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Selected herbal extracts improve diabetes associated factors in 3T3-L1 adipocytes

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Abstract

Owing to the current worsening situation of the increasing burden of diabetes around the world including Malaysia it is worthwhile to discover non-pharmacological prevention or treatment for it. In order to have a useful explanation of the efficacy of herbs or nutrients for diabetes; it is desirable to know the effect on the balance between the adipogenesis, adipolysis and glucose uptake in the adipose tissues. Therefore, four herbs namely Orthosiphon stamineus (Cat whisker) (OS), Peronema canescens (Sungkai) (PC), Momordica charantia (Bitter gourd/bitter melon) (MC) and Pithecellobium jiringa (Jering) (PJ) were screened for their antidiabetic properties in in vitro model 3T3-L1 adipocytes. Water extracts of these herbs were prepared and evaluated for their effects on cell proliferation, adipogenesis, adipolysis and glucose uptake in 3T3-L1 preadipocytes cells. The aforementioned extracts promoted cell proliferation at a dose of 0.25mg/ml which showed more than 90% viability after 48 hours of treatment. The result of this study indicates that OS extracts significantly (P<0.001) increased adipogenesis whereas PC, MC and PJ extracts were not effective compared to control. The extracts from all four plants caused increased lipolysis compared to control. The Extract from OS and PJ significantly (P<0.05) stimulated glucose uptake in the cells whereas PC, MC were not effective. When the glucose consumption was compared to control it was significantly (P<0.001) increased for all extracts in the medium. The present study provides some important baseline data on the biochemical aspects of the effect induced by the herbs and suggestive of possessing antidiabetic properties which can be exploited for diabetes prevention and associated metabolic dysfunctions.

Keywords: Herbal extracts, 3T3-L1 adipocytes, adipogenesis, adipolysis, glucose uptake
1. Introduction

According to the current data available in the world literature, non-communicable diseases (NCD) are increasing day by day; therefore, an enhanced number is reported in the articles appearing in the literature. Similarly, the cost of treatment is rising on families, health departments and governments thus contributing to global cost of treatment of the NCDs. Among the NCDs diabetes is the leading one and causing higher rates of morbidity and mortality adding to the misery of the communities. Therefore, an emphasis should be given on the alternative treatment/prevention of the NCDs which would be less expensive and easily available made from local ingredients. Since ancient time, medicinal plants have been used almost in all cultures as sources of medicine. It has been estimated that about 80-85% of population in developed and developing countries depend on traditional medicine for their primary health care needs. This involves the use of plant extracts or their active compounds as a major part of traditional use (Elujoba et al., 2005; Muthu et al., 2006; Tomlinson & Akerele, 1998). World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs and plants be further investigated (Bailey & Day, 1989). The World Health Organization (2002) has also realized that an effective health agenda for developing countries never be achieved by western medicine alone and need to be complemented with alternative medicine including traditional herbal medicine. In order to achieve the goal of primary health care, WHO advised developing countries of the world to make use of their medicinal plants (WHO, 2002). It is reported that sufferers of some chronic diseases from developed countries are also choosing herbal remedies as an alternatives compared to commercial synthetic drugs (Calixto, 2000). This interest might be because of several factors which includes the followings: There are some beliefs that phyto-medicine is used for the treatment of certain diseases where conventional medicine fails. Studies performed by Iwu, Duncan, & Okunji, (1999) reports that medicinal plants are gentle, affective and often specific in their function to organ or systems of the body. According to Bnouham et al., (2006), Most of the new compounds isolated from plants have hypoglycaemic effects show equal and sometimes possess more antidiabetic activity compared to commercial drugs. Medicinal plants are usually less expensive and easy to find than synthetic drugs. Modern drugs usually give greater and quicker effects than medicinal plants but pose a higher degree of side effects. Medicinal plants usually considered as natural, non-toxic, lack adverse effects (Aronson, 2008; Haq, 2004). Some of the plants may delay the development of diabetic complications (Chauhan et al., 2010). Ethno-pharmacological surveys reports that more than 1200 plants have been used as traditional medicine for their hypoglycaemic activity (Dey et al., 2003; Grover et al., 2002; Marles & Farnsworth, 1995). Most of these plants have been evaluated using animal models in order to confirm their hypoglycaemic activity (Gupta et al., 2005; Kesari et al., 2006) as well as in human (Herrera-Arellano et al., 2004; Jaouhari et al., 1999; Jayawardena et al., 2005). Some of these plants have also been studied for their bioactive compounds (Grover, et al., 2002; Jayawardena, et al., 2005; Kesari et al, 2005). The most studied and commonly used medicinal plants for blood glucose lowering properties have been tested and confirmed in different parts of the world include Allium cepa (Onion), Allium sativum (Garlic), Aloe vera, Cinnamomum tamala, Coccinia indica, Gymnema sylvestre (Gurmar), Momordica charantia, Murrayi koningii, Ocimum sanctum, Panax (Asian) Ginseng, Trigonella foenum-graecum (Fenugreek) and Syzigium cumini (Bailey & Day, 1989; Bnouham, et al., 2006; Grover, et al., 2002). There are several mechanisms of action involved in which these medicinal plants or herbs act to control blood glucose level (Tanira, 1994). Some hypothesis related to their effects on improved glucose homeostasis by increasing peripheral utilization of glucose and hepatic glycogen or decrease glycogenolysis and inhibition of intestinal glucose absorption. Other mechanisms may involve the effects on the activity of pancreatic β cells by regeneration of damaged cells and increase insulin synthesis or secretion. Apart from those, Bnouham, et al., (2006) and Tanira, (1994) report that i) mimicking the action of insulin by acting like insulin, ii slowing down the absorption of carbohydrates from the gut iii) increase insulin sensitivity by enhanced glucose uptake by adipose and muscle cells and iv) alter the activity of some enzymes involved in glucose metabolism. Therefore this project was designed to study the effect
of various herbs namely and a common ingredient of spices cinnamon for the efficacy of diabetes and hyperlipidaemia in in vitro model adipose cell lines (American Type cultured 3t3-L1 Cells).

