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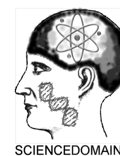

Changes in Erythrocytic Membrane Free Energy of Albino Rabbits Administered Ethanol Leaf Extract of *Spondias mombin* Linn

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Research Article

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ABSTRACT

Aims: To determine the effect of ethanol extract of *Spondias mombin* Linn on membrane potential energy, the intracellular and extracellular concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} in an easily accessible model cell, erythrocyte, was studied. The effect on the first approximation estimates of erythrocytic free energy changes (ΔG) was calculated.
Study Design: Randomized study.
Place and Duration of Study: Department of Biochemistry, Federal University of Technology, Owerri, Nigeria between January 2010 and February 2011).
Methodology: Twenty-five female albino rabbits (1.47 ± 0.17 kg) of the same age set were divided into 5 groups of 5 animals. The first group served as the baseline control, the groups II and III animals were intraperitoneally administered 1ml of 750 mg/kg body weight of *S. mombin* extract daily for 7 and 14 days respectively, while groups IV and V animals were intraperitoneally administered 1ml of 0.14 IU/kg body weight of oxytocin drug ((Pitocin®, USA) daily for 7 and 14 days respectively. Plasma and lyzed red blood cells obtained were analyzed for extracellular and intracellular erythrocyte concentrations of sodium, potassium, calcium and magnesium using standard methods.
Results: The study showed that both the extract and oxytocin administrations significantly ($p<0.05$) reduced intracellular [Mg^{2+}] and extracellular [K^+] and [Ca^{2+}], with a concomitant increase in intracellular [Ca^{2+}]. Both treatment with the extract and oxytocin, did not significantly ($p>0.05$) alter [Na^+] and extracellular [Mg^{2+}]. On the other hand, while the extract did not affect intracellular [K^+], oxytocin significantly ($p<0.05$) reduced it. The results also indicated that while the extract significantly ($p<0.05$) increased the [K^+]-based estimated ΔG, oxytocin treatment reduced it. On the other hand, both the extract and oxytocin caused a non-significant ($p>0.05$) drop in the Ca^{2+} based ΔG, with no significant ($p>0.05$) alteration of the [Na^+] and [Mg^{2+}] based estimated ΔG. The pole reversal

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observed with the study of the Ca^{2+}-based ΔG indicates depressive effect of both the extract and oxytocin on Ca^{2+} ATPase pump.
Conclusion: The observations above indicate that, while the use of the extract might enhance energy generation and conservation, the use of oxytocin might elicit exhaustive utilization of energy.

Keywords: Membrane potential; erythrocyte; hog plum; oxytocic activity; cations.

1. INTRODUCTION

Spondias mombin Linn (Fam. Anacardiaceae) is a fruitiferous, medicinal plant naturally grown in the rainforest and coastal areas of America and West Africa. It is called hog plum in English, and in Nigeria, it is known by various names such as 'jikara' (Igbo), 'Tsardar masar' (Hausa), and 'iyeye' or 'akika' (Yourba). The tree is about 18.3m in height and 1.5m in girth when fully grown. The leaf has a common stalk 12-24 inches long, 5-8 pairs of opposite leaflets, and an odd terminal one (Igwe et al., 2010). The leaves have a folk reputation for use as an oxytocic agent, particularly for the expulsion of retained placenta in women and domestic animals, when normal delivery is delayed or difficult, and as an astringent in postpartum medication (Nworu et al., 2007). The high success rate claimed in the use of the plant in obstetrics raises hope for the possibility of discovering a novel oxytocic agent or a lead compound. This has led to many researches and publications on the oxytocic, abortifacient, and related activities of the plant extract (Offiah and Anyanwu, 1989; Nworu et al., 2007; Chukwuma and Isek, 2008; Igwe et al., 2011a; Igwe, 2011).

