In vivo and *in vitro* anti-natriuretic activity of twigs fraction from *Dorstenia picta*: A possible mechanism

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Abstract: The present study examine the *in vivo* effects of *Dorstenia Picta* (*D. picta*) on urinary volume and sodium excretion in streptozotocin-induced diabetic rats, and determine a possible mechanism by which the extract increased sodium transport in A6 cells monolayers. Administration of the plant extract at the dose of 150 mg/kg during two weeks decreased urinary volume and sodium excretion. *In vitro* study showed that, apical application of the plant extract at the dose of 100 µg/mL does not significantly increase sodium transport, whereas basolateral administration provoked a significant ($P<0.05$) increase of sodium transport in a concentration-dependent manner. The plant extract increases the sodium transport by 69.93% versus 55.41% for insulin and 78.44% for adenosine after 30 min. Preincubation of A6 cells monolayers with inhibitor of all adenosine receptors completely suppressed adenosine and plant extract stimulated sodium transport. Interesting is that, the A_1 inhibitor receptor (DPCPX), at 100 nM completely abolished the effect of plant extract. The plant extract increased sodium transport by increase PI3-kinase activity and this effect is strongly inhibited by LY-294002. These data also suggest that, the twigs methanol fraction from *Dorstenia picta* increase sodium transport via PI 3-kinase pathway and requires A_1 adenosine receptor.

Keywords: Adenosine receptor, diabetes, *Dorstenia picta*, phosphatidylinositol 3-kinase, sodium transport.

INTRODUCTION

Several experimental models are used to demonstrate the various complications related to diabetes mellitus; including streptozotocin-induced diabetic model. Complications of diabetes mellitus include retinopathy, neuropathy and nephropathy **(**Kalantarinia and Okus, 2006; Tiwari *et al*., 2007). Hyperglycemia observed in diabetics causes a loss of water followed by a loss of electrolytes (Na^+ and K^+). One important function of the kidneys is to regulate the amount of sodium in the body. This regulation occurs at the distal tubule and precisely at the collecting duct which is the target of some hormones including insulin. This hormone has been shown to increase sodium transport via phosphatidylinositol 3-kinase pathway (Record *et al*., 199; Blazer-yost *et al*., 2003). Some antidiabetic agents exert their activities by mimicking the effect of insulin on several biochemical and cellular assays (Bei *et al*., 1999; Sakurai *et al*., 2002).

Plants of Moraceae family are commonly used in the Cameroonian traditional medicine in the case of diseases including hypertension and diabetes mellitus (Ngueguim *et al.,* 2006; Ngueguim *et al*., 2007). In previous studies, twigs fraction of *Dorstenia picta* (*D. picta*) has been shown to have antidiabetic activities (Ngueguim *et al*., 2007). The aim of the present study was to investigate the effects of twigs methanol fraction from *Dorstenia picta* on urinary volume and sodium excretion. Furthermore, the signalling pathway triggered was investigated.

MATERIALS AND METHODS

Plant material

The twigs of *Dorstenia picta* were collected from Ngoumou (centre region of Cameroon) in August 2005. The botanical identification of the plant was carried out by Dr. zapfack Louis of the Department of Plant Biology and Physiology, Faculty of Science, University of Yaounde I. The voucher specimen (N°57063) was deposited at the National Herbarium, Yaoundé, Cameroon.

Preparation of the plant extract

The air dried tiwgs of *D. picta* were powdered (2 kg) and macerated at room temperature in two liters of methanol for 48 hours. The mixture was filtered and concentrated under reduced pressure. The residue obtained (200 g) after concentration was successively partitioned in hexane and ethyl acetate. The remaining residue (neither soluble in hexane nor ethyl acetate) constitutes the methanol residue extract of *D. picta* (60 g).

Chemicals

All the chemicals were purchased from Sigma-Aldrich Chemical Co (St Louis, MI, USA).

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Cell culture and treatment

The A6 cells used in this study were obtained from Prof W. Van Driessche (Department of Physiology, Katholiek Universiteit te Leuven, Belgium. They were grown on permeable sterilized culture plate insert (Millipore Corporation, Bedford, MA 01730 U.S.A) in humidified incubator with 1 % CO_2 in O_2. The growth medium was a mixture of 34% Ham's F- 12, 34% Leibovitz's L-15, 20 % water, 10% FBS, 3.8 mM L-glutamine, 87 IU penicillin, 87 µg/mL streptomycin and 8 mM $NaHCO_3$. The osmolality of the growth medium was 260 mosmol/kg H_2O. Cells were seeded and the growth medium was renewed twice weekly and used after 7-14 days of growth.

Amiloride was added to the apical bathing medium at a final concentration of 100 µM. The *D. picta* extract was added at the basolateral bathing medium at a final concentration of 100 µg/mL, unless otherwise indicated. Insulin (100 nM), adenosine (100 µM), dissolved in distilled water were also added basolaterally, adenosine antagonist: the selective A_1 inhibitor 1,3-Dipropyl-8-cyclopentylxanthine (DPCPX, 100 µM), the selective A_{2a} inhibitor 1,3,7-Trimethyl-8-(3-chlorostyryl) xanthine (CSC, 100µM), the selective A_{2b} 8-[4-[((4-Cyanophenyl)carbamoylmethyl)oxy]phenyl]-1,3-di(n-propyl) xanthine (MRS 1754, 100 µM), the selective A_3 inhibitor 3-Ethyl-5-benzyl-2-methyl-4-phenylethynyl-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (MRS1191, 100 µM) were dissolved in DMSO and were added basolaterally while the PI 3-kinase inhibitor LY-290042 (50 µM, Sulfophenyl) theophylline hydrate ; inhibitor of all adenosine receptors (SPT, 100 µM) were dissolved in DMSO and added to both bathing media.