2. Materials and methods

Four dried herbs namely herbs namely Orthosiphon stamineus (Cat whisker) (OS), Peronema canescens (Sungkai) (PC), Momordica charantia (Bitter gourd) (MC) and Pithecellobium jiringa (Jering) (PJ) were purchased from local supplier at Kuantan, Pahang, Malaysia. The cell lines namely 3T3-L1 preadipocytes (derived from mouse embryonic fibroblast) were purchased from American Type Culture Collection (Manassas, VA). Fifty gram of dried and powdered herbs materials were soaked in 100 ml of distilled water and stirred using incubator shaker for two days for 24 hours at 1000 rpm and 60 °C. This procedure was repeated for three times using the same spice and herb materials. The extracts were then combined and filtered through Whatman filter paper. The extracts were placed in a freezer at 4 °C overnight followed by freeze drying for 1 week. The crude extracts were weighed and stored at -80°C for further analysis. The 3T3-L1 preadipocytes were grown in Dulbecco’s Medium Eagle Medium (DMEM) and supplemented with 10 % fetal bovine serum (FBS) and 1 % penicillin (10,000 U/ml) and 1 % streptomycin (10μg/ml). The cells were sub cultured every 2-3 days after reaching confluent. From the cultured cells the supernatant were removed and discarded and were washed two times with phosphate-buffered saline A (PBSA). This step was conducted to remove traces of serum that might inhibit the action of the trypsin. The cell counting and viability were performed as described by Taher, (2005) and Patel et al., (2009) using hemocytometer. Cell viability was assessed by using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay as described previously with slight modification by Mosmann, 1983. The MTT is only cleaved by all living and metabolically active cells, but not by dead cells. The amount of formazan formed is directly proportional to the cell number over a wide range, using a homogenous cell population. Activated cells produce more formazan than resting cells, which could allow the measurement of activation even in the absence of proliferation. These properties are all consistent with the cleavage of MTT only by active mitochondria. An increase in number of living cells results in an increase in the total metabolic activity in the sample. This increase directly correlates to the amount of purple formazan crystals formed as monitored by the absorbance (Gomez et al., 1997). The absorbance reading correlates with viable cell number and metabolic activity of the cells. Adipogenesis and adipolysis assay kits (adipogenesis assay kit item number 10006908 and adipolysis assay kit, item number 10009381 from Cayman Chemical Company, 1180 East Ellsworth Road, Ann Arbor, Michigan 48108, USA) were used to assess the effect of the extract on lipid synthesis and degradation in the cultured 3T3-L1 cells lines. These kits methods allow screening of compounds involved in lipid storage and metabolism in the cultured 3T3-L1 cell lines. Glucose uptake activity was analyzed by measuring the uptake of radiolabelled glucose. This was performed based upon previously described methods with modification by Roffey et al., (2007). Samples from each lysate were counted using liquid scintillation Counter (Packard Tricarb 2700 TR/SL liquid scintillation analyzer, Packard Instrument Co.). Glucose uptake was measured in triplicate. Furthermore, Glucose oxidase assay was performed by using Glucose Assay Kit (Sigma-Aldrich, Inc). The collected data was compiled and statistically analysed using SPSS (Version 12.0). In most cases, descriptive statistic was applied and is represented as mean and standard deviation (S.D.) of the mean. Furthermore, one-way analysis of variance (ANOVA) was used wherever appropriate. The data was considered statistically different at 95% confidence interval using Turkey’s post hoc test.
3. Results

