease in transit time in the calcium chloride pre-treated normal rats and this is comparable to the effect observed in Nifedipine treated group. The significant reduction in GITT produced by MEMSL in the normal rats reflects a strong possibility of MEMSL acting as calcium channel antagonist through the voltage gated calcium channel which may be due to the presence of alkaloids, saponins, cardenolides. There is the possibility of the extract acting as an inhibitor of potassium channel at higher concentration as observed in glibenclamide treated groups.

Keywords*: Musa Sapientum*, Gastrointestinal transit time, Alloxan diabetes

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INTRODUCTION

Diabetes mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Tiwari and Rao, 2002). Hyperglycemia, pre and postprandial, has been reported to be the most significant manifestation of diabetes mellitus and amelioration of this hyperglycemia results in reduction of symptoms or complications of diabetes mellitus (Reddy et al, 2006). There are several reports on gastrointestinal transit disorders in diabetes mellitus (Feldman and Schiller, 1983; Wegener et al, 1990; Kawagishi, 1992; Chang, 1995). *Musa sapientum* (Banana) is a large tropical plant with a succulent pseudo-stem. Different parts of this plant have been studied, and this plant has been reported to have antidiabetic properties among others (Morton, 1987; Oke et al 1999, Pari and Umamaheswari, 2000; Dhanabal et al., 2005; Ingale et al, 2009; Adewoye et al, 2009).

Insulin, a drug of choice in the management of diabetes mellitus produced acceleration of small intestinal transit in normal mice, independent of blood glucose level (Reddy et al, 2006). Glibenclamide, a sulphonylurea was reported to close ATP-sensitive k^+ channels resulting in increase in transit time (Santos and Rao, 1999). Though various

parts of *Musa sapientum* have been shown to possess antidiabetic properties, its effect on transit time and the mechanism of action is however yet to be elucidated. Since gastrointestinal transit time affect rate of food digestion and absorption and musa sapientum has been reported as a hypoglycemic plant, the mechanisms that may be involved in moderating hyperglycaemia could include the modulation of gastrointestinal transit time.

This study was therefore carried out to investigate the effect of methanolic extract of *Musa sapientum leaves* (MEMSL) on gastrointestinal transit time in alloxan induced diabetic rats and the possible mechanism of its action.

MATERIALS AND METHODS

Animals

Fifty five adult male albino Wistar rats with an average weight of 190.0±0.3gm were used in the study. They were obtained from the Central Animal House, College of Medicine, University of Ibadan. The animals were allowed to acclimatise for two weeks before commencing the study. They were fed on commercial rat pellets and had access to drinking water *ad libitum.*

Plant Collection and Extraction

Fresh *Musa sapientum* leaves were collected and identified (Identification No. UIH - 22304) at the Department of Botany and Microbiology, University of Ibadan. The fresh leaves of *Musa sapientum* were washed with water, air dried at room temperature and ground into powder. 100g of dried powder of MEMSL was dissolved in 1 liter of methanol (Analar, GmBh, Germany) and allowed to stand in the dark for 72 hours. The solution was filtered through 0.45µ filter and the filtrate evaporated to dryness in a water bath at 45^{o}C. The methanolic extract was stored at 4^{o}C until use.

Phytochemical Screening

Phytochemical analysis of Musa sapientum leaves was done in the Department of Pharmacognosy, University of Ibadan using standard phytochemical screening methods.

Experimental Design

Preliminary toxicity test of the extract was carried out using the Organization for Economic Cooperation and Development (OECD) standard for toxicity (Botham, 2004) the extract at 1500mg/kg was well tolerated in experimental animals. The extract was administered orally at a dose of 250mg/kg and 500mg/kg respectively (Adewoye et al, 2009). All animals were fasted for 24hrs prior to the beginning of the experiment.

The animals were divided into eleven groups of five rats each: group 1 - non-diabetic untreated rats (control); group 2 - non-diabetic rats treated with 250mg/kg MEMSL; group 3 – non diabetic rats treated with 500mg/kg MEMSL; group 4 - diabetic untreated (diabetic control); group 5 diabetic treated with 250mg/kg MEMSL; group 6 - diabetic treated with 500mg/kg MEMSL and group 7 diabetic treated with 5mg/kg bodyweight (standard recommended dosage) Glibenclamide; group 8 were normal rats treated with 50mg/kg nifedipine; group 9 were nomal rats treated with calcium chloride($CaCl_2$)(10mg/kg); group 10 and 11 were pretreated with $CaCl_2$ and 10minutes after treated with 250mg/kg and 500mg/kg MEMSL. All treatments were administered orally using orogastric tube.