In vivo studies

Induction of experimental diabetes in rats

Diabetes mellitus was induced by intravenous (penile vein) injection of 55 mg/kg of streptozotocin in 0.9% sterile sodium chloride solution to non-fasted rats anesthetized with ether. Control group received normal saline through the same route. Four days later, diabetes was confirmed in streptozotocin-treated rats by measuring fasting blood glucose levels. Rats with blood glucose levels greater than or equal to 300 mg/dL were considered as diabetic. Rats were kept for 14 days before the beginning of the treatment to stabilize the diabetic condition and to allow a permanent and chronic hyperglycaemia (Jyoti *et al*., 2002).

Treatment

Forty rats were divided into five groups of 8 rats each: group I made of normal rats received the vehicle (10 mL/kg of distilled water), group II served as diabetic control received also the vehicle, group III was administered insulin subcutaneously (10 UI/kg), groups IV and V were treated with methanol-derived extract from *Dorstenia picta* twigs, at the doses of 75 and 150 mg/kg respectively (Ngueguim *et al*., 2007). Fourteen days after injection of streptozotocin, substances were given daily during two weeks. At the end of treatment, rats were individually housed in metabolic cages for 12 hours. Then urines were collected for sodium excretion and urinary volume measurement. Concentration of sodium was determined by flame photometry (Ratnasooriya *et al*., 2004).

Electrophysiological measurements

A6 cells monolayer, grown on filter inserts were incubated overnight in serum free-medium before electrophysiological measurement. They were used only if their electrical transepithelial resistance was > 4.200 $\Omega.cm^2$ and their transepithelial potential difference > 30 mV. Resistance and potential difference were measured using an epithelial voltohmmeter (EVOM) with chopstick electrodes made of Ag-AgCl pellet (Markadieu *et al*., 2004). Sodium transport was expressed as the current (I_{Na+}) in $\mu A/cm^2$.

Immunodetection of protein kinase B (PKB)

Immunodetection of PKB on Thr308 and Ser 473 residues was performed on cell extracts. Cells were grown for a minimum of 10 days on 100 mm transwell inserts incubated in the presence of insulin or *D. picta* extract for 5 min. A6 cells were scraped in ice-cold lysis buffer (15 mM KCl, 10 mM Tris-HCl, 2 mM EDTA, 1mM sodium orthovanadate, 0.1% β-mercaptoethanol, 300 µg/mL pefabloc and 10 µg/mL leupeptin, pH 7.4) and harvest quickly at 4°C. Cell extracts were maintained at 4°C for 1h and centrifuged (13.000 rpm); the pellet was then discarded. The SDS-PAGE was performed as previously described (Markadieu *et al*., 2004). The extracts were disolved in sample buffer (10 mMTris.HCl, 1.5% SDS, 0.6% DTT, 6% glycerol, 0.1% bromophenol blue, pH 6.8) and denaturated by heat at 95°C for 3 min. Proteins were separated on 7.5% acrylamide gels and transfered to nitrocellulose membrane. This membrane was blocked and incubated overnight with a primary antibody at 4°C. The membrane was washed (three times) and incubated for 1h at room temperature with the appropriate peroxidase labeled secondary antibody (DAKO). Detection was performed by exposure to enhanced chemiluminescence (Amersham).

Immunoprecipitation

Cell lysates were incubated overnight with anti-phosphotyrosine antibodies and subsequently with protein A-agarose for 2 hours. The beads were washed three times in PBS and the collection was done after centrifugation. Samples were boiled and loaded onto 7% acrylamide gels. Membranes were probed with anti-p85 antibody.

PI3-kinase assay

PI3-kinase assay was carried out by the method described by Markadieu *et al*. (2004).

Briefly, samples were collected and washed in 50 mM Tris-HCl buffer (pH 7.4). Phosphatidylinositol (10 µg) and phosphatidylserine (20 µg) were dissolved in chloroform (10 µL). The mixture was dried at room temperature and dissolved in 50 mM Tris-HCl. The solution was homogenized before addition of radioactive ATP (4 µci, [γ-^{32}P] ATP) in kinase buffer (pH 7.4). This solution was mixed with the sample and incubated for 30 min at 37°C. The reaction was stopped with Blye & Dyer. The mixture was centrifuged and the organic phase was collected, concentrated and spotted on oxalate-precoated TLC plate. Radioactive spots were visualized and the phosphatidylinositol 3-phosphate (PIP_3) was detected.

STATISTICS ANALYSIS

The results are presented in the form of means ± SEM. The comparison between the means of control groups, treated was done by the ANOVA's test followed by Dunnett's test. P<0.05 was considered as significant.