As mentioned earlier, herbs namely *Orthosiphon stamineus* (Cat whisker) (OS), *Peronema canescens* (Sungkai) (PC), *Momordica charantia* (Bitter gourd/bitter melon) (MC) and *Pithecellobium jiringa* (Jering) (PJ) were extracted using hot distilled water and the final yield recorded is presented in the Table 1. Among the plant extracted, M. charantia yielded the highest crude extracts and O. stamineus the lowest with 20.0 and 5.40 % respectively. These extracts were used for the treatment cultured adipocytes.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Vernacular name</th>
<th>Final yield (g)</th>
<th>Final yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Orthosiphon stamineus</em> (OS)</td>
<td>Cat whisker</td>
<td>2.72</td>
<td>5.40</td>
</tr>
<tr>
<td><em>Peronema canescens</em> (PC)</td>
<td>Sungkai</td>
<td>4.10</td>
<td>8.20</td>
</tr>
<tr>
<td><em>Momordica charantia</em> (MC)</td>
<td>Bitter gourd</td>
<td>9.78</td>
<td>20.0</td>
</tr>
<tr>
<td><em>Pithecellobium Jiringa</em> (PJ)</td>
<td>Jering</td>
<td>3.75</td>
<td>7.50</td>
</tr>
</tbody>
</table>
3.1 Cell Growth, Viability and Cell Proliferation

It was observed that 3T3-L1 preadipocytes showed a maximum growth and viability on fourth day after the subculture. The cells growth as well as viability start to decline after fourth day of the subculture as indicated in the Figure 1 and 2 respectively.

The results show that the effective concentration of extracts was 0.25 mg/ml (Fig. 3.3). However, when the highest concentration (1.0 mg/ml) of extracts was applied to culture, the number of live cells decreased. This indicated that the extracts at concentration of 1.0 mg/ml might be potentially toxic to 3T3-L1 adipocytes. The concentration of 0.25 mg/ml extracts resulted in the maximum cell growth with approximately 90% of viability, after applied for 48 hours as indicated in the Figure 3. Thus, the MTT assay showed that the cells well tolerated the extracts at concentration of 0.25 mg/ml. This maximum tolerated concentration was chosen for this study.

![Figure 1](image.png)

*Fig. 1 Growth of 3T3-L1 preadipocytes treated with extracts. The data are means ± S.D. of triplicate experiments.*
Fig. 2 Cell viability of 3T3-L1 preadipocytes treated with extracts. The data are means ± S.D. of triplicate experiments.
Fig. 3 Effect of extracts on the adipose cells (3T3-L1 adipocytes) viability at a concentration range of 0.031 – 1.00 mgs assessed by using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay.
3.2 Lipid Droplet Formation, Adipogenesis and Adipolysis

After day 11, preadipocytes differentiation was terminated and stained with Oil Red O. The lipid droplets were viewed in all extract induced adipocytes. The Fig 4 (A&B) show fully differentiated adipocytes before performing Oil Red O staining.

