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Glucose-6-phosphate dehydrogenase Activity in albino rats treated with aqueous extract of fresh leaves of *Senna hirsuta*

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Abstract

In Eastern Nigeria, various parts of *Senna hirsuta* have been successfully by herbal medicine practitioners for management and treatment of many diseases, such as malaria, dysentery, typhoid fever, etc. This present research assessed the effect of aqueous extract fresh leaves of *Senna hirsuta* on glucose - 6 - phosphate dehydrogenase (G6PD) activity in albino rats. The research was performed with twenty-five (25) adult male albino rats placed in five groups (A, B, C, D and E) with five rats in each group. The animals in groups A, B, C, and D were administered orally with aqueous extract of fresh leaves of *Senna hirsuta* using doses of 200, 400, 600 and 800mg/kg body weight respectively, for seven consecutive days, while group E served as the control. There was a decrease in physical activities, feed and water intake, in the treated groups compared with the control. The serum total protein concentrations in the test groups did not vary significantly ($P > 0.05$) with those of the control. The G6PD activity in the treated groups was significantly lower ($P < 0.05$) than that of the control. These effects were found to be dose dependent. The results of this research suggest that the aqueous extract of fresh leaves of *Senna hirsuta* contain chemical compound(s) capable of inhibiting the activity of G6PD, and this may partly responsible for their utilization in management and treatment of malaria.

Key-Words: Malaria, Serum, Total protein, Albino rats, *Senna hirsuta*

Introduction

Medicinal plants are commonly used in modern medicine and pharmacology. These are plants in which one or more of their organs contain substances that can be used for therapeutic purposes or may be precursors for the synthesis of useful drugs, some of the parts of plants that produce certain substances that have therapeutic effects are the seeds, the roots, the bark, leaves, flower etc [1].

Medicinal plants are of great value in the treatment and cure of diseases. Over the years, scientific research has expanded the knowledge of medicinal plants and new drugs in the western world, as people are becoming aware of the potency and side effects of synthetic drugs, there is an increasing interest in plant-based medications. The future development of the pharmacognostic analysis of herbal drugs is largely dependent upon reliable methodologies for correct identification, standardization and quality assurance of herbal drugs.

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Describing herbal drugs in a systematic manner is based on multiple approaches of pharmacognostic, taxonomic and chemical analysis, including documentation of their biological and geographical source, cultivation, collection, processing, morphological, microscopic and chemical characters [2].

All plants produce chemical compounds as part of their normal metabolic activities. These can be divided into primary metabolites, such as fat and sugar found in all plants, and secondary metabolites such as alkaloids and tannins, found in the smaller range of plants, some only in a particular genus or species[3]. The secondary metabolites are responsible for most pharmacological activities of plants [4].

Senna hirsuta, is a large genus of flowering plants in the legume family *Fabaceae*. This diverse genus is native throughout the tropics with a small number of species in temperate regions. The number of species is estimated to be from about 260 to 350[5][6]. It is a large upright herbaceous plant becoming slightly woody with Age and growing 0.5 – 0.3m tall, the stems, leaves and pods are all densely covered with long pale grayish-white colored hairs, with yellow flower of five sepals, reproduction is by seed only. For

centuries it has been used as a remedy for high blood pressure, lowering cholesterol levels, typhoid fever, malaria, diarrhea, skin rashes among others [7].

Despite being a disease that is both preventable and curable, malaria still has an enormous harmful impact on human health. Different species of anopheles mosquito are responsible for transmitting the pathogens that causes malaria. Malaria is caused by protozoan parasites of the genus *Plasmodium* of which five species are now known to infect humans [8]. Malaria parasites exhibit complex lifecycles, with clinical symptoms arising from cycles of erythrocytic invasion, growth and division, followed by cell lysis and reinvasion. *P. falciparum* is especially dangerous as this species is able to remodel the erythrocyte to enable infected cells to adhere to endothelial surfaces, thus progressively blocking microcapillaries in the major organs, including the brain. In this way, the parasite reduces passage of infected erythrocytes through the spleen, which is able to detect and destroy such compromised cells [9].

