World Journal of Medical Sciences 2 (1): 28-32, 2007
ISSN 1817-3055

Evaluation of the Effects of Intake of Extract of Unripe Pawpaw (Carica Papaya) on Liver Function in Sickle Cell Patients

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Abstract: Extract of unripe pawpaw (Carica papaya) has traditionally been used as an antisickling agent by some sickle cell patients in Western Nigeria. An evaluation of the effects of intake of the extract on functions of the liver of sickle cell patients categorized into three according to their age, 2 to <6years (x 5.2), 6 to <12years (x 9.7) and, 12yreas and above (x 21.4) was carried out using standard techniques. Total and conjugated bilirubins (TB and CB) were significantly reduced ($p<0.05$) during intake than before intake of the extract. Total proteins (TP), albumin (Alb) and globulin (glb) were significantly increased ($p<0.05$) but within the reference range during intake than before intake. The valves of aspartate and alanine transaminases (AST and ALT) and cholesterol were within the reference range before and during the intake of the extract. Alkaline phosphatase (alk. Phos) was significantly increased ($p<0.05$) but within reference range during intake than before intake of the extract. Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) were within the reference range before and during intake of the extract. We concluded that the extract of unripe pawpaw has no adverse effect on liver functions.

Key words: Alkaline phosphatase · antisickling agent · bilirubin · prothrombin · transaminases · unripe pawpaw

INTRODUCTION

The liver is the largest organ of the body and is responsible for producing most of the endogenous energy sources used by the body. The liver is the principal organ for the metabolism of carbohydrates, protein, lipids, porphyrins and bile acids. It is capable of synthesizing most body proteins except the immunoglobulins, which are produced by the lymphocytic plasma cell system. The liver is also the major site for storage of iron, glycogen, lipids and vitamins. The liver plays an important role in the detoxification of xenobiotics and excretion of metabolic end products such as bilirubin, ammonia and urea [1-3].

Sickle cell disease is a life-long haemolytic anaemia caused by a single point mutation in the beta (β) chain of haemoglobin (Hb). A single nucleotide substitution (GTG for GAG) in the sixth codon of the globin gene results in the substitution of valine for glutamic acid on the surface of the variant-globin (s globin) chain [4]. This change allows HbS to polymerize when deoxygenated, since valine can dock with complimentary sites on adjacent globin chains. The polymerization of deoxygenated HbS is the primary indispendable event in the molecular pathogenesis of sickle-cell disease. It is dependent on intraerythrocytic HbS concentration, degree of cell deoxygenation, pH and the intracellular concentration of HbF [5]. The polymer is a rope-like fibre that aligns with

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others to form a bundle, distorting the red cell into classic crescent or sickled forms. These shapes interfere with a critical erythrocyte feature; its deformability [6]. These rigid sickle cells are responsible for the vaso-occlusive phenomenon that are characteristic of this disorder. The presence of other haemoglobins in the red blood cell, such as haemoglobin F, haemoglobin A, haemoglobin C and haemoglobin D, in that order, has antisickling effect on the polymerization of HbS [7, 8].

Carica papaya is used in many countries in different ways. It is eaten as ripe fruit and as an ingredient in jellies, preserves, juice as a beverage; young leaves, shoots and fruits cooked as a vegetable. Latex used to remove freckles, bark for making rope, leaves as a soap substitute to remove stains and flowers eaten in Java [9]. Papain, the proteolytic enzyme from carica papaya is used in medicine for the treatment of dyspepsia, digestive disorders and reducing enlarged tonsils. The root infusion is used for syphilis in Africa, leaf smoked for asthma relief in various remote areas. Flowers have been used for jaundice and inner bark used for sore throat[9]. Annonaceous acetogenins derived from the extracts of the twigs of the pawpaw tree may be good chemotherapeutic agents for cancer as these compounds inhibit enzymes necessary for metabolic processes in tumour cells [10-15].

The extract of unripe pawpaw has been reported to possess antisickling properties [16] and it is being used as an antisickling agent by some sickle cell patients in Western Nigeria. The minimum concentration of the extract that achieved maximum antisickling effect was established to be lg/ml of saline and the antisickling agent was found to be located in the ethyl acetate fraction of the extract [17]. The extract of unripe pawpaw has been shown to possess no adverse effect on the functions of liver, kidney and bone marrow in rats [18]. Although liver function was reported to be normal in seven-year old sickle cell children that ingested the extract for seven days [16], data on safety on long term ingestion of the extract on liver function and in different age groups of sickle cell patients are lacking, hence the present study is designed to investigate the effect of ingestion of extract of unripe pawpaw on liver function of sickle cell patients of different age groups over a relatively long period.