RESULTS

In vivo studies

Effects of *D. picta* extract on natriuresis and urinary volume

The effect of twigs methanol fraction of *Dorstenia picta* on urinary volume is shown in fig. 1a. Two weeks after administration of the plant extract, there was a significant decrease in urinary volume by 33.19% and 57.77 % (compared with diabetic control rats) at the doses of 75 and 150 mg/kg respectively. Insulin (10UI/kg) also significantly (p<0.05) reduced urinary volume by 49.39%. Fig. 1b shows a significant (p<0.01) increased in urinary sodium (66.33 %) in diabetic rats compared to the normal control rats. However in the presence of plant extracts (75 mg/kg and 150 mg/kg), urinary sodium excretion was significantly reduced by 35.26 % (p<0.05) and 43.99 % (p<0.01) respectively when compared with diabetic control rats. Treatment of diabetic rats with insulin (10 UI/kg) showed a significant (p<0.05) decrease in urinary sodium content (30.87 %).

In vitro studies

Effects of the *D. picta* extract on sodium transport

Addition of the *D. picta* extract at the final concentration of 100 µg/mL to the basolateral side of A6 monolayers (but not to the apical side), induced a significant (p<0.001, n=3) increase in sodium transport. The increase of sodium transport reaches a maximum after 30 minutes and then goes back to the baseline current within about one hour (fig. 2a). Increasing the concentration of *D. picta* extract (from 6.25 to 100 µg/mL) added to the basolateral medium induced a concentration dependent rise in sodium transport which reached 69.86% at 100 µg/mL (fig. 2b). Added fifteen minutes after the stimulation of the sodium current by the extract, Amiloride (100 µM) completely abolishes the sodium current (fig. 2c).

Comparison of the plant extract with insulin

The results show that, like plant extract, at different concentrations (1 nM-100 nM) insulin induced a similar dose effect response. At the maximal dose, insulin induced a significant increase in sodium transport by 51.38 % (fig. 3a). However the decrease in current observed within 60 min after the maximal value was slow in the case of insulin and quite quicker with *D. picta* extract. The transient nature of the increase in sodium transport induced by the extract could be related to its degradation as sequential additions "on top of each other" induced a nearly stable increase, more comparable to that observed in the case of insulin(fig.3b). Quite interestingly,

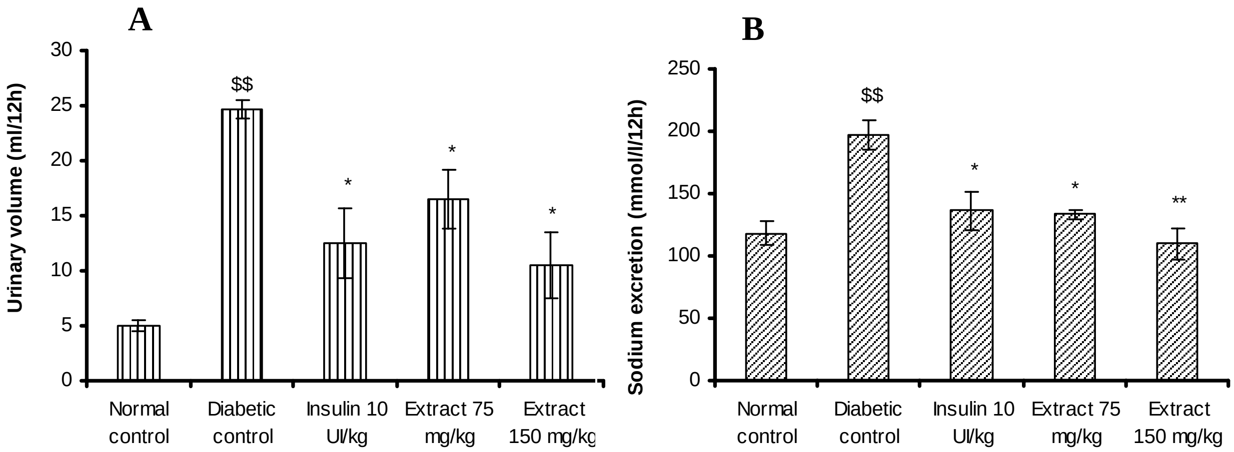

Fig. 1: Effect of *Dorstenia picta* extract on urinary volume (A) and sodium (B) excretion in streptozotocin-induced diabetic rats. Each bar represents mean±SEM, n=8, * p<0.05); ** p<0.01) compared to diabetic control, $$, p<0.01) compared to normal control.