As mentioned earlier on that plants extracts were evaluated for their activities on adipogenesis (the induction of differentiation of pre-adipocytes into adipocytes). The absorbance reading at 492 nm for differentiated cells was typically around 0.2-0.4. For of control and un-differentiated cells, the absorbance was around 0.05. From the test, it is evident that replacing insulin with extracts exhibited the activity in promoting cell differentiation which is indicated by the accumulation of the lipid droplets. The results for adipogenesis are expressed as absorbance values. Theoretically, higher the lipid droplets formation the higher is the optical density and therefore, the plants with more formation of lipid droplets results higher absorbance would be effective in the induction of differentiation of pre-adipocyte to adipocyte. Insulin treatment exhibited higher lipid droplets formation followed by O. stamineus, and M. charantia whereas P. canescens and P. jiringa moderately induced the differentiation. Control (without treatment) showed no differentiation as indicated by the lipid droplet formation and absorbance reading. There was significant difference for insulin and O. stamineus on the undifferentiated cells when compared with the control (differentiated cells). However, there was no difference for other extract when compared with control (differentiated cells) as indicated in the Fig 4 & 5.

(A) Fully differentiated preadipocytes before Oil Red O stained viewed at magnification X 20 and (B) magnification X 40 showing lipid droplets formation/accumulation.
(C) Positive control (insulin)  
(D) Negative control (without treatment)  
(E) Orthosiphon stamineus  
(F) Peronema canescans  
(G) Momordica charantia  
(H) Pithecellobium jiringa
Fig. 4 Effect of extracts on adipocytes differentiation viewed at magnification X 20 and the red areas showed lipid droplets. (C) Insulin (D) without treatment (E-H) treated with plants extracts.

![Graph showing optical density for lipogenesis (492 nm) for different plants extracts.](image)

Fig. 5 The effect of plants extracts on the adipogenesis in 3T3-L1 adipocytes. Differentiating cells were treated for 48 hours with 0.25 mg of extracts in adipocyte-induction media. After day-11 post-confluent, adipocyte differentiation was terminated and stained with Oil Red O and extracted with either dye solution or isopropanol. The absorbance was measured with UV spectrophotometer at 492 nm. Data are means ± S.D. of three observations.
The aforementioned extracts effect was evaluated on adipolysis which is the hydrolysis of triacylglycerol to release free fatty acid and glycerol. The results were recorded from the quantitative measurement of glycerol release in the differentiated 3T3-L1 adipocytes into the culture medium using the glycerol release assay in response to isoproterenol.

Fig. 6 Effect of extracts on adipolysis (breakdown of lipids) in 3T3-L1 adipocytes observed with glycerol release assessment with UV spectrophotometer. Data are means ± S.D of three observations, expressed as glycerol concentration (µg/ml).

Compared to control both insulin and plants significantly increased adipolysis activity in cells treated with the extracts but lower than isoproterenol. Insulin and O. stamineus had similar action whereas P. canescens, M. charantia and P. jiringa extracts caused significantly higher glycerol release as shown in the Fig. 6.
3.3 Effect of Extracts on Glucose Uptake and Glucose Consumption

Furthermore, the extracts were also evaluated for the glucose uptakes in the cells both by isotopic as well as by glucose oxidase methods and the results are shown in the Figures 7-8. There was significantly (P<0.001) increased glucose uptake activity in cells treated with Sodium Orthovanadate compared to control and the extracts. Similar in effect the O. stamineus and P. jiringa significantly (p < 0.05) enhanced glucose uptakes and the other two extracts were not different to control. While assaying the glucose consumption with glucose oxidase assay, it was found that insulin showed significantly (p < 0.001) higher consumption of glucose followed by O. stamineus (p < 0.001), M. charantia (p < 0.001), P. canescens (p < 0.001), and P. jiringa (p < 0.01) compared to control.

![Fig.7 Effect of extracts on glucose uptake in 3T3-L1 adipocytes after treatment for 48 hours with 0.25mgs in the induction media and assessed by radiolabelled assay. After day-11 post-confluent, adipocyte differentiation was terminated and assayed for 2-deoxy-D-[1, 2-3H]-glucose uptakes then, measured by using liquid scintillation counter. Data are means ± S.D. of three observations, expressed as disintegration per minute (DPM).]
Fig. 8 Effect of extracts on glucose consumption by 3T3-L1 adipocytes after treatment for 48 hours with 0.25mgs in the induction media and assessed by using glucose oxidase method. The 3T3-L1 adipocyte cells were cultured in 96 well plates until reached 90% confluent Glucose remaining in the medium was measured with UV spectrophotometer. Data are means ± S.D. of three observations, expressed as glucose concentration (mg/ml).
4.0 Discussion