Oxytocic activity, directly or indirectly, enhances uterine muscle contraction to elicit placenta expulsion. Thus, rhythmic uterine contractions play an important role in parturition. For uterine or any other smooth muscle to contract or relax, ions must flow through ion channels into or out of the muscle cells (McKillen et al., 1999; Igwe, 2011). Ion channels are 'gated' signal transducers that provide a regulated path for the movement of inorganic ions across the plasma membrane in response to various stimuli (Wray et al., 2003). The precisely timed opening and closing of ion channels and the resulting transient changes in membrane potential underlie the electrical signaling necessary for stimulation of muscle to contract. Furthermore, many hormones and chemical agents, including phytohormones, exert their effects by altering the membrane potentials of their target cells (Igwe et al., 2011a; Nelson and Cox, 2005).

Uterine myocyte excitability depends on the movement of Na^+, Ca^{2+}, and Cl^- ions into the cytosolic compartment from the extracellular space, and of K^+ ions in the opposite direction (Sanborn, 1995). The ionic current that maintains the resting membrane potential of cells and the changes that occur in response to pharmacologic and signaling molecules constitute the complex electrophysiologic network that controls the contractile activity of the uterus (Matthew et al., 2004). Rhythmic oscillations, termed 'slow waves' which occur in the levels of the resting membrane potential reflect the distribution of Ca^{2+}, Na^+, K^+ and Cl^- ions between the intracellular and extracellular spaces and this, in turn, reflects the permeability of the plasma membrane to each of these ions (Sanborn, 2000). Of these relevant ions, the largest electrochemical gradient in the resting state exists for Ca^{2+}, which has 104 greater concentration in the extracellular space as compared with the cytosolic compartment (Aguilar and Mitchell, 2010). Although variations in ionic current of Mg^{2+} may not have direct

effect on the maintenance of resting membrane potential, but being an antagonist of Ca^{2+} activity, may have a role to play in cell's resting state maintenance.

In pursuance of the oxytocic properties of *S. mombin*, the effect of the ethanol leaf extract on the intracellular and extracellular erythrocytic concentrations of Na^{+}, K^{+}, Ca^{2+} and Mg^{2+} was studied. First approximation estimates of the changes in free energies and hence membrane potential changes across the erythrocytic cells were calculated. This might give the first insight into the effect of the plant extract on the distribution of these cations, and polarization/depolarization of erythrocyte, a model cell that is readily available.

2. MATERIALS AND METHODS

2.1 Plant Material

Apparently healthy leaves of *Spondias mombin* were obtained from their natural habitat in the bushes in and around Obinze and Iheagwa villages of Imo State. The leaves were duly authenticated at the Department of Crop Science, Federal University of Technology, Owerri.

2.1.1 Preparation of plant material

Apparently healthy fresh leaves of *S. mombin* were plucked out from the stem stalks, rinsed in clean water and air-dried at room temperature for 10 days to achieve a constant weight. The dried leaves were ground into fine powder with a mechanical grinder (Heman, Japan). About 1.4 kg of the powder was weighed and soaked in 3.5 liters of 75 % ethanol, covered with aluminum foil. The mixture was stirred every 3 hours for proper mixing and allowed to stand for 24 hours. The resulting solution was then filtered. The filtrate was subjected to a slow but complete solvent evaporation using a regulatory hot plate (Techmel, USA) at a temperature of 40-60℃. The extract in the filtrate yielded 27.71g. This was packaged in an air-tight container, labeled and stored below 4℃ in a freezer.

2.2 Animals

Twenty-five female virgin albino rabbits (1.47 ± 0.17 kg) of the same age set were obtained locally from Owerri, Imo State. The animals were housed in stainless steel cages under standard laboratory condition of 12 hours light/dark cycle. They were allowed access to feed (Guinea Feed Nigeria Ltd) and water *ad libitum.*

2.3 Experimental Design

The twenty-five rabbits were randomly divided into 5 groups of 5 animals each:

Group 1 – the baseline control that were not administered any drug and were sacrificed on the zero day.
Group 2 – administered 1ml (750 mg/kg body weight) of *S. mombin* extract daily for 7 days.
Group 3 – administered 1ml (750 mg/kg body weight) of *S. mombin* extract daily for 14 days.
Group 4 – administered 1ml (0.14 IU/kg body weight) of standard oxytocin drug (Pitocin®, USA) daily for 7 days.
Group 5 – administered 1ml (0.14 IU/kg body weight) of standard oxytocin drug (Pitocin®, USA) daily for 14 days.