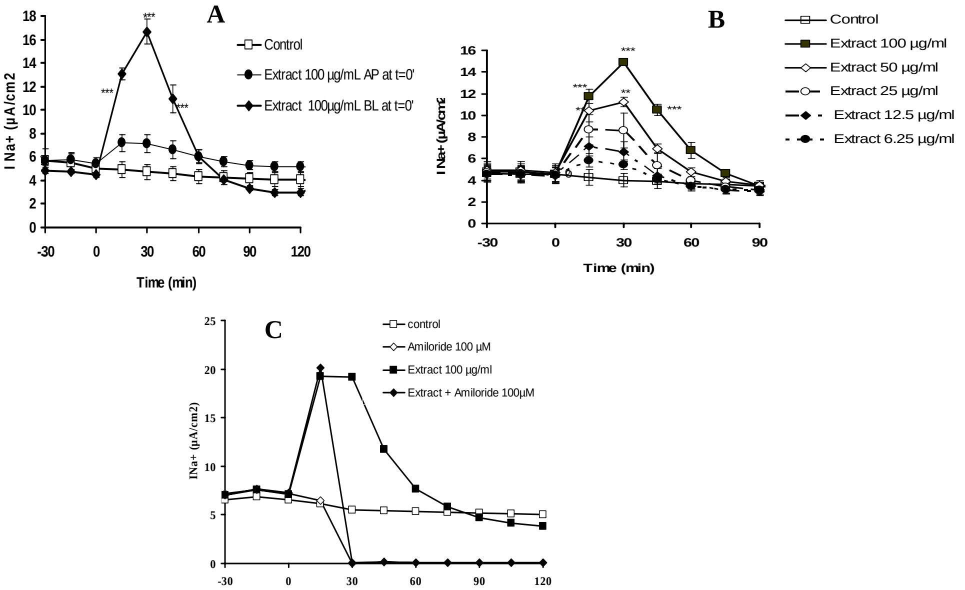

Fig. 2: Effects of *D. picta* extract on the sodium transport. A: Basolateral (Bl) and apical (Ap) addition of plant extract on sodium transport B: Dose response curves for extract stimulated sodium transport. C: Amiloride suppressed plant extract stimulated sodium transport. Extract added at time t=0, amiloride added into bathing medium at t=15 min. Each point represents the mean ± SEM, n=3, *** p< 0.001).

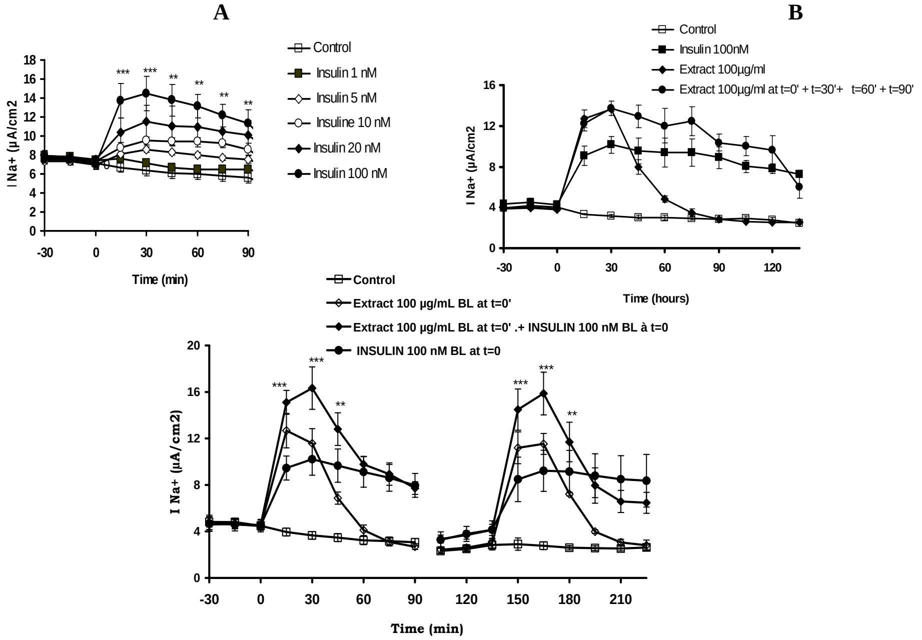

Fig. 3: Effects of *D. picta* extract and insulin on the sodium transport. A: Dose response curves for insulin. B: Effects of sequential addition of the *D. Picta* every 30 min. C: Effect of addition of insulin 100 nM together with plant extract 100 µg/ml. Extract and insulin were added into basolateral bathing medium at t=0. Each point represents the mean ± SEM, n=3, *(p<0.05); ** (p<0.01); *** (p<0.001) compared to the extract only. In between the two stimulations: wash of both media and rest for one hour.

the addition of the *D. picta* extract together with insulin, both at their maximal effective concentrations, induced a significant increase in sodium transport than any of them alone, but not reaching the sum of both of them (fig. 3c). Furthermore such stimulations were reproduced after washing the basolateral medium and adding insulin, the extract or both together again. Thus, repetitive stimulations indicate their complete reversibility and reproducibility (at least twice on the same monolayer) which is compatible with exocytic insertion and endocytic retrieval of sodium channels (ENaCs) as suggested for insulin (Blazer-Yost *et al*., 2004).

Comparison of plant extract with adenosine

Adenosine is well known to stimulate sodium transport (Lang *et al*., 1985; Casavola *et al.,* 1996). We have compared the effect of adenosine with plant extract on sodium transport. When added in basolateral medium at different concentration (0.1-100 µM), adenosine increased sodium transport in a concentration dependent manner (fig. 4a). At the maximal dose (100 µM), the maximal effect was obtained at 30 min and tends to go back to the baseline current. Adenosine (100 µM) added together with the plant extract at the basolateral medium did not reaching the sum of both of them (fig. 4b) suggesting a common pathway of these substances on sodium