MATERIALS AND METHODS

Preparation of extract for ingestion: Matured fresh unripe pawpaw was plucked, peeled cut into pieces and the cream-coloured seeds inside discarded. One hundred grammes was soaked in 100ml of water for 72 hours at room temperature. The extract was sieved into a clean bottle.

Category of patients: Patients were categorized into three based on their age as follows: There were fifteen patients in each group.

Category one
Children: 2 to <6years-One teaspoonful (5 ml) of extract of unripe pawpaw was ingested 3 times daily for 6 months.

Category two
Children: 6 to <12years: Two teaspoonful (10ml) of the extract of unripe pawpaw was ingested 3 times daily for 6 months.

Adults: 12 years and above-Three teaspoonful (15ml) of the extract was ingested 3 times daily for 6 months.

Collection of specimen: Ten millilitres of blood was collected from each of the patients before extract ingestion through clean venepuncture, 4.5 ml of blood was dispensed into 0.5ml 3.8% Sodium citrate bottle for PT and APTT; and 5ml of blood was dispensed into lithium heparin bottle for other liver function tests. Blood collection was repeated 24hours, 1 week, 2 weeks, 3 weeks, I month, 2 months, 3 months, 4 months, 5 months and 6 months after daily ingestion of the extract throughout the period of the study. Blood samples were analysed on the day of blood collection.

Analytical procedure: Total bilirubin, conjugated bilirubin, total protein albumin, globulin, AST, ALT, Alk. Phos and cholesterol were estimated using standard techniques [19] PT and APTT were performed using standard techniques [20].

Statistics: The mean and standard deviation and the level of significance for the differences between means were computed by students test SPSS 6.

RESULTS

The effects of intake of extract of unripe pawpaw on liver function tests in sickle cell patients of different age groups were presentesd in tables 1, 2 and 3. In all ages the plasma levels of total and conjugated bilirubins after 6 months of intake were significantly lower ($p<0.05$) than

Table 1: Effect of intake of extract of unripe pawpaw on liver function test in sickle cell patients (2- <6 years)

	TB	CB	TP	ALB	Glob	AST	ALT	AlK Phos	Chole		
Reference	UmoI L^{-1}	UmoI L^{-1}	g L^{-1}	g L^{-1}	g L^{-1}	IU L^{-1}	IU L^{-1}	IU L^{-1}	mmoI L^{-1}	PT sec	APTT sec
range	up to 20	5	58-80	35-50	20-45	up to 18	up to 22	60-170	2.5-6.5	11-14	30-40
Intervals											
Before Intake	19.80±9.70	9.73±5.87	60.33±6.52	36.26±3.17	24.07±4.74	14.60±4.22	12.07±4.96	78.20±6.91	3.01±0.27	12.53±0.94	33.72±2.60
24 hours after	16.53±7.84	8.27±4.15	60.53±5.64	40.07±2.81	20.46±4.00	13.80±3.97	11.27±4.73	79.73±7.87	2.97±0.22	12.79±0.68	35.07±2.56
1week after	12.40±3.40	5.67±2.50	64.07±4.88	41.20±2.40	24.87±4.52	13.13±3.20	10.53±3.98	86.33±12.53	3.10±0.25	12.02±0.52	33.51±2.42
2 weeks after	10.47±1.77	4.47±1.92	64.40±6.23	42.13±3.92	25.27±4.20	14.72±2.66	10.16±3.10	89.13±16.43	3.08±0.25	12.82±0.77	34.77±1.77
3 weeks after	9.87±1.64	4.47±1.96	64.33±6.64	44.67±4.64	24.67±3.75	10.87±2.72	9.89±2.05	91.33±14.26	3.15±0.22	12.27±0.46	33.53±2.48
1 month after	9.00±2.07	4.00±1.46	68.20±5.21	43.53±3.98	24.67±2.89	10.00±2.07	11.03±2.01	94.27±14.00	3.25±0.26	12.35±0.51	33.87±2.69
2 months after	8.93±2.22	3.07±1.67	72.13±6.29	43.87±11.96	24.93±3.86	10.80±2.11	10.91±1.95	99.20±16.03	3.30±0.23	13.02±0.83	34.84±2.27
3 moths after	7.80±1.32	2.60±0.99	70.07±5.66	44.93±4.37	25.13±3.81	11.61±1.80	11.23±1.80	100.33±14.89	3.37±0.25	13.03±0.64	34.01±2.80
4 months after	6.80±1.01	2.47±0.92	72.60±5.70	46.60±3.60	25.00±4.51	11.35±1.94	10.92±1.66	104.27±15.22	3,32±0.18	12.75±0.74	34.55±2.45
5 months after	7.87±2.07	2.67±1.35	72.60±3.94	46.67±3.20	24.93±3.08	13.36±1.75	9.99±1.51	106.13±15.13	3.47±0.29	13.01±0.56	34.92±2.12
6 months after	7.33±2.19	2.53±1.19	71.40±3.83	46.40±4.16	25.00±3.42	12.63±2.00	10.37±1.77	108.67±14.97	3.45±0.32	12.24±0.51	35.01±2.17