Estimation of Gastrointestinal Transit time

Charcoal meal marker was freshly prepared by dispersing 10% (w/v) activated charcoal in 5% (w/v) gum acacia mucilage in distilled water and triturated well. Each rat received 4% charcoal meal (10ml/kg p.o.) orally through a metal oral cannula 1 hr after their respective treatments. After 10min, animals were sacrificed by cervical dislocation, the abdomen was then cut open; the leading marker was identified and tied immediately with a cotton thread to avoid movement of the marker. The entire length of the small intestine was isolated by cutting at the pyloric and ileocecal ends. The distance travelled by charcoal meal and the total length of the intestines was measured in cm(s). The gastrointestinal transit time was expressed as percentage (%) of the distance travelled by the charcoal meal to length of the intestine (Sandhiya et al, 2008; Tembhurne and Sakarkar, 2009).

$$\% \text{ Transit} = \frac{\text{Distance travelled by charcoal meal}}{\text{Total length of small intestine}} \times 100$$

Induction of Diabetes mellitus

The experimental rats were made diabetic by a single intraperitoneal injection of 120mg/kg bodyweight of alloxan dissolved in normal saline (Schuzdelski 2001). Blood samples were obtained from small cuts at the tip of the tail unto a glucometer test trip. The samples were analyzed using a blood glucose meter. The glucose meter utilized the glucose oxidase principle of glucose analysis. Diabetes was confirmed 48 hours after alloxan injection by a sustained blood glucose level between 250 and 360mg/dl. Rats with sustained high blood glucose within this range after five days were selected for the diabetic groups.

Statistical Analysis

Data obtained are expressed as the mean ± SEM. Student t-test and two-way analysis of variance (ANOVA) was used to assess the level of statistical significance. The level of significant difference between the groups was evaluated at $P \leq 0.05$.

RESULTS

Phytochemical screening of Musa sapientum

The phytochemical screening of Musa sapientum revealed the presence of alkaloids, cardenolides and saponins. The presence of anthraquinones and tannins was however not established.

Effect of MEMSL on GITT in normal animals

In normal animals treated with 250mg/kg and 500mg/kg MEMSL, significant ($P \leq 0.05$) decrease, equivalent to 34.47% and 33.62% in GITT respectively was observed when compared with control animals. This decrease was dose dependent (Fig 1). Rats treated with nifedipine only (a calcium channel blocker) showed a significant decrease in GITT similar to the reduction observed in the normal groups treated with the extract (Fig.2). In animals treated with calcium chloride only (a calcium ion channel modifier), a significant increase in GITT was observed compared with control. However, when

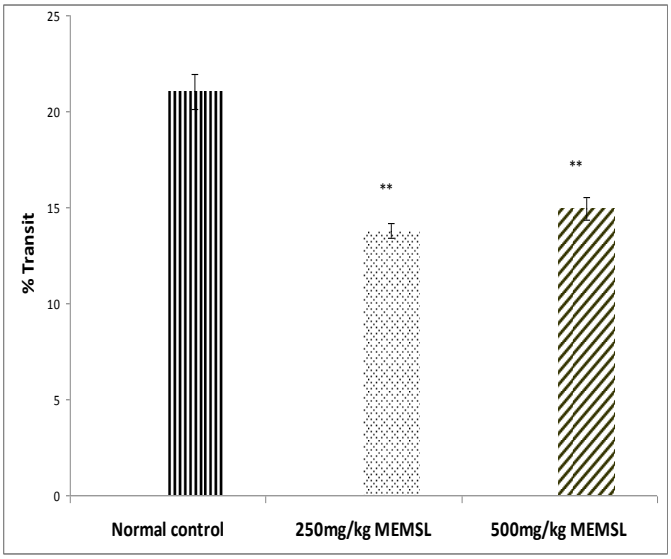

Figure1.

Effect of Methanolic extracts of *Musa sapientum* leaves on gastrointestinal transit time in normalanimals. Values are Mean ± SEM. ** = values that are significantly different from corresponding values obtained in control animals (* = P<0.05; ** = P<0.01).