The *S. mombin* extract and oxytocin drug doses used were decided based on our earlier toxicological studies (Igwe, 2011; Igwe et al., 2011b). The animals were intraperitoneally administered 1 ml of their respective drugs/extracts reconstituted in distilled water, once a day for the stipulated number of days.

2.4 Sample Collection

At the end of each experimental period, 5 fasting animals were randomly selected from each group, anaesthetized with chloroform soaked in cotton wool, and about 5 ml of blood collected by cardiac puncture from each animal. The blood sample was dispensed, into a lithium heparin bottle. The blood sample was centrifuged (Teco, USA) at 4000 rpm for 5 minutes, its plasma separated, stored frozen at 0℃ for 24 hours and used for determination of extracellular cation concentrations. While the packed red cells were washed thrice with normal saline, completely drained and blotted dry with filter paper, lysed with 1 ml of distilled, deionized water, and stored frozen for determination of intracellular cation concentrations.

The sodium and potassium concentrations of the plasma and lyzed red blood cells were determined by ion selective electrode method using Humalyte machine (Human, Germany). Also, the intracellular and extracellular concentrations of calcium and magnesium concentrations of the plasma and lysed red cells were determined by atomic absorption spectrophotometry (Perkin Elmner, USA).

2.5 Estimated Free Energy Change

The free energy required to transport the cations across the erythrocyte membrane was estimated using extracellular and intracellular cation concentrations determined above. The first approximation estimates of the free energy change were calculated for each cation using the Nernst equation as described by Bronk (1973):

$$\Delta G_m = \frac{RT}{n} \ln \frac{[\text{cation}]_{\text{intracellular}}}{[\text{cation}]_{\text{extracellular}}}$$

Where: R = gas constant = $8.315 JK^{-1}mol^{-1}$; n = Number of charge; T = Absolute temperature (approximate temperature of Owerri = 30℃ + 273 = 303K).

3. RESULTS AND DISCUSSION

3.1 Results

There was no statistically significant ($p>0.05$) reduction in erythrocytic intracellular sodium concentration. Similarly, administration of oxytocin standard drug did not significantly ($p>0.05$) cause any change in the intracellular sodium concentration when compared with the baseline values (Table 1). The administration of *S. mombin* extract caused a statistically non-significant ($p>0.05$) time-dependent progressive increase in extracellular sodium concentration, varying from a baseline value of 125.80 ± 4.82, through 135.60 ± 6.15 for the 7th day to 138.60 ± 4.10 mmol/l for the 14th day. Similarly, administration of oxytocin standard drug did not significantly ($p>0.05$) cause any change in the extracellular sodium concentration when compared with the baseline values. The table also shows that the free energy change and hence, the membrane potentials for Na^+ transport across the membrane were not significantly ($p>0.05$) increased by treatment with either the extract or oxytocin.

Table 2 shows that administration of *S. mombin* extract caused a statistically non-significant ($p>0.05$) increase in intracellular erythrocytic potassium concentration. Similarly, oxytocin drug administration did not significantly ($p>0.05$) reduce intracellular potassium concentration at the 7th day of treatment (18.83 ± 2.65 mmol/l). However, the reduction in intracellular K^+ concentration became significant by the 14th day of treatment (18.30 ± 1.16 mmol/l) in comparison with the corresponding value of the extract treated (26.65 ± 4.14 mmol/l) group. Statistically significant ($p<0.05$) time-dependent decreases in extracellular erythrocytic potassium concentrations were observed after administration of *S. mombin* leaf extract. These varied from a baseline value of 6.47 ± 0.64 mmol/l through 5.26 ± 0.40mmol/l for the 7th day to 5.48 ± 0.37 mmol/l of the 14th day for the extract administered groups. On the other hand, oxytocin administration did not significantly ($p>0.05$) reduce the plasma potassium concentration. The table also presents the effects of the extract and oxytocin on the free energy required for transport of K^+ across the erythrocyte membrane. It shows that the extract, when compared to the baseline, elicited a statistically significant ($p<0.05$; $\chi^2=132.90$) increase in potential energy available to the cell and, hence increased cellular polarization. On the other hand, oxytocin caused a significant ($p<0.05$; $\chi^2=37.93$) reduction in free energy of the cell, and hence reduction in the membrane potential and relative cellular depolarization, in comparison with that obtained for the baseline.