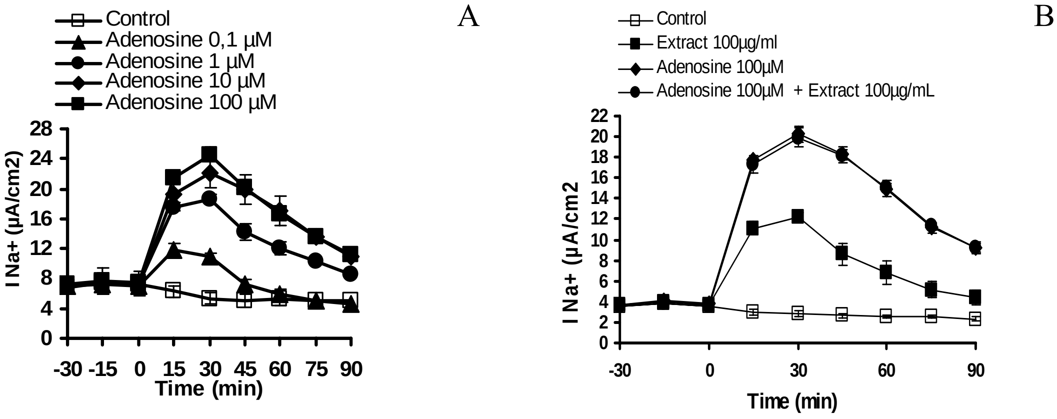

Fig. 4: Comparison of the effect of plant extract with adenosine. A: Dose response curves for adenosine on sodium current. B: Effect of adenosine and/or *D. picta* extract on sodium transport. Extract and adenosine were added into basolateral bathing medium at t=0. Each point represents the mean ± SEM, n=3.

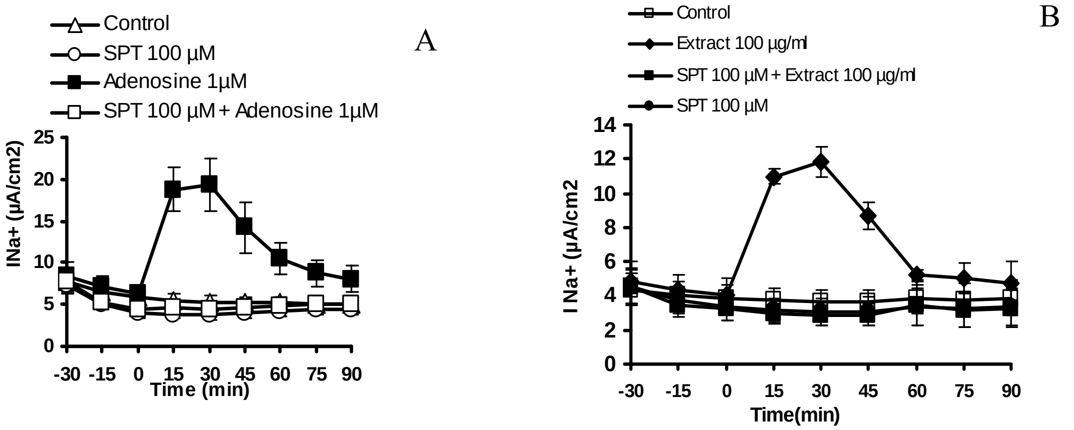

Fig. 5: Effect of SPT on adenosine (A) and *D. picta* extract (B) stimulated sodium current. Extract and adenosine were added into basolateral bathing medium at t=0, SPT was added bilaterally at t=-30. Each point represents the mean ± SEM, n=3.

transport. To determine whether *D. picta* extract stimulated-sodium transport act as adenosine, an inhibitor of all adenosine receptors (SPT) and selective adenosine antagonist receptors (DPCPX, CSC, MRS1754 and MRS1191) were used. Preincubation of A6 cells monolayers with SPT completely suppressed stimulation of sodium transport induced by adenosine (fig. 5a) or plant extract (fig. 5b). On the other hand, preincubation of A6 cell with selective adenosine antagonist receptors showed that, stimulation of sodium transport was completely inhibited by the selective A_1 receptor antagonist (fig. 6). The selective MRS1754 A_{2B}, CSC A_{2A} and MRS1191 A_3 receptor antagonists provoked a reduction of 72.76%, 33.81% and 8.06% of sodium transport respectively. Thus, DPCPX and MRS 1754 were more potent antagonists in the present study. Furthermore, neither CSC (fig. 7a), nor MRS1191 (fig. 7b) have an effect on adenosine and plant extract stimulated-sodium transport. However preincubation of A6 cell with MRS1754 (fig. 7c) and DPCPX (fig. 7d) at the final

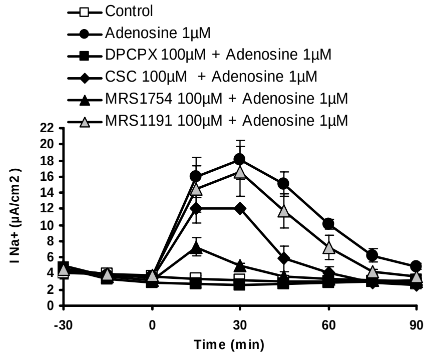

Fig. 6: Effect of different adenosine antagonist receptor on adenosine stimulated sodium transport. Adenosine was added into basolateral bathing medium at t=0, DPCPX, CSC, MRS1754, MRS1191 were added into basolateral bathing medium at t=-30. Each point represents the mean ± SEM, n=3.