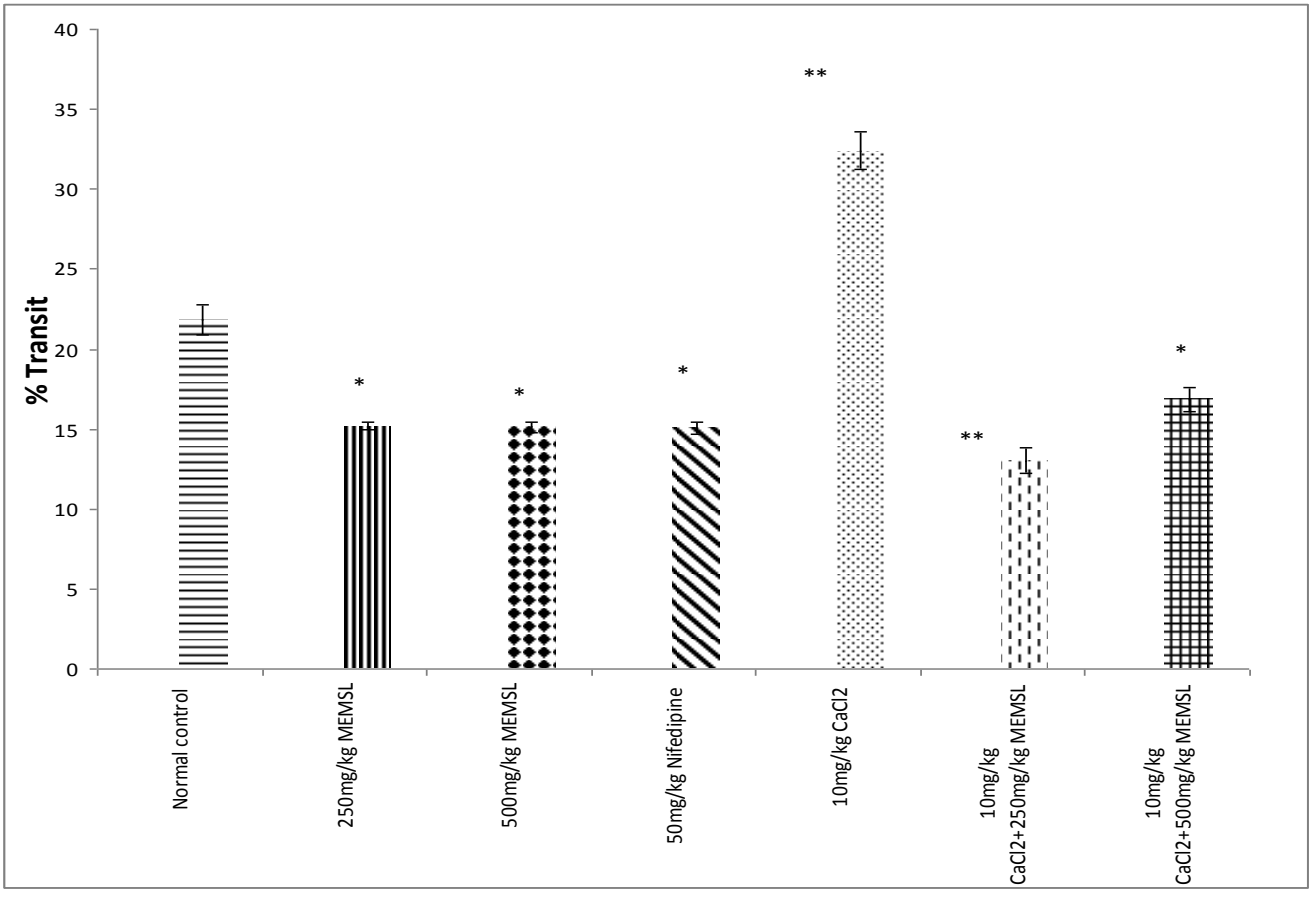

Figure2.

Mechanistic study of the effect of Methanolic extract of *Musa sapientum* leaves on gastrointestinal transit time in normal animals. Values are Mean ± SEM. ** = values that are significantly different from corresponding values obtained in control animals (* = P<0.05; ** = P<0.01).