Table 3 shows that the intracellular erythrocytic calcium concentration of the *S. mombin* extract and oxytocin treated groups were significantly ($p<0.05$) increased after 7 (4.63 ± 1.31 and 4.54 ± 1.05mmol/l) and 14 (3.86 ± 1.04 and 4.27 ± 0.69 mmol/l respectively) days of administration when compared with the calcium concentration of the baseline (0.95 ± 0.10mmol/l) group. The extracellular calcium concentrations of the extract and oxytocin-treated animals were significantly ($p<0.05$) reduced by the treatments, in comparison with that of the baseline group. The table also shows that both the extract and oxytocin caused a non-significant ($p>0.05$) drop in the Ca^{2+} estimated free energy change across the erythrocyte cell membrane. Both treatment also led to non-significant ($p>0.05$) charge (pole) reversals on the erythrocyte membranes, which were slightly more pronounced at the 7th day than after 14 days of treatment.

Table 4 shows that intracellular Mg concentrations of the *S. mombin* extract treated group were significantly ($p<0.05$) reduced with progressive administration of the extract. Similarly, administration of oxytocin significantly ($p<0.05$) reduced intracellular Mg concentration, but only at the 7th day of treatment. The extracellular Mg concentrations of the extract groups increased progressively but non-significantly ($p>0.05$) when compared with the baseline value of 0.95 ± 0.25 mmol/l. On the other hand, treatment with oxytocin increased significantly ($p<0.05$) extracellular Mg concentrations but only at the 7th day (1.70 ± 0.36 mmol/l) of treatment. The results also shows that both the extract and oxytocin caused a non-significant ($p>0.05$) decrease in the Mg^{2+} estimated free energy change.

Table 1. Effects of the extract and oxytocin on change in free energy for transport of Na^+

	Baseline	Extract		Oxytocin	
	Day 0	**Day 7**	**Day 14**	**Day 7**	**Day 14**
Extracellular concentration (mmol/l)	125.80 ± 4.82	135.60 ± 6.15	138.60 ± 4.10^{8*}	121.20 ± 5.26	125.40 ± 7.13
Intracellular concentration (mmol/l)	5.91 ± 1.20	5.28 ± 0.91	5.56 ± 0.74	4.81 ± 0.89	4.86 ± 0.41
Ratio $([Na^+]_{in}/[Na^+]_{out})$	0.047	0.039	0.040	0.040	0.039
In $([Na^+]_{in}/[Na^+]_{out}]$	-3.06	-3.25	-3.22	-3.22	-3.25
ΔG_m $(Jmol^{-1})$	-7.71	-8.18	-8.10	-8.13	-8.19

**Significant (p<0.05) increase in comparison with the baseline value per row*

Table 2. Effects of the extract and oxytocin on free energy change for transport of K^+

	Baseline	Extract		Oxytocin	
	Day 0	**Day 7**	**Day 14**	**Day 7**	**Day 14**
Extracellular concentration (mmol/l)	6.47 ± 0.64	5.26 ± 0.40	5.48 ± 0.37	6.10 ± 1.02	5.96 ± 0.67
Intracellular concentration (mmol/l)	23.30 ± 4.72	27.85 ± 5.70	26.65 ± 4.14	18.83 ± 2.65	18.30 ± 1.16
Ratio $([K^+]_{in}/[K^+]_{out})$	3.60	5.29	4.86	3.09	3.07
In $([K^+]_{in}/[K^+]_{out}]$	1.28	1.67	1.58	1.14	1.11
ΔG_m $(Jmol^{-1})$	3.22	4.21	3.98	2.85	2.82
Difference in ΔG_m	0.00	-0.99	-0.76	0.37	0.40