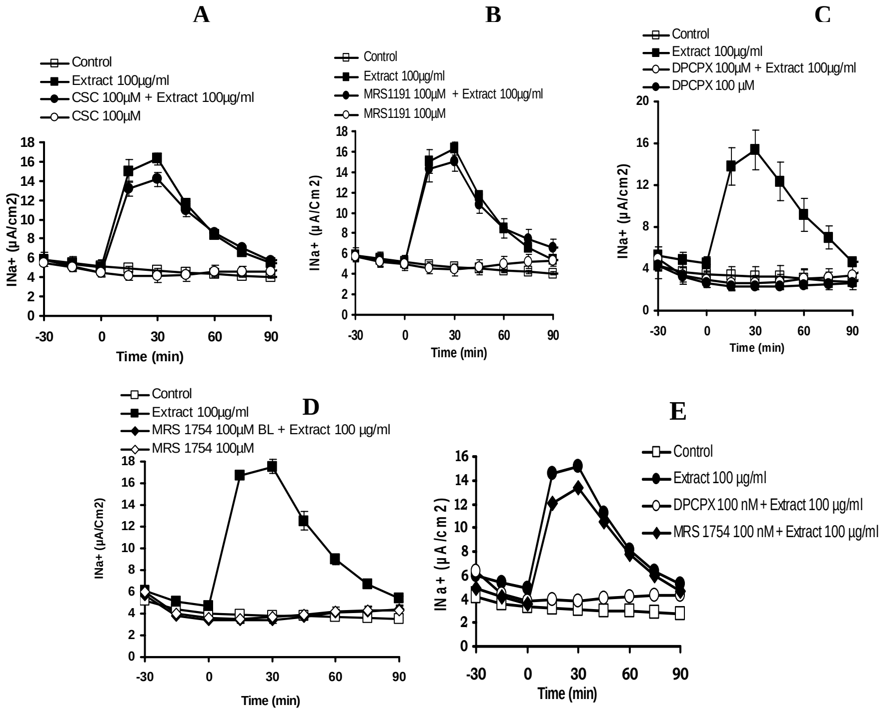

Fig. 7: Effect of different adenosine antagonist receptors on *D. picta* extract stimulated sodium transport. Extract was added into basolateral bathing medium at t=0, DPCPX, CSC, MRS1754, MRS1191 were added into basolateral bathing medium at t=-30. Each point represents the mean ± SEM, n=3.

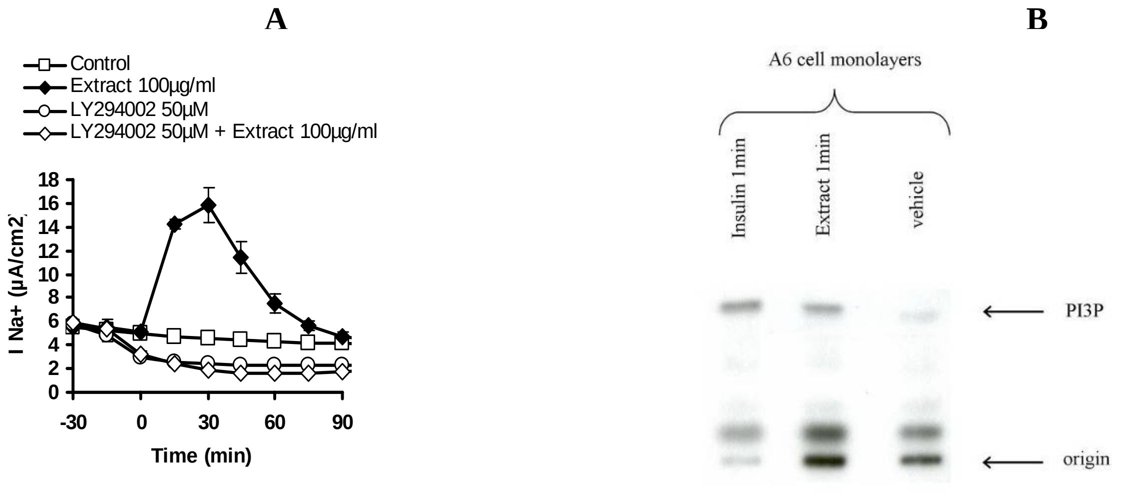

Fig. 8: Effect of the *D. picta* extract on PI3-kinase activity. A: Effect of LY-294002 on sodium transport stimulated extract. B: Effects of insulin and the *D. picta* extract on PI3kinase. LY-294002 was added bilaterally at time t= -30 min. The plant extract (100µg/ml). was added into basolateral bathing medium at time t = 0 min. Each point represents the mean ± SEM, n=3.

concentration of 100 µM completely abolished adenosine and plant extract stimulated sodium current. Interesting is that, the A_1 inhibitor (DPCPX), at 100 nM completely abolished the effect of plant extract on sodium transport while, at the same concentration the MRS1754 was completely ineffective on plant extract stimulated sodium transport (fig. 7e). This suggests that adenosine A_1 inhibitor is more potent antagonist of the plant extract than MRS1754.

Effect of the *D. picta* extract on PI3-kinase activity

LY-294002 is a specific inhibitor of PI3-Kinase (Paunescu *et al.*, 2000). Some authors established that, insulin increase sodium transport in A6 cell monolayers by activating PI3-kinase pathway (Record *et al.*, 1998). Therefore, in the present study, the involvement of this signalling pathway was investigated. Preincubation of A6 cell with the PI 3-kinase inhibitor at the final concentration of 50 µM (added in the both bathing medium) prevent the effect of plant extract on sodium transport (Figure 8a). The effect of the plant extract was directly evaluated on PI3-kinase activity by estimating the quantity of PI3P generated from phosphatidylinositol (PI) and ATP. Like with the insulin pre-treatment; the amount of PI3-^{32}P was higher in the case of plant extract than the control (fig. 8b).

Phosphorylation of protein kinase B (PKB) by the plant extract

An useful and quite general readout of PI 3-kinase stimulation lies in the activation of a downstream effector, Akt/PKB. We therefore investigated whether exposure to the *D. picta* extract induced the phosphorylation of PKB. The immunoblots demonstrate PKB phosphorylation on both residues, serine 473 and threonine 308, although the phosphorylation was much pronounced in the case of insulin treatment (fig. 9).

