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Full Length Research Paper

Antisickling properties of the fermented mixture of *Carica papaya* Linn and *Sorghum bicolor* (L.) Moench

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The antisickling properties of fermented mixture of dried unripe fruit pulp of *Carica papaya* and dried *Sorghum bicolor* leaves, mixed in equal proportions in distilled water, was carried out using sodium metabisulphite sickled red blood cells and the result presented. Equal weight of dried *C. papaya* fruit pulp and *S. bicolor* leaves were fermented together in distilled water at room temperature and the aque-ous extract obtained and used for antisickling assays. The extract gotten from the materials incubated for 5 days indicated as SP5, was found to have the highest antisickling properties with 93% inhibitory and 84% reversal activities. The concentration of the day 5 extract was further varied. 0.2 ml was found to be the optimum volume of the test extracts.

Key words: Antisickling, reversal, inhibitory, *Carica papaya, Sorghum bicolor,* sickle cell anaemia.

INTRODUCTION

Sickle cell disease was first discovered by a Chicago physician, Dr. James B. Herrick in 1904 when he exami-ned a 20 year-old black student from the West Indies (Hammerschmidt, 2002). Normal red blood cells move through small blood vessels (capillaries) in the body to deliver oxygen and food nutrients. Sickled red blood cells however, tend to obstruct the blood flow in the capillaries, thus causing poor blood microcirculation (Kuyper et al., 1994). The red cell membrane of sickle cell haemoglobin (HBSS) are osmotically and mechanically more fragile than those of Haemoglobin AA. Hence, sickle RBC are easily destroyed and removed from circulation in the spleen thus causing anaemia and subsequent spleno-megally (Written, 1989). Red blood cells of the patients have a short life span of about 2 weeks as opposed to 120 days in normal subjects, thereby causing chronic anaemia. Patients suffer from painful crisis, acute chest syndrome and malfunctioning of organs including the spleen, heart and brain, as well as from degeneration of the bone (Written, 1989).

Sorghum bicolor (L) Moench (Family: Poaceae) is the dominant cereal crop in the Guinea and Sudan savanna zones of West Africa. It is a summer annual, coarse, erect plant with much variability in growth characteristics. Leaves are broad and coarse, similar in shape to those of corn but shorter and wider (Purseglove, 1972) . In Nigeria, guinea corn (*Sorghum* spp) is the main food crop of the northern states, but it is grown as far south as Ibadan in Nigeria, latitude 7° to 22°N (Phillips, 1964). Sorghum is reported to be antiabortive, cyanogenic, demulcent, diu-retic and emollient. Sorghum is a folk remedy for cancer, epilepsy, flux, and stomach ache (Duke and Wain, 1981). Traditionally in Nigeria, *S. bicolor* (L) Moe-nch is used as a blood builder and used in the treatment of sickle cell crisis. A 4 - 5 day cold infusion of a mixture of *S. bicolor* leaves and *Carica papaya* unripe fruit pulp is used by the Yorubas (western Nigeria) to alleviate bone pains (Elu-joba, 2001 personal communication). Figure 1

C. papaya Linn. (Family: Caricaceae) is a perennial, herbaceous plant, with copious milky latex, reaching to 6 - 10 m in height; the stem up to 10 inches thick, simple or branched above the middle and roughened with leaf scars (Williams, 1961). The unripe fruit is used tradi-tionally among the Yoruba tribe of Nigeria for treating jaundice and for the management of sickle cell anaemia (Elujoba, 2001, personal communication).

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The unripe fruit contains glycine, phenylalanine and tryptophan with reported antisickling properties (Pizzorno and Murray, 1985; Ekeke and Shode, 1990).

The objective of this work was to establish the antisickling potentials of the fermented mixture of dried *C. papaya* unripe fruit pulp and dried *S. bicolo*r leaves, thereby justifying the ethnomedicinal claim for its use in the management of sickle cell anaemia by the Yoruba tribe of Nigeria

MATERIALS AND METHODS

Fermentation of the mixture of *C. papaya* fruit and *S. bicolor* leaves

15 g of dried powdered unripe fruit of pawpaw and 15 g of sorghum leaves were accurately weighed into each of the 16 conical flasks (8 flasks in duplicates) and 300 ml distilled water was added to each flask. These were then incubated at room temperature. After 24 h incubation period, two of the incubates were filtered and the filtrate boiled while the residue was discarded. Thereafter, it was allowed to cool and then refrigerated for later use. The extracts from the mixture were tagged SP1-SP7 for 24 -168 h.

Collection of blood

Fresh blood samples were collected from confirmed sickle cell patients every week at the OAUTHC Haematology Outpatient Clinic. 5 ml each of fresh blood samples from sickle cell anemia patients in steady state, between the ages of 12 and 23 years (both sexes) were drawn from the veins by vein-puncture, into EDTA (Ethylene diaminotetraacetic acid) bottles. The blood was mixed carefully and properly (fresh blood samples were always used within the first 48 h of collection).