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme that catalyzes the first reaction in the pentose phosphate pathway, providing reducing power to all cells in the form of NADPH (reduced form of nicotinamide adenine dinucleotide phosphate). NADPH enables cells to counterbalance oxidative stress that can be triggered by several oxidant agents, and to preserve the reduced form of glutathione [10]. Since red blood cells do not contain mitochondria, the pentose phosphate pathway is their only source of NADPH; therefore, defense against oxidative damage is dependent on G6PD [11]. The most common clinical manifestations of G6PD deficiency are neonatal jaundice and acute hemolyticanemia, which in most patients is triggered by an exogenous agent. The striking similarity between the areas where G6PD deficiency is common and *Plasmodium falciparum*malaria is endemic provides circumstantial evidence that G6PD deficiency confers resistance against malaria. The highest frequencies are detected in Africa, Asia, the Mediterranean region, and in the Middle East; owing to recent migrations. However, the disorder is also found in North and South America and in northern European countries [12].

Many of the medicinal applications of parts of *Senna hirsuta* have not been scientifically investigated. The present study evaluated effect of aqueous extract of the leaves on G6PD activity, as a possible contributing explanation to their claimed antimalarial property.

Material and Methods

Collection of animals

Twenty-five adult male albino rats, weighing 95 – 120g, were collected from pharmacy department of University of Nigeria, Nssuka in a large cage and were transported to the animal house of Biochemistry Department, Ebonyi State University.

Collection of plant leaves:

Fresh leaves of *Senna hirsuta* were obtained from Ezza –Ofu Inyimagu Izzi local Government Area, Ebonyi State,Nigeria, and authenticated by Professor S.C. Onyekwelu of Applied biology department of Ebonyi State University.

Preparation of Extract

The method of extraction used by Agbafor [13] was adopted, utilizing distilled water as solvent.The extract was concentrated using rotor evaporator to get gel-like dark brown extracts.

Experimental Animals and Handling

Ethical approval for use of animals in research was given by Ebonyi State University Research and Ethics Committee.

Twenty-five adult male albino rats, used in the research, were randomly distributed into five groups (A, B, C. D and E), and allowed to acclimatize for seven days before administration commenced. Groups A, B, C and D were orally administered 200, 400, 600 and 800mg/kg body weight the extract respectively for seven consecutive days, while group E, the control, was given distilled water. All the animals were allowed free access to feed and water.

Collection of Specimen from the Animals

After treatment, the animals were fasted overnight and were scarified under mild anesthesia using chloroform before blood was collected by cardiac puncture. The blood was transferred to sterile screw capped tubes without anticoagulant, left at the room temperature for coagulation, and centrifuged for 10 min at 1500 x g to obtain the serum.

Measurement of Parameters

Serum activity of G6PD was measured according to the method of Tian et al [14], while Lowry [15] method was adopted to determine total protein.

Statistical analysis

Data generated were expressed as mean ± SD. Statistical significance of difference was determined using the program SPSS 12 (SPSS, USA) by performing one-way ANOVA with post-hoc comparisons between the control group and each of the treated groups by Ducan's multiple comparison test. A p-value less than 0.05 was considered statistical significant.

Results and Discussion

Within the seven days of treatment, there was an obvious decrease in physical activities of the albino rats after the administration of the aqueous extract of fresh leaves of *Senna hirsuta.*A reduction in feed and water intake was also noticed after the administration of the extract as compared to that of the control (data not shown).The reason for this observation is yet not fully understood. However, it could be attributed to the changes in metabolic activities of the treated animals elicited by constituents of the extract. The average body weight of groups given the extract decreased throughout the period of administration, while that of the control increased (tables 1). This decrease in body weight may be due to the observed decrease in feed and water intake.

Similar observations have been reported by Agbafor [13]; Agbafor and Akubugwo [16]; Agbafor et al [4].

As shown in table 2, the decrease in serum total protein of the test groups relative to the control was not significant ($P>0.05$). This suggests that the chemical constituent of the drug at the doses administered may not have a significant effect on the rate of protein biosynthesis and degradation. This result is in line with the result obtained by Agbafor et al [4].