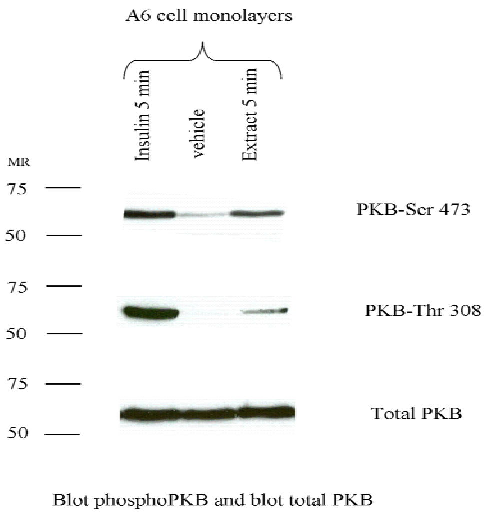

Fig. 9: Effect of the plant extract on the phosphorylation of PKB on the residue of serine 473 and threonine 308.

DISCUSSION

The results indicate that, administration of twigs methanol fraction of *Dorstenia picta* during two weeks reduced urinary volume and sodium excretion in streptozotocin-induced diabetic rats. All doses have shown a significant reduction in these two parameters. The higher dose (150 mg/kg) was the most potent. Preliminary phytochemical analysis of this plant extract revealed the presence of several compounds among which triterpenes, and saponins (Ngueguim *et al*., 2007). These compounds have been shown to significantly reduce diuresis and natriureris (Diniz *et al*., 2009).

The A6 cell line derived from *Xenopus leavis* kidney cells has been widely exploited to elucidate cellular and molecular mechanisms involved in sodium transport (Paunescu *et al*., 2000; Blazer-yost *et al*., 2003; Markadieu *et al*., 2004; Tiwari *et al*., 2007). In the present study, the treatment of A6 cell with plant extract added at basolateral side increase sodium current. The extract response was amiloride sensitive as demonstrated by adding the amiloride fifteen minutes after the stimulation of the sodium current by the plant extract. Interesting was that no significant effect was observed when the extract is injected in the apical side, suggesting that the mechanism which initiated the natriferic effect of the extract is at basolateral side. Similar results were obtained with *Nigella arvensis* in sodium transport by using patch clamp technique (Atia *et al*., 2002). This natriferic effect of the plant extract was dose-dependent and partially potentialised in the presence of insulin, when added together. In the second step of the experiment, the ability of the plant extract and adenosine to stimulated sodium current was compared. Adenosine is known to stimulated sodium transport (Lang *et al*., 1985). Like plant extract, adenosine increases sodium transport in concentration-dependant manner. In addition, added together in bathing medium, adenosine and plant extract, did not shown an additive effect. These results suggest a common pathway of these two substances on sodium transport.

Adenosine is known to interact with four cell surface receptor subtypes know as A_1, A_{2A}, A_{2B} and A_3, which are coupled to different G-proteins (Fredholm *et al*., 2001). To further elucidate the action mechanism of the plant, different adenosine antagonist receptors were used. Neither A_{2A} nor A_3 adenosine antagonist receptors prevent plant extract stimulated sodium current. However A_1 and A_{2B} completely inhibited the sodium transport stimulated by the plant extract. These results suggest that the plant extract could possible act via A_1 and A_{2B} Adenosine receptors. A_1 antagonist adenosine receptor, DPCPX completely abolished the effect of plant extract on sodium transport at 100 nM while at the same concentration, the A_{2B} agonist was ineffective. This suggests that, A_1 adenosine receptor is more selective than the A_{2B} adenosine receptor.

The fact that insulin stimulated sodium transport via PI3-kinase pathway (Record *et al*., 1998; Blazer-yost *et al*., 2003), prompt us to investigate the effect of *D. picta* extract in this pathway. This investigation was done by using a specific inhibitor of PI-3kinase (LY-294002) (Vlahos *et al*., 1994). Pretreatment of A6 cell with this inhibitor completely abolished sodium transport when the cells are stimulated by the extract or insulin. In addition as indicated by the amount of PIP3 generated (Markadieu *et al*., 2005), the plant extract or insulin increased the activity of this enzyme. These results suggest that the plant extract stimulated sodium transport required PI-3kinase activity like insulin.

Since the activity of PI-3kinase is related to the activity of PKB, the effect of plant extract on PKB phosphorylation was also explored. The results revealed that, the plant extract induced PKB phosphorylation on both serine 473 and threonine 308. However, it is well known that, the phosphorylation of this enzyme is not lead to the stimulation of sodium transport in A6 cell (Arteaga *et al*., 2005).

In conclusion, we have shown in the present study that the twigs methanol fraction from *Dorstenia picta* reduced urinary volume and natriuresis in streptozotocin-induced diabetic rats. This plant extract increases sodium transport via A_1 adenosine receptor and trigged PI-3kinase pathway.

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REFERENCES

Arteaga MF and Canessa CM (2005). Functional specificity of sgk1 and AKT on ENaC activity. *Am. J. Physiol. Cell Physiol*., **289**: 90-96.

Atia F, Mountian I, Simaels J, Waelkens E and Driessche V (2002). Stimulatory effects on Na^+ transport in renal epithelia induced by extract of *Nigella arvensis* are cause by adenosine. *J. Ex. Biol*., **205**: 3729-3737.

