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Effects of *Croton penduliflorus* Methanolic Extract on Intestinal Enzymes and Protein Content in Pregnant Rats

¹T.O. Oyesola, ²F.S. Oluwole and ¹O.A. Oyesola

¹Department of Physiology, School of Basic Medical Sciences, College of Health Sciences, Igbinedion University Okada, Okada, Edo State, Nigeria
²Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria

Abstract: The seeds of *Croton penduliflorus* (Family Euphorbiaceae) are often used as a purgative. The physiological effects of the methanolic extract on some intestinal disaccharide splitting enzymes were investigated in pregnant rats using an *in vivo* study. The extract was administered orally at a dose of 550 mg kg⁻¹ body weight during the three phases of pregnancy. The extract caused a significant increase in maltase activity in the three phases of pregnancy, a significant increase in total protein concentration in early and late pregnancy and a significant increase in albumin concentration in early and mid pregnancy ($p < 0.01$). The extract also caused a significant increase in sucrase activity in early pregnancy and in lactase activity in mid pregnancy. The present data suggest that increase activity of disaccharidase brush border enzymes most especially sucrase show that the extract might be having hyperplastic (growth) effect on the small intestinal enzyme activities, there is possibility of increased nutrients to the pregnant rats and fetuses.

Key words: *Croton penduliflorus*, pregnancy, sucrase, maltase, lactase

INTRODUCTION

In recent years, renewed interest has been shown in the use and efficacy of medicinal plant as means of alleviating as well as treating of specific disease conditions. As a result of this, there has been increased awareness by government, scientific and medical communities of the importance of medicinal plants as a therapeutic and essential pivot in health care programmes especially in developing countries.

Among the medicinal plant preparation in current and common use in Nigeria is the ground form of the seed of *Croton penduliflorus* of the family of Euphorbiaceae. It is often used as a purging nut and possesses inflammatory, vesicant and contraceptive properties (Bablola, 2009).

The plant is used extensively as a remedy for several stomach complaints (Adesogan, 1981), *Croton penduliflorus* in small doses causes diuresis and is a powerful diaphoretic. Externally, the oil is used as liniment for acute rheumatism arthritis, neuralgia and diseases of the joints (Gills, 1992). *Croton penduliflorus* possesses drastic purgative properties. This purgative property was isolated and identified in the seed oil as white

Corresponding Author: Oyesola Olusoji Adebuseye, Department of Physiology, School of Basic Medical Sciences, College of Health Sciences, Igbinedion University Okada, Okada, Edo State, Nigeria
Tel: +2347033139101

crystals (Asuzu *et al.*, 1988, 1990). Its chronic use was reported unsafe during pregnancy especially in the late phase (Asuzu *et al.*, 1990). The plant also was found to cause gross and histopathological changes in stomach, duodenum, ileum and colon (Asuzu *et al.*, 1989).

As reported by Ming-Fen *et al.* (1997), total intestinal lactase and sucrase activities decrease with age in a manner that likely involves a posttranscriptional process. The age-related decline in disaccharidase activity, if extrapolated to humans, may have important implications for the digestion of carbohydrate contained in the diet of the elderly. Diet, genetic and hormonal factors have been found to alter the structure and activities of intestinal enzymes in rats (Taylor and Mary, 1989; Infante *et al.*, 2008; Zarin and Mobarham, 1987). The enzyme activity concentrations (lactase, maltase, sucrase) were also reported to be much lower in chronic diarrhea (Marcellus *et al.*, 2003). Younaszai and Ranshaw (1976), reported that intestinal enzymes were increased with pregnancy and in both pregnant and non-pregnant rats, diabetes was associated with marked increase in specific and total activities of the three mucosal disaccharides.

While, croton seeds and its oil have been used in the treatment of a wide range of disorders in the past, both in pregnant and non pregnant individual; its effect on the gastrointestinal tract has not been documented with pregnancy. It is therefore of great interest to study the effect of the methanolic seed extract on intestinal tract enzymes and total protein contents in pregnant rats.