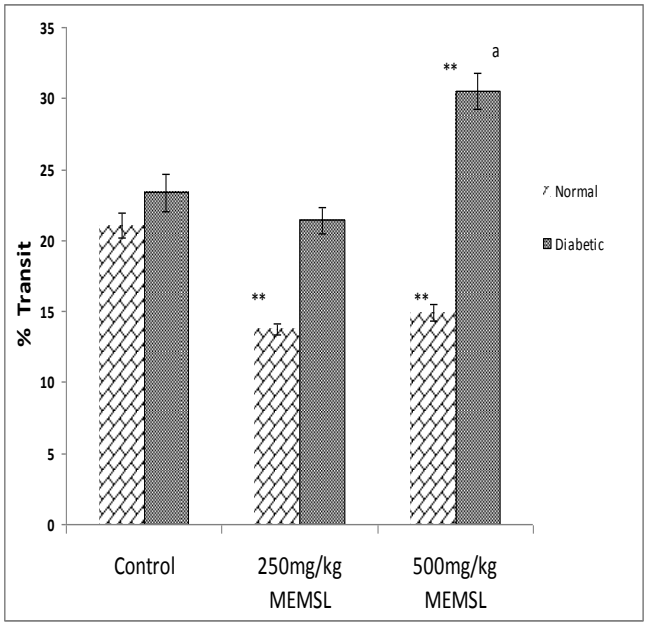

Fig.3. Effect of Methanolic extract of *Musa sapientum* leaves on gastrointestinal transit time in normal animals and diabetic animals. Values are Mean ± SEM. ** = values that are significantly different from corresponding values obtained in normal control animals (* = P<0.05; ** = P<0.01). [a] = values that are significantly different from corresponding values obtained in diabetic control animals (a = P<0.05)

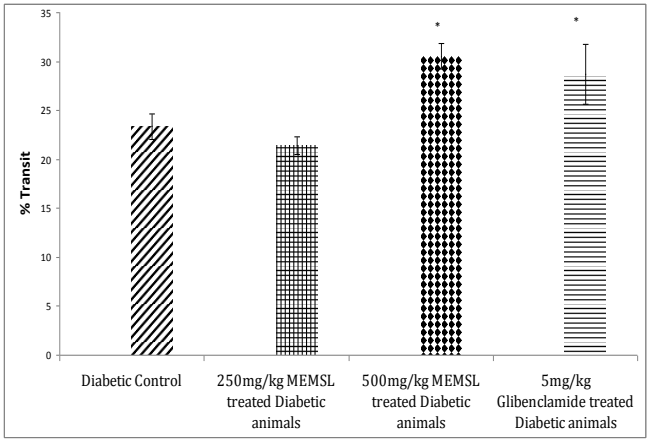

Fig. 4 Effect of Methanolic extract of *Musa sapientum* leaves on gastrointestinal transit time in Diabetic animals. Values are Mean ± SEM. * = values that are significantly different from corresponding values obtained in diabetic control animals (* = P<0.05).

animals were pretreated with $CaCl_2$ and 10minutes later given 250mg/kg and 500mg/kg of extract respectively, significant ($P \leq 0.05$) decrease in GITT was observed compared with control (Fig. 2).

Effect of MEMSL on GITT in diabetic animals

In the diabetic control groups, a slight but insignificant increase in GITT was observed when compared with the normal group (fig 3). There was an insignificant decrease in GITT in diabetic rats treated with 250mg/kg MEMSL while a significant increase in GITT was observed in diabetic animals treated with 500mg/kg of extract (fig 3). It was also observed that in animals treated with 5mg/kg glibenclamide only (a standard anti-diabetic drug), a significant increase in gastrointestinal transit time was observed when compared with the diabetic controls (fig 4)

DISCUSSION

About 76% of diabetic patients suffer from gastrointestinal disorders (Reddy et al, 2006). Chesta, 1990, reported delayed stomach to caecum transit time in diabetes. Anjaneyulu and Ramarao (2002), reported that gastrointestinal complications such as changes in transit time may occur due to increased cholinergic and decreased beta-adrenergic receptor activities in diabetes mellitus and that gastrointestinal disorders seem to be the consequence of altered innervations of a receptor system in diabetes. Oyebola and Alada, (1993) reported the effects of adrenergic receptor blockers on hyperglycemia and Alada and Oyebola, (1997) reported the role of adrenergic receptors in glucose uptake by the canine gut. These reports show the regulatory role of gastrointestinal tract and the modulatory roles of its receptors on blood glucose. Moderation of various gastrointestinal activities relating to blood glucose including gastrointestinal transit of food substances may be involved in management of diabetes mellitus. Gastrointestinal complications have been widely reported in diabetes mellitus while information on the effect of diabetes on intestinal motility remains inconclusive (Perusicova, 2004). Some researchers report decreased motility resulting in gastropariesis, constipation (Rayner et al, 2001), while others report increased motility resulting in diabetic diarrhea (Perusicova, 2004)