Bei Z, Gino S, Deborah S, Zhihua L,Yan Z, Inmaculada R, Dolores V, Diez MT, Pelaez F, Ruby C, Kendall RL, Mao X, GrifÞn P, Calaycay J, Zierath, Juleen R, Heck JV, Smith RG and Moller ED (1999). Discovery of a small molecule insulin mimetic with antidiabetic activity in mice. *Sciences*, **284**: 274-277.

Blazer-yost BL, Esterman MA and Vlahos CJ (2003). Insulin stimulated trafficking of ENaC in renal cells requires PI3-kinase activity. *Am. J. Physiol. cell Physiol*., **284**: 1645-1653.

Blazer-Yost BL, Vahle JC, Byars JM and Bacallao R (2004). Real-time three dimensional imaging of lipid signal transduction: apical membrane insertion of epithelial Na^+ channels. *Am. J. Physiol. Cell Physiol.*, **287**: 1569-1576.

Casavola V, Guerra L, Reshkin SJ, Jacobson KA, Verrey F and Murrer H (1996). Effect of Adenosine on Na^+ and Cl^- Currents in A6 Monolayers. Receptor Localisation and Messenger Involvement. *Memb. Biol.*, **151**: 237-245.

Diniz LR, Santana PC, Ribeiro AP, Portella VG, Pacheco LF, Meyer NB, César IC, Cosenza GP, Brandão MG and Vieira MA (2009). Effect of triterpene saponins from roots of *Ampelozizyphus amazonicus* Ducke on diuresis in rats. *J. Ethnopharmacol.*, **123** (2): 275-279.

Fredholm BB., Ijzerman AP., Jacobson AK, Klotz NK and Linden J (2001). International union of pharmacology XXV Nomenclature and classification of adenosine receptor. *Pharmacol. Rev.*, **53**: 527-552.

Jyoti M, Vihas TV, Ravikumar A and Sarita (2002). Glucose lowering effect of aqueous extract of *Enicostemma Littorale* Blume in diabetes: a possible mechanism of action. *J. Ethnopharmacol.*, **81**: 317-320.

Kalantarinia K and Okusa MD (2006). The renin-angiotensin system and its blockade in diabetic renal and cardiovascular disease. *Curr Diab Rep.*, **6**: 8-16.

Lang MA, Preston AS, Handler JS and Forrest JNJ (1985). Adenosine stimulates sodium transport in kidney A6 epithelia in culture. *Am. J. Physiol.*, **249**: 330-335.

Markadieu N, BLero D, Boom A, Erneux C and Beauwens R (2004). Phosphatidylinositol 3,4,5-triphosphate: an early mediator of insulin stimulated sodium transport in A6 cell. *Am. J. Physiol. Renal Physiol.*, **287**: 319-328.

Markadieu N, Crutzen R, BLero D, Erneux C and Beauwens R (2005). Hydrogen peroxide and epidermal growth factor activated phosphatidylinositol3-kinase an increase sodium transport in A6 cells monolayer. *Am. J. Physiol. Renal Physiol.*, **288**: 1201-1212.

Ngueguim TF, Dimo T, Kouambou N C, Tchamadeu MC, Dzeufiet DPD, Beauwens R and Kamtchouing P (2006). Antidiabetic activity of *Dorstenia picta* aqueous twigs extract in streptozotocin induced diabetic rats. *Pharmacologyonline,* **3**: 900-911.

Ngueguim TF, Dimo T, Dzeufiet DPD, Vouffo B, Dongo E, Renaud B, Assongalem AE, ZaPfacck L and Kamtchouing P (2007). Anti-diabetic activities of methanol-derived extract of *Dorstenia picta* twigs in streptozotocin-induced diabetic rats. *As. J. of Trad. Med.*, **2**(4): 140-148.

Paunescu TG, Blazer-yost BL,Vlahos CJ and Helman SI (2000). Ly-294002 inhibitable PI3-kinase and regulation of baseline rates of Na^+ transport in A6 epithelia. *Am J. Physiol. Cell Physiol.,* **279**: 236-247.

Ratnasooriya WD, Pieris KPP, Samaratunga U and Jayakody JAC (2004). Diuretic activity of *Spilanthes acmella* flowers in rats. *J. Ethnopharmacol.*, **91**: 317-320.

Record RD, Froelich LL, Vlahos CJ and Blazer-yost BL (1998). Phosphatidylinositol 3-kinase activation is required for insulin stimulated sodium transport in A6 cell. *Am. J. Physiol. Endocrinol. Metab.*, **274**: 611-617.

Sakurai T, Nishimura T, Otake N, Xinsheng Y, Abe K, Zeida M, Nagasawa H and Sakuda S (2002). Assamicin I and II novel triterpenoid saponin with insulin-like activity from aesculus assamica. *Griff. Biorg. Med. Chem. Lett.*, **12**(5): 807-810.

Tiwari S, Riazi S and Ecelbarger CA (2007). Insulin's impact on renal sodium transport and blood pressure in health, obesity. *Am. J. Physiol. Renal Physiol.*, **4**: 974-984.

Vlahos CJ, Matter WF, Hui KY and Brow F (1994). A specific inhibitor of phosphatidylinositol 3-kinase 2-(4-morpholinyl-4H-1-benzopyran-4-ane (LY294002). *J. Biol Chem.*, **269**: 5241-5248.