MATERIALS AND METHODS

Plant Material

The seeds of *Croton penduliflorus* were bought at the popular Kings' market in Ibadan, Oyo State, Nigeria and were ground with laboratory mortar and pestle in the laboratory of the Department of Physiology, University of Ibadan, Nigeria. Methanolic extract was obtained after extraction in methanol using Soxhlet apparatus and later defatted in hexane. The extraction was done in the Chemistry Department, University of Ibadan, Nigeria. The seed was verified and confirmed as herbarium specimen FHI 91302 of Federal college of Forestry, Herbarium Section, Jericho, Ibadan, Nigeria.

Work Duration

The study was conducted between March 2001 to September 2001 in the Department of Physiology, University of Ibadan, Nigeria.

Animals

Forty-eight female albino rats of the Wistar strain weighing between 180 and 220 g were used for the study. Non-pregnant rats and pregnant rats which were mated in the animal house of the Department of Physiology, University of Ibadan were used. They were divided into 4 groups. Vaginal smear were taken daily and first appearance of sperm in the vaginal smear was taken as day one of pregnancy. The rats were studied in early, mid and late phases of pregnancy that is, days 1-7, 8-14 and 15-21, respectively. The rats were given the extract orally between days 3-6, 11-13 and 18-20, respectively at 550 mg kg⁻¹ b.wt. They were however allowed access to food and water.

Preparation of Intestinal Mucosal Homogenate

The animals used were killed by a blow in the head. The rats were laid on their backs to the dissecting board with their limbs tied. The abdomen of each animal was opened up after an incision along the mid line. The intestines were carefully brought out. A specific length

of the small intestine was excised, spread on the dissecting board and was opened up using a small dissecting pair of scissors. The intestinal mucosa of each rat was scraped off using a piece of glass slide and homogenized by using one part of the scraped intestinal mucosa to four parts of cold normal saline. The homogenizing flask containing the intestinal mucosa solution was chilled with crushed ice during homogenization. After the stage of homogenization, the net solution was centrifuged using a laboratory centrifuge to remove nuclei and large cell debris. The supernatant was decanted and stored for enzyme assay and chemical analysis. The supernatant was assayed for sucrase, lactase and maltase according to the method of Dahlqvist (1964), the total protein content was assayed using the Biuret method and the albumin content using the (bromocresol green, BCG) binding method (Doumas *et al.*, 1971). The assay was done in the Biochemistry Department of University of Ibadan, Nigeria.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA). Results were presented as Mean±SD. p-values <0.05 and p-value <0.01 were regarded as statistically significant using SPSS-10.

RESULTS

From Table 1, there was a significant increase in sucrase activity in the early, mid and late ($p<0.01$) pregnancy while the significant increase in lactase activity was observed in the mid pregnancy ($p<0.01$). For maltase activity, there was no significant change in the enzyme levels during three phases of pregnancy.

Upon administration of the extract, there was a significant increase in sucrase activity in the mid and late pregnancy ($p<0.01$) when compared with the control (non-pregnant rats treated with methanolic extract of *Croton penduliflorus* MECP). Lactase activity increases significantly only in late pregnancy ($p<0.01$), while there were significant increases in maltase activity in early, mid and late pregnancy ($p<0.05$).

Table 2 shows that there was no significant change in the concentration of total protein in the three phases of pregnancy but the concentration of albumin increased significantly in late pregnancy ($p<0.01$). This value doubles that recorded for the control group.

The extract caused a significant increase in total protein concentration in late pregnancy ($p<0.01$). The significant increase in albumin concentration was observed in early and mid pregnancy but was reduced in late pregnancy ($p<0.01$) (Table 1).