The reduction observed in gastrointestinal transit time in normal rats treated with 250mg/kg and 500mg/kg MEMSL was comparable to the effect of Nifedipine (a dihydropyridine voltage gated L type channel blocker). In this study, phytochemical screening of the crude extract of Musa sapientum leaves showed the presence of alkaloids and saponins which can affect the electrical activities of the smooth muscles of the gastrointestinal wall. Francis et al (2002) and Alexander et al (2008) reported that alkaloids in addition to causing dryness of the mucosa in the upper gastrointestinal tract also antagonize the muscarinic acetylcholine receptors thus preventing the binding of acetylcholine. Since acetylcholine is the transmitter responsible for the peristaltic and segmentation movements in the small intestine, blockage of such receptors could cause delay, slowing down of activities or blocking of the

smooth muscle contraction. Also, saponins are known to possess the ability to block membrane ion channels (Kai et al, 2008), however, the mechanism by which it does this is yet to be fully elucidated. The combined effects of the alkaloids and saponins in the extract could have accounted for the observed decrease in GITT in the normal and increase in the $CaCl_2$ pretreated animals when treated with the extract. On the other hand, increased concentrations of alkaloids and saponins have been reported to cause increased electrical activity of membranes leading to increased intestinal motility (Gogelein and Huby, 1984; McManus et al, 1993; Alexander et al 2008). The higher dose of the extract could not be unconnected with a change in the concentration of saponins and alkaloids present in the extract; it is therefore possible that the concentration of alkaloids and saponins in the high dose (500mg/kg) could be one of the reasons for the increased intestinal motor activity displayed in GITT in the diabetic group treated with the extract. This increase was comparable to the effect of glibenclamide (5mg/kg) on diabetic animals (Fig.4). Glibenclamide is an ATP gated K+ channel inhibitor (Santos and Rao, 1999)

Calcium ions play a vital role in the excitation and contraction of smooth muscles (Orlov and Postnov, 1982; Carafoli, 1991; Sandhiya et al, 2008). The administration of nifedipine produced a decrease in GITT while calcium chloride produced increased GITT in the animals. The results obtained in normal rats treated with both 250mg/kg and 500mg/kg of the extract was comparable to the results obtained from nifedipine treated rats which suggests the possibility of the extract at these doses in normal rats acting in a similar manner to nifedipine. This observation was further substantiated when groups 9, 10 and 11 were pretreated with $CaCl_2$ and in addition treated with 250 and 500mg/kg of the extract. A reduction in transit time was observed which implies that the extract most likely blocked the agonistic action of calcium chloride through calcium channels thereby reducing the transit time that would have increased in the presence of calcium chloride (McManus et al, 1993). Glibenclamide treated diabetic group produced significant increase in transit time contrary to the inhibitory effect observed in control group (normal rats) treated with glibenclamide. The diabetic group treated with MEMSL also produced significant increase in transit time compared to control group treated with MEMS. Glibenclamide functions by closing the ATP-sensitive K^+ channel thereby blocking the hyperglicaemic reduction in transit time (Santos and Rao, 1999). Similar effect observed in this study with MEMSL showed a possible involment of the ATP-sensitive K^+ channels in its mechanism of action.

In conclusion, methanolic extract of *Musa sapientum* leaves at 250mg/kg and 500mg/kg caused a significant decrease in gastrointestinal transit time in normal rats while in diabetic rats, the extract at 500mg/kg caused significant increase in gastrointestinal transit time, mimicking the effect of glibenclamide (an ATP gated K^+ channel inhibitor). These effects may be due to the extract at low dose acting as a possible Ca^{2+} inhibitor, hence, the inhibition on gastrointestinal motility. The involment of the ATP-sensitive K^+ channel or adrenergic receptor in the mechanism of action of MEMS is not impossible (Santos and Rao, 1999; Oyebola and Alada,1993). However, there is need for further studies on the biological activities of this plant in order to ascertain its effect on K^+ channel and how it affects gastrointestinal transit time in diabetes.

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