The MTT method is used to measure cytotoxicity, proliferation or activation of the cells. The main advantages of the colorimetric assay are its rapidity and precision, convenient and the lack of any radioisotope (Mosmann, 1983 & Sylvester, 2011). The MTT gives a yellowish aqueous solution which on reduction by dehydrogenases and presence of reducing agents in metabolically active cells yields a water insoluble purple formazan. The lipid soluble formazan product can be extracted with organic solvents and estimated by spectrophotometry. The amount of MTT formazan formed during a given exposure is directly proportional to the number of viable cells (Mosmann, 1983; Sylvester, 2011). The finding of this study showed that there was reduced adipocytes viability with increasing concentrations of the extracts. Similar effects have been previously reported when adipocytes were tested with bitter gourd (Popovich et al., 2010). In this study, a maximum cell growth of approximately 90% viability at concentration of 0.25mg/ml was chosen in order to avoid toxic effect to the adipocytes. The summary of the extract is indicated in the Table 2 and the results are discussed in following sections;

<table>
<thead>
<tr>
<th>Activities</th>
<th>O. stamineus</th>
<th>P. Canescens</th>
<th>M. charantia (MC)</th>
<th>P. jiringa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipogenesis</td>
<td>↑***</td>
<td>↓***</td>
<td>_</td>
<td>↓***</td>
</tr>
<tr>
<td>Adipolysis</td>
<td>↑***</td>
<td>↑***</td>
<td>↑***</td>
<td>↑***</td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>↑**</td>
<td>↑**</td>
<td>↑**</td>
<td>↑**</td>
</tr>
<tr>
<td>Glucose Consumption</td>
<td>↑***</td>
<td>↑***</td>
<td>↑***</td>
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</tbody>
</table>