Inhibitory antisickling assay

HbSS whole blood (0.2 ml) was pipetted into test tubes (in duplicates); 0.2 ml phosphate buffered saline solution and 0.2 ml of the extracts were added. The mixture was overlaid with liquid paraffin (1 ml) and incubated in a thermostated water bath at 37°C for 4 h. Freshly prepared 2% w/v sodium metabisulphite solution (0.6 ml) was carefully added under the liquid paraffin to the incubation mixture after the 4 h incubation period. The final mixture was thoroughly and carefully mixed by rolling the test tubes between the palms of the hand. The mixture was incubated further for another $1^1/_2$ h at 37°C in a water bath. The liquid paraffin was carefully removed with a Pasteur pipette and the resultant mixture was fixed in 3 ml of 5% v/v buffered formalin.

The experiment was set up in duplicate with negative control where 0.2 ml phosphate buffered saline solution was used in place of the extracts plus two positive controls, of 0.2 ml vanillic acid and 0.2 ml siculine syrup®.

The percent inhibitory activity for each sample was calculated from the results appropriately and presented as a duplicate means for all the samples including the experimental controls.

Reversal antisickling assay

HbSS whole blood (0.2 ml) was placed in a test tube (in duplicate), 0.2 ml phosphate buffered solution was added and the mixture was overlaid with 1 ml liquid paraffin. 2% w/v sodium metabisulphite solution (0.6 ml) was introduced gently under the liquid paraffin. The mixture was thoroughly and carefully mixed by rolling the test tube between the palms of the hands before incubating at 37°C in a thermostated water bath for $1^1/_2$ h. 0.2 ml of the extracts was added under the liquid paraffin (carefully as before) and incubated further for 6 h. The experiment was set up in duplicates with a negative control where 0.2 ml phosphate buffered saline solution was used in place of the extracts. After incubation, the liquid paraffin layer was carefully removed with Pasteur pipette and 3 ml of 5% v/v buffered formalin solution was added. The mixture was thoroughly mixed to ensure proper fixation (Kiernan, 2000). The counting of cells was carried out as described earlier.

The experiment was set up in duplicates with the negative control where 0.2 ml phosphate buffered saline solution was used in place of the extracts plus two positive controls of 0.2 ml parahydroxylbenzoic acid (PHBA) and 0.2 ml siculine syrup®. The percentage reversal activity for each sample was calculated and presented as duplicate means of all the samples including the experimental controls.

Counting of cells

Slides were prepared from fixed cells after centrifugation. The fixed cell mixtures were each centrifuged and their supernatants decanted. With a capillary tube, a drop or two was applied on a microscope slide, carefully covered with a cover slip and with a high power objective (x 100) of the microscope, 400 cells (both sickled and unsickled erythrocytes) were counted and the percentage sickled cells recorded.

Varying the concentrations of fermented plant materials

The volume of the extracts used for the antisickling assay was varied from 0.1 to 0.5 ml. The day 5 (SP5) extract was used for this experiment, thereby increasing the concentration of the fermented extracts present in the incubated mixture. The antisickling assays were carried out for both the inhibitory and reversal activities.

RESULTS

The inhibitory experiment of the mixture of sorghum and pawpaw, when soaked in equal ratio of 1 g pawpaw to 1 g sorghum (1:1) with 20 ml distilled water at room temperature for 1 to 7 days (SP1 - SP7), gave the results as shown in Table 1. The incubate on day 5 (SP5) gave the highest inhibitory activity. The mixture of dried pawpaw fruit and sorghum leaves incubated for five days (SP5) gave the highest reversal activity as shown in Table 2, Plate 1, 2 and 3). The inhibitory activities of the varied concentrations the fermented mixture of dried *C. papaya* unripe fruit pulp and *S. bicolor* leaves from 0.1 to 0.5 ml is shown in Table 3. The reversal activity of the varied concentrations of the fermented mixture of dried *C. papaya* unripe fruit pulp and *S. bicolor* leaves (1:1) mixture from 0.1 to 0.5 ml is as shown in Table 4.

DISCUSSION AND CONCLUSION

The inhibitory and reversal antisickling activities of the extract of the fermented mixture of *C. papaya* and *S. bicolor* gave a 93 and 84% activities respectively Tables 1 and 2). Thus confirming the ethnomedical report that the Yorubas use the extract from the fermented mixture of sorghum leaf and unripe pawpaw fruit for the treatment of sickle cell anaemia (Elujoba, 2001, personal communication). The results of the varied concentration of the test extract shows that there is no significant difference in

Table 1. Inhibitory activities of the fermented mixture of dried *C. papaya* unripe fruit pulp and *S. bicolor* leaves.