Table 2: Effect of intake of extract of unripe pawpaw on liver function test in sickle cell patients (6<12years)

	TB	CB	TP	ALB	Glob	AST	ALT	AlK Phos	Chole		
	UmoI L^{-1}	UmoI L^{-1}	g L^{-1}	g L^{-1}	g L^{-1}	IU L^{-1}	IU L^{-1}	IU L^{-1}	mmoI L^{-1}	PT sec	APTT sec
	up to 20	5	58-80	35-50	20-45	up to 18	up to 22	60-170	2.5-6.5	11-14	30-40
Intervals											
Before Intake	75.73±31.86	33.07±12.37	65.80±5.43	40.40±3.31	25.40±4.66	15.67±3.40	16.53±4.22	97.40±22.46	3.23±0.37	13.05±0.74	34.91±3.44
24 hours later	74.07±32.27	34.53±15.18	65.67±5.23	39.67±3.22	26.00±3.87	16.33±3.55	16.60±3.94	98.07±22.79	3.23±0.40	13.19±0.55	35.79±3.18
I week after	18.00±4.26	7.33±2.52	67.47±4.49	40.80±3.26	26.67±3.58	15.60±3.62	17.40±4.30	100.93±22.91	3.20±0.43	13.16±0.44	34.80±2.17
2 weeks after	13.93±3.03	6.00±1.73	68.20±4.84	42.27±2.87	25.93±4.30	14.93±3.34	15.47±3.18	103.00±22.60	3.26±0.42	12.97±0.75	35.02±2.73
3 weeks after	13.33±3.89	5.47±1.36	69.33±5.34	42.20±2.48	27.13±4.93	14.60±3.33	15.47±3.38	106.00±22.98	3.20±0.46	13.29±0.51	34.76±2.29
1 month after	11.47±3.38	5.07±1.00	70.47±4.84	42.93±2.05	27.53±4.07	14.87±4.41	15.40±3.91	108.20±22.94	3.11±0.44	13.25±0.59	35.01±2.03
2 months after	11.13±3.14	4.07±0.07	71.27±4.46	43.73±2.19	27.60±3.74	14.40±3.33	15.13±5.14	109.40±21.99	3.16±0.50	12.97±0.53	35.04±2.54
3 months after	9.87±2.59	3.60±1.06	73.20±4.60	44.47±1.60	29.07±3.40	14.27±4.17	15.07±4.59	111.27±23.36	3.24±0.48	13.07±0.58	34.81±2.84
4 months after	10.02±2.37	3.40±0.63	72.13±3.87	44.27±1.22	27.87±3.74	13.33±4.56	14.87±4.56	114.27±23.35	3.17±0.47	13.05±0.67	36.64±1.48
5 months after	9.40±3.68	3.07±0.88	73.40±3.33	44.67±2.41	28.73±3.37	13.36±4.96	14.00±4.02	117.20±23.95	3.23±0.45	12.93±0.43	34. 47±2.40
6 months after	9.00±2.88	2.80±0.94	73.60±2.85	46.33±6.98	27.27±2.58	13.53±4.55	14.13±3.85	118.47±24.92	3.32±0.46	13.28±0.48	34.37±1.88

Table 3: Effect of intake of extract of unripe pawpaw on liver function test in sickle cell patients (12 years and above)