The observed effect of the extracts on adipogenesis in adipocytes is of clinically importance for glucose homeostasis and energy storage. Thus, it might lead to more effective strategies for the treatment of diabetes and metabolic diseases (Lefterova and Lazar, 2009). Conversion to adipocytes can happen spontaneously but the process is enhanced by the addition of combination of dexamethasone, isobutylmethylxanthine (IBMX) and insulin (Rosen et al., 1978). Within 3 days exposure to inducers, the cells undergo mitotic clonal expansion which is required for differentiation (Tang et al., 2003). The Oil Red O is a lipophilic dye that may indicates the adipogenesis stage by staining intracellular lipid droplets. The Oil Red O staining was performed to evaluate the degree of differentiation at day 11 post-differentiation. The quantity of lipid in the adipocytes stained that with Oil Red O appeared to be proportional to the level of cell differentiation. It shows that the Oil Red O specifically stains both triglycerides and cholesteryl oleate, as reported by Ramirez et al., (1992). Thus, high dense colour suggests increased differentiation as well as triglycerides accumulation. Conversely, less colour intensity indicates decreased lipid accumulation and inhibition of differentiation which was observed in this study as indicated in the Figure 4. The differentiation is accompanied by an increase of 10 to 50 fold in the specific enzymes activities involved in fatty acid and triglycerides synthesis. Similarly, specific insulin receptors present in cells before the onset of differentiation increases by 25 fold during conversion to adipocytes (Smith et al., 1988). The results of this study show that replacing insulin with studied extracts exhibited an activity in promoting cell differentiation which was indicated by the accumulation of lipid droplets. Insulin acts as an essential regulator of the differentiation of 3T3-L1 adipocytes and accelerates the differentiation process (Smith et
al., 1988; Kletzien et al., 1992; Guller et al., 1988). Similar, observations were reported by Taher et al., (2004) that induction of adipocyte differentiation by water extract of cinnamon shows insulin like activity in adipogenesis. Another study showed a consistent result to this study in which it was observed that replacing insulin with cinnamon extract results in fully differentia ted adipocytes. This might be due to the presence of polyphenols which is the compound from water extract of cinnamon (Cao et al., 2010). Current observations on the effect of M. charantia fruit extract on muscle cells showed an up-regulation of Pparg, a key transcriptional factor in adipogenesis regulation (Kumar et al., 2009). Another study also reported on in vitro study that a number of polypeptides from M. charantia seeds have the insulin-like activities of stimulation of adipogenesis and inhibition of corticotropin-induced adipolysis. The mechanism is suggested to be involved in the interaction of the peptides with adrenergic or corticotropin receptors (Ng et al. 1986). The M. charantia extract increases the adipogenesis activity and might be due to the presence of charantin and polypeptides as hypoglycaemic compounds (Raman and Lau., 1996). Study on L. speciosa extract reports inhibition the adipocyte differentiation activity (Liu et al., 2001) and has been attributed to ellagitannins, an active compound from water extract of L. speciosa (Bai et al., 2008). However, Bernlohr & Simpson, (1996) reported absence of lipid accumulation in preadipocytes stage. This is consistent with previous study which showed that there were several compounds to be involved in the stimulation of adipolysis in differentiated 3T3-L1 and primary human adipocytes which include isoproterenol and tumour necrosis factor-α (TNF-α). Isoproterenol is a non-selective antagonist of the beta-adrenergic class of GPCRs, which stimulate cAMP levels in adipocytes which results in phosphorylation of perilipin (Robidoux, Martin, & Collins, 2004). Perilipin is located at the surface of the lipid droplets (Sztalryd et al., 2003) which results in the release of the hormone-sensitive lipase (HSL) to the surface of the lipid droplets (Zhang et al., 2003). The HSL then cleave triglycerides into their constituent fatty acids and free glycerol, which can be assayed as a marker of adipolysis. Glycerol generated by triglyceride breakdown is released into the extracellular spaces through aquaporin adipose, which has glycerol permeability (Kishida et al., 2000). Study on primary human adipocytes treated with M. charantia extract for 48 hours demonstrated consistent result with our finding which showed reduction in lipid contents, perilipin mRNA expression and increased adipolysis as measured by the release of glycerol (Pratibha et al., 2010). However, insulin showed a decreased in adipolysis activity. This has been reported in a previous study which shows that treatment of the adipocytes with isoproterenol increase glycerol release of the adipocytes but insulin is suppressed this causes increase in adipolysis (Hattori et al., 2003). The observed effect of the extracts on the uptake and glucose consumption can be of clinical significance and has been reported elsewhere (Roffey et al., 2006). Phytochemical compounds such as flavonoids, phenolic acids and tannins have been implicated in hypoglycaemic activity and these compounds have been found in extracts. Another study reported that water and methanol extract of L. speciosa leaves stimulate glucose uptake in 3T3-L1 adipocytes in a manner similar to insulin (Liu et al., 2001). Ellagitannin named ‘Lagerstroemin’ had been identified in the water extract of L. speciosa as the activators of glucose transport in fat cells observed with a glucose uptake assay (Hayashi et al., 2002). It is also consistent with the study by Cheng and Liu, (2000) revealed that O. stamineus extract which are rich in phenolic compound namely caffeic acid, has been reported to increase glucose uptake in rat myocytes. Previous studies reported that in vitro study with M. carantia fruit extracts caused a significant increase of glucose uptake in muscle but no effect in adipose tissue (Meir and Yaniv 1985; Welihinda and Karunanayake 1986). Another study revealed that the treatment of a combination of 0.2 mg/ml M. charantia water extract and 0.5 nm insulin was associated with significant increase in glucose uptake. However, there was no effect on glucose uptake in the absence of insulin in adipocytes exposed to the water extracts (Roffey at al., 2007) but no effect with M. charantia extract on glucose consumption by adipocytes due to loss of triterpenoids from extracts during preparation (Kaur et al., 2011). This is because, previous study by Tan et al., (2008) revealed that the bioactive components namely cucurbitane triterpenoids present in M. charantia extract have been associated with increased AMP-activated protein kinase, key target mediating glucose uptake. The vanadate stimulates the rate of 2-deoxyglucose uptake to the same extent as insulin in adipocytes. This effect was suggested that vanadate induced the recruitment of Glut-4 in the plasma similar to insulin (Paquet et al., 1992) and same in adipocytes (Grisouard et al., 2010). The study showed an increase of cellular glucose uptake after 24 and 48 hours treatment with metformin but did not
significantly increase glucose uptake with just 3 hours incubation time. Furthermore, incubation with 0.001 and 0.1 mM metformin also did not potentiate the glucose uptake compared to 1 and 10 mM metformin which induced glucose uptake activity (Grisouard et al., 2010).

5.0 Conclusion

The present study provides some important baseline data on the biochemical aspects of the effect induced by the herbs and suggestive of possessing antidiabetic properties which can be exploited for diabetes prevention and associated metabolic dysfunctions.

6.0 Acknowledgements

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