Table 1: Comparison of intestinal enzyme activities during administration of methanolic extract of *Croton penduliflorus* in pregnant rats

Phase of pregnancy	Sucrase	Lactase	Maltase
Control	0.852±0.095 ^a	0.117±0.011 ^a	2.020±0.069
Control/extract	0.697±0.017 ^b	0.117±0.011	2.012±0.078 ^b
Early	1.190±0.019 ^a	0.224±0.098	1.728±0.044
Early/extract	0.865±0.094	0.295±0.104	2.290±0.046 ^{ab}
Mid	1.538±0.073 ^a	0.280±0.029 ^a	1.940±0.071
Mid/extract	1.607±0.102 ^b	0.132±0.015	2.233±0.025 ^{ab}
Late	1.640±0.131 ^a	0.164±0.028	1.910±0.175
Late/extract	1.531±0.080 ^b	0.170±0.009 ^b	2.412±0.046 ^b

Values are Mean±SEM; Control are non pregnant rats; ^{ab} $p<0.05$; ^a $p<0.01$ when compared with control

Table 2: Comparison of total protein and albumin concentration in pregnant rats during administration of methanolic extract of *Croton penduliflorus*

Phase of pregnancy	Total protein (g L ⁻¹)	Albumin (g L ⁻¹)
Control	49.667±5.333	11.333±0.422 ^a
Control/extract	44.833±3.390 ^b	11.333±0.4222 ^b
Early	35.400±7.827	12.400±0.980
Early/extract	66.000±8.357	19.000±1.225 ^b
Mid	49.000±8.143	13.200±1.200
Mid/extract	58.500±5.801	23.000±1.000 ^b
Late	44.000±4.025	26.800±1.800 ^a
Late/extract	169.333±19.592 ^b	15.667±1.282 ^b

Values are Mean±SEM; Control are non pregnant rats; ^{a,b}p<0.01

DISCUSSION

Enzymes are energized protein molecules necessary for life as they catalyze and regulate nearly all biochemical reactions that occur within the human body. Enzymes turn the food we eat into energy and unlock this energy for use in the body. Their presence and strength can be noticed in all body functions including pregnancy.

Among the intestinal brush border enzyme are sucrase, maltase and lactase which break down disaccharides to monosaccharides like glucose, galactose and fructose that are readily absorbed.

This experimental study shows that in pregnancy there was significant increase (p<0.01) in sucrase activity in those animals that were not treated with MECP compared with those female rats treated with MECP (Table 1) in early phase of pregnancy.

The lactase activity increased in the early phase was significantly increased (p<0.01) in the mid phase of pregnancy before it declined during the late phase of pregnancy, this is consistent with the findings of Sangild and Elnif (1996), that lactase activity was found to increase initially and then decline following the level of cortisol in the body.

Upon administration of MECP, the lactase activity in early phase of pregnancy increased but was not significant. MECP was found to decrease the level of lactase (p<0.01) at mid pregnancy.

MECP caused a significant increase in maltase activity, (p<0.01) in the three phases of pregnancy when compared with animals that were not treated with MECP. This may partly be due to the level of secretion of thyroxine (T₄), which often increases during the first trimester of pregnancy and then plateau thereafter.

The total protein concentration of the intestinal mucosal was relatively unchanged in its concentration during pregnancy. This may probably be due to the unchanged level of secretion of Growth Hormone (GH) throughout the three phases of pregnancy. The increase observed upon administration of MECP was significant especially in the late phase of pregnancy (p<0.01). The observation of low mucosal protein may be as a result of the increase in enzyme specific activity in agreement with Zarbin and Mobarham (1987). This implies that since the intestinal mucosal enzymes are synthesized from the protein content of the intestine and their levels were found to increase, it may be an indication that as the level of enzymes increase the protein is being depleted.

The albumin concentration increased significantly in the late phase of pregnancy, but upon administration of MECP, albumin concentration increased significantly in early and mid pregnancy (p<0.01) but in late pregnancy it was reduced significantly (p<0.01).

The increase in the activities of maltase and sucrase due to pregnancy and MECP are expected to promote digestion and absorption of sugar in food. This thus makes more food available to the pregnant rats and their fetuses. The increase in enzyme activity enhances the process of digestion and absorption in a more effective manner.

In conclusion, MECP has been found a factor that can increase intestinal enzyme activity in pregnant rats and affects the protein content significantly in the late phase of pregnancy similar to the report of Taylor and Mary (1989), Infante *et al.* (2008) and Zarlín and Mobarham (1987) that diet, genetic and hormones alter the structure and activities of intestinal enzymes in rats.

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