**Significant (p<0.05) increase in comparison with the baseline value per row*

Table 3. Effects of the extract and oxytocin on free energy change for transport of Ca^{2+}

	Baseline	Extract		Oxytocin	
	Day 0	Day 7	Day 14	Day 7	Day 14
Extracellular concent ration (mmol/l)	3.41 ± 0.91	1.03 ± 0.18**	1.85 ± 0.54**	1.26 ± 0.28**	2.03 ± 0.96
Intracellular concentration (mmol/l)	0.95 ± 0.10	4.63 ± 1.31*	3.86 ± 1.04*	4.54 ± 1.05*	4.27 ± 0.69*
Ratio ($[Ca^{2+}]_{in}/[Ca^{2+}]_{out}$)	0.28	4.50	2.09	3.60	2.10
In ($[Ca^{2+}]_{in}/[Ca^{2+}]_{out}$]	-1.28	1.50	0.74	1.28	0.74
ΔG_m ($Jmol^{-1}$)	-1.61	1.91	0.96	1.61	0.96

***Significant (p<0.05) reduction in comparison with the baseline value; *Significant (p<0.05) increase in comparison with the baseline value per row.*

Table 4. Effects of the extract and oxytocin on free energy change for transport of Mg^{2+}

	Baseline	Extract		Oxytocin	
	Day 0	Day 7	Day 14	Day 7	Day 14
Extracellular concentration (mmol/l)	0.90 ± 0.25	1.60 ± 0.33	1.56 ± 0.74	1.70 ± 0.36*	0.96 ± 0.24
Intracellular concentration (mmol/l)	5.10 ± 0.54	3.30 ± 0.09**	3.19 ± 0.22**	3.90 ± 0.46**	4.51± 0.72
Ratio ($[Mg^{2+}]_{in}/[Mg^{2+}]_{out}$)	5.67	2.06	2.04	2.29	4.70
In ($[Mg^{2+}]_{in}/[Mg^{2+}]_{out}$]	1.73	0.72	0.71	0.83	1.55
ΔG_m ($Jmol^{-1}$)	2.18	0.91	0.90	1.05	1.95

***Significant (p<0.05) reduction in comparison with the baseline value; *Significant (p<0.05) increase in comparison with the baseline value per row.*

3.2 Discussion

Sodium and calcium are the principal extracellular cations, while potassium and magnesium are the principal intracellular cations. Maintenance of these variations in the distribution of the cations, inside and outside the cell, helps to create a potential difference that is harnessed by the body for various purposes including initiation and potentiation of muscle contraction, signaling across membranes and potential energy generation and conservation.

Administration of *S. mombin* extract did not significantly affect intracellular concentrations of Na^+ and K^+, but increased extracellular Na^+ with a concomitant time-dependent reduction in K^+ extracellular concentration. This reduction in extracellular [K^+] may give credence to the reported diuretic potential of the plant in ethnomedical practice (Akubue et al., 1983). This is further substantiated by the report that the extract is a rich source of potassium (Igwe et al., 2010), yet extracellular [K^+] was reduced by the extract's administration, indicating its increased loss through urine. Furthermore, the extract caused a significant increase in the intracellular [Ca^{2+}], while significantly reducing the extracellular [Ca^{2+}]. The extract as well as oxytocin standard drug had inverse effects on intracellular and extracellular [Mg^{2+}] compared to their effects on [Ca^{2+}]. These inverse effects on intra- and extracellular [Mg^{2+}] by both the extract and oxytocin may be a reflection of the tendency of the body to balance cations level in the intra- and extracellular fluids. Mg^{2+} is a critical cation and cofactor in numerous intracellular processes. It is a cofactor for adenosine triphosphate; an important membrane stabilizing agent; required for the structural integrity of numerous intracellular proteins and nucleic acids; a substrate or cofactor for important enzymes such as adenosine triphosphatase, guanosine triphosphatase, phospholipase C, adenylate cyclase, and guanylate cyclase; a required cofactor for the activity of over 300 other enzymes; a regulator of ion channels; an important intracellular signaling molecule; and a modulator of oxidative phosphorylation. It is also intimately involved in nerve conduction, muscle contraction, potassium transport, and calcium channels (Quamme, 1993; Romani et al., 1993). The kidney occupies a central role in Mg^{2+} balance in the body through renal excretion. Therefore, factors that modulate and affect renal Mg^{2+} excretion can have profound effects on Mg^{2+} balance (McLean, 1994). Thus, the apparent hypermagnesaemia created by the administration of the extract may lead to increased urinary excretory potential and consequently increased diuresis.