Incubation period	% Sickled	% Inhibition
Day 0 (SP0)	73	13
Day 1 (SP1)	52	39
Day 2 (SP2)	22	74
Day 3 (SP3)	18	79
Day 4 (SP4)	11	88
Day 5 (SP5)	6	93
Day 6 (SP6)	7	92
Day 7 (SP7)	7	92
PBS	84	0
Vanillic acid	40	52
Siculine®	15	82

Table 2. % Reversal activities of the fermented mixture of dried *C. papaya* unripe fruit pulp and *S. bicolor* leaves.

Incubates	% Sickled	% Reversal
Day 0 (SP0)	81	8
Day 1 (SP1)	71	20
Day 2 (SP2)	58	34
Day 3 (SP3)	40	55
Day 4 (SP4)	29	68
Day 5 (SP5)	15	84
Day 6 (SP6)	15	83
Day 7 (SP7)	16	83
Phosphate buffer saline	88	0
p- hydroxyl benzoic acid	44	50
Siculine®	15	83

Table 3. Inhibitory activities of varied concentrations of the fermented mixture of dried *C. papaya* unripe fruit pulp and *S. bicolor* leaves.

Volume (ml)	% Sickled	% Inhibition
0.1	28	67
0.2	6	93
0.3	5	94
0.4	4	95
0.5	Clumped	Clumped
PBS (0.2)	84	0

Table 4. Reversal activities of the varied concentrations the fermented mixture of dried *C. papaya* unripe fruit pulp and *S. bicolor* leaves

Volume (ml)	% Sickled	% Reversal
0.1	36	60
0.2	15	84
0.3	14	84
0.4	14	84
0.5	Clumped	Clumped
PBS	89	0

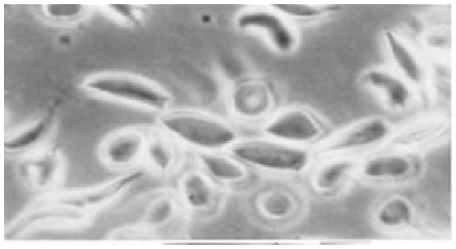

Plate 1. Untreated Control showing irreversibly sickle cells.

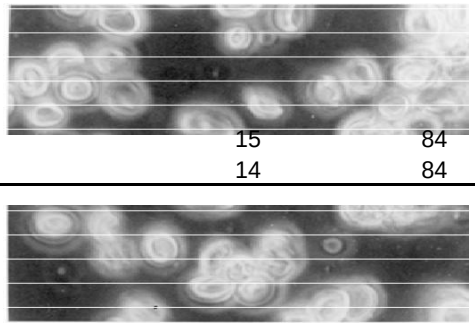

Plate 2. Red blood cells of the inhibitory activity of the day 5 fermented mixture of dried *C. papaya* unripe fruit pulp and *S. bicolor* leaf with 93% Inhibition.

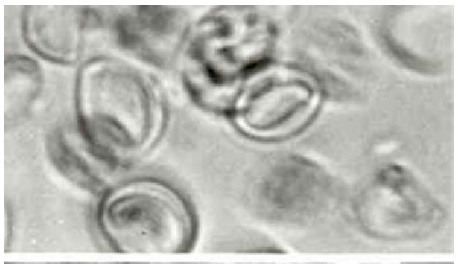

Plate 3: Reversed red blood cells by day 5 extract of the fermented mixture of dried *C. papaya* unripe fruit pulp and *S. bicolor* leaves (83% reversal).

the activities of the higher concentrations i.e. 0.3 - 4 ml when compared with 0.2 ml; hence 0.2 ml is the optimum volume for this experiment (Tables 3 and 4). Fermenta tion was carried out at room temperature by endogenous enzymes present in the plant materials.

The effect of cyanate on a sickling has been reported; cyanate improves red cell survival, increase haemoglobin levels and prevents most of the minor painful episodes ofsickle cell anaemia (May et al., 1972) . It can thus be suggested that cyanate, which is found to be present in *S.bicolor,* might be one of the antisickling agents present. Iron has also been reported by Duke (1992) to have anti-anaemic properties and this is found to be present in *S. bicolor.* The amount of tannin present in *S*. *bicolor* in relations to the bioavailability of iron was assessed by Radhakrishnan and Sivaprasad (1980) in normal and anaemic subjects and they concluded that in anaemic subjects, iron availability was lower with high tannin sorghum. Traditionally, sorghum is believed to be a blood builder possibly because of the presence of blood- red coloured pigments hence its use as antianaemic drug.

C. papaya had been reported to possess antisickling properties suggesting that the active compound(s) preventing and reversing sickling could be organic acids, produced after hydrolysis of corresponding esters in the fruit (Thomas and Ajani, 1987). Also reported are amino acids, glycine, phenylalanine and tyrosine which have been reported to possess antisickling properties (Duke, 1992).

The combination of these two plants could have synergistic effect since one or more components of each have antisickling properties hence the high values for both the reversal and inhibitory activities.

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