	TB	CB	TP	ALB	Glob	AST	ALT	AlK Phos	Chole		
	UmoI L^{-1}	UmoI L^{-1}	g L^{-1}	g L^{-1}	g L^{-1}	IU L^{-1}	IU L^{-1}	IU L^{-1}	mmoI L^{-1}	PT sec	APTT sec
	up to 20	5	58-80	35-50	20-45	up to 18	up to 22	60-170	2.5-6.5	11-14	30-40
Intervals											
Before intake	49.00±9.52	24.73±9.18	63.13±5.89	39.40±3.89	23.73±4.40	13.80±3.10	15.00±3.83	90.60±14.66	3.20±0.34	12.93±0.61	32.87±2.58
24 hours later	47.07±9.59	23.67±8.13	62.47±6.10	39.47±3.91	23.00±4.21	13.67±2.94	14.13±4.16	91.47±14.74	3.24±0.32	12.51±0.53	32.99±2.45
I week after	18.40±7.13	9.20±4.66	64.67±5.54	40.80±3.97	23.87±4.47	13.27±3.22	14.73±3.69	95.20±15.17	3.25±0.31	12.66±0.71	33.16±2.44
2 weeks after	13.27±3.53	6.33±1.88	66.40±5.47	41.73±3.56	24.67±3.64	11.87±3.96	14.13±3.29	100.33±16.42	3.32±0.34	12.83±0.68	33.40±2.44
3 weeks after	12.73±2.15	5.33±1.18	66.53±5.85	42.33±3.11	24.20±4.89	12.53±3.93	13.93±3.45	102.07±17.17	3.30±0.40	12.93±0.54	33.54±1.81
1 month after	11.47±1.81	5.20±1.08	68.47±6.32	42.67±3.06	25.93±4.56	12.27±4.37	13.07±4.56	104.00±17.75	3.31`±0.38	13.00±0.51	34.73±2.13
2 months after	10.33±1.54	4.40±0.99	69.20±6.63	43.47±3.11	25.80±5.02	12.13±3.42	12.73±4.17	107.80±18.70	3.30±0.37	12.83±0.63	34.40±1.56
3 months after	9.53±1.46	3.47±0.83	70.40±6.79	44.39±2.99	25.07±5.82	11.53±4.55	12.53±4.94	110.47±18.50	3.28±0.33	13.17±0.49	34.57±2.02
4 month after	9.53±1.77	3.40±0.63	72.13±3.87	44.27±1.22	27.87±3.74	13.33±4.56	14.87±4.56	114.27±23.35	3.17±0.47	12.96±0.43	34.23±1.91
5 month after	9.93±1.94	3.67±0.82	70.47±4.67	45.07±3.73	25.40±3.91	11.47±4.76	12.20±5.01	114.13±18.63	3.39±0.28	13.01±0.54	34.23±1.59
6 months after	10.07±1.62	3.20±0.77	72.20±3.39	45.87±3.68	26.33±3.77	11.53±5.19	12.87±5.00	115.73±18.02	3.41±0.27	13.07±0.53	35.07±2.12

values obtained before extract intake. The values of total proteins, albumin and globulins were significantly higher ($p<0.05$) after 6 months of extract intake than the values obtained before intake. The values of AST, ALT, alk.phos and cholesterol were all within the reference range before and after extract intake for 6 months PT and APTT were within the reference range before and after extract intake for 6 months.

DISCUSSION

The reduction in plasma bilirubin levels soon after the commencement of intake of the extract of unripe pawpaw provided scientific justification for its use by herbalists as a treatment for jaundice. There were hyperbilirubinaemia in the age group 6 to 12 years and adult before intake of the extract, the bilirubin levels were on the high side in children of 2 to 6 years in all the age groups, the bilirubin levels returned to normal levels within a week of intake of extract.

This was maintained throughout the period of the study. The reduction and maintainance of bilirubin levels within the reference range was an indication that there was no haemolysis of red cells. The exact mechanism of antihaemolytic action of the extract is not yet known.

The plasma concentration of total protein were increased although within the reference range, after intake of the extract for 6 months. This increase was majorly due to increase in albumin, an indication that the synthetic function of the liver was improved. The normal PT and APTT that were recorded before intake and throughout the study period also showed that the synthetic function of the liver was not impaired because most of the coagulation factors are synthesized by the liver. The plasma levels of AST and ALT before extract intake and throughout the study period remained normal, a pointer that the extract did not damage liver cell. The plasma levels of alkaline phosphatase were increased although within the reference range, after intake of the extract. Since other liver function tests were normal, the increase in alkaline phosphatase might be due to increase bone activities. There were slight increase in cholesterol levels but they were within the reference range.

From our findings, it was shown that the extract of unripe pawpaw had no adverse effect on liver function; the active agent in the extract is being purified and characterized.

ACKNOWLEDGEMENT

We are grateful to late Professor K.D. Thomas of Chemical Pathology Department, Obafemi Awolowo University, Ile-Ife, Nigeria who initiated this work but did not live to see it to completion.

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