Unlike the extract, oxytocin administration reduced the concentrations of intracellular [K^+], explaining its action through efflux of K^+ and influx of Ca^{2+} into the cell. Meanwhile, the significant increase in intracellular [Ca^{2+}] elicited by both the extract and oxytocin indicates activation of the Ca^{2+} pump which is necessary for the maintenance of the action potential required for initiation of muscle contraction (McKillen et al., 1999).

Contraction of smooth muscle requires an increase of intracellular Ca^{2+} concentration. This increase is derived from Ca^{2+} entry from the extracellular fluid, as well as Ca^{2+} release from the sarcoplasmic reticulum (SR). Correspondingly, relaxation is initiated by the removal of intracellular Ca^{2+}, either to the extracellular fluid or to the SR. Transport of Ca^{2+} out of the cell is an active process that depends on the activity of the plasma membrane Ca^{2+}-ATPase (PMCA) and the Na^+-Ca^{2+} exchanger, both of which have been found in smooth muscle cells of different species (Shmigol, et al., 1999). Another Ca^{2+} extrusion mechanism involving SR has been suggested (Wray et al., 2003). It has been reported that the SR acts as a "superficial buffer barrier", by taking up a fraction of the Ca^{2+} that enters the cell through the plasmalemma before it reaches the contractile machinery. In order for the SR to buffer calcium on a steady state basis, the accumulated Ca^{2+} is released from the SR into the

narrow space between the SR and plasmalemma (referred to as 'vectorial Ca^{2+} release'), from where the Na^{+}- Ca^{2+} exchanger and PMCA complete the extrusion process.

The absence of SR and hence "vectorial Ca^{2+} release' in erythrocytes, the model cell studied in this research work may explain the significantly higher concentration of Ca^{2+} observed in the animals administered both the extract and oxytocin. Furthermore, increased erythrocyte $[Ca^{2+}]$ and decreased Ca^{2+}-ATPase activity represent the abnormal metabolism of Ca^{2+} in several disease conditions (Devi et al., 2000). This could also have elicited the charge reversal observed, although statistically non-significant, from the study of the membrane potential of the cells using the extracellular and intracellular $[Ca^{2+}]$. A further study of the change in free energy of the red cells using Na^{+} and Mg^{2+} concentrations showed no significant variations in comparison with their baseline control values. However, using the commonly and widely accepted cation, K^{+} for change in free energy calculation (Bronk, 1973), the extract was found to have elicited a significantly higher cellular polarization than that of the baseline group. This indicates that the use of the extract enhances energy generation and conservation. On the other hand, oxytocin administration caused a significant drop in the K^{+}-based estimated free energy change of the studied red blood cells in comparison with those of the baseline animals, indicating a more exhaustive utilization of energy. These observations point to the possible advantages of the extract, and probable differences in the mode of actions of the extract and oxytocin hormone in muscle contraction and induction of labour, characteristics that have been associated with them (Nworu et al., 2007; Offiah and Anyanwu, 1989).

4. CONCLUSION

In conclusion, *S. mombin* leaf extract administration increased intracellular $[Ca^{2+}]$ and $[K^{+}]$ which may give the plant its reported oxytocic effect, and the energy generation and conservation potential observed in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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