There was a significant decrease ($P<0.05$) in the activity of glucose-6-phosphate dehydrogenase in the groups administered the extract relative to the control (table 2). Research is in progress to identify the possible reason(s) for this observation. However, the chemical constituents of the extract may be responsible for this reduction in the enzyme activity. This action of the extract may be a contributing factor to the utilization of leaves by herbal medicine practitioners in treatment/management of malaria. *In vitro* studies show that the malaria parasite, *Plasmodium falciparian* is inhibited in G6PD deficient erythrocytes. The parasite is very sensitive to oxidative damage and is killed by a level of oxidative stress that is tolerable to a G6PD deficient human host [11]. Hence, G6PD deficiency confers malaria resistance to G6PD deficient individuals. Similar results were documented by previous workers [17][18] on pathological effects on body organs caused by oral administration of artemether and some other anti-malarial agents.

G6PD plays an important role in the cellular response to the oxidant stress in *E. coli* [19]. Agents responsible for production of oxygen radicals induce G6PD and eight other genes required to protect cells from free radical damage [20]. In G6PD-deficient individuals, the NADPH production is diminished and detoxification of H_2O_2 is inhibited and therefore cellular damage results, lipid peroxidation leading to breakdown of erythrocyte membranes and oxidation of proteins and DNA [10]. The antimalarial drug, primaquine is believed to act by causing oxidative stress to the parasite. It is ironic that antimalarial drugs can cause human illness through the same biochemical mechanism that provides resistance to malaria. Divicine also acts as antimalarial drug, and ingestion of fava beans may protect against malaria [21].

Conclusion

Aqueous extract of fresh leaves of *Senna hirsta* possess chemical constituents that inhibit activity of G6PD. This inhibition may be partly responsible for application of the leaves in treatment of malaria. However, investigation of antimalarial action of the leaves is in progress in our laboratory.

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Table1: Changes in average body wt of the rats (g) during seven days of extract administration

NO OF DAYS	BODY WEIGHTS(g)				
	A	B	C	D	E
1	95.54 ± 2.91	89.12 ± 2.40	93.60 ± 5.18	120.60±5.18	87.23 ± 5.75
2	97.65 ± 4.66	86.12 ± 6.49	88.60 ± 3.30	113.22 ± 3.68	90.33 ± 7.37
3	95.45 ± 3.73	82.98 ± 3.17	85.67 ± 4.95	103.54 ± 4.10	95.35 ± 6.45
4	92.50 ± 4.90	78.32 ± 3.47	80.67 ± 7.89	97.61±5.08	98.32 ± 3.95
5	91.80 ± 6.84	75.54 ± 7.89	76.54 ± 6.73	90.33±5.06	104.00 ± 6.94
6	88.98 ± 5.18	71.45 ± 2.28	72.10 ± 7.89	88.60 ± 4.17	107.50 ± 2.70
7	84.75 ± 3.49	67.50 ± 7.89	65.76 ± 6.28	85.35 ± 5.25	112.67 ± 2.89

All values are mean ± standard deviation. N = 5.
Key: Group A = 200mg/kg body weight.
Group B = 400mg/kg body weight.
Group C = 600mg/kg body weight.
Group D = 800mg/kg body weight.
Group E = 0.2ml distilled water.

Table 2: Average enzyme activity and total protein concentration in the animals after seven days of extract administration

Animal Group	Average Enzyme Activity (U/l)	Total Protein (mg/ml)
A	5.57 ± 0.46 [a]	0.68 ± 0.04[a]
B	4.06 ± 0.12 [a]	0.63 ± 0.06 [a]
C	2.31 ± 0.21[b]	0.52 ± 0.11 [a]
D	1.80 ± 0.26[b]	0.50± 0.05[a]
E	9.04 ± 0.53 [c]	0.76 ± 0.03 [a]

Values are mean ± SD. N = 5. Values in the same column bearing different superscripts differ significantly ($P<0.05$).
Key: Group A = 200mg/kg body weight.
Group B = 400mg/kg body weight.
Group C = 600mg/kg body weight.
Group D = 800mg/kg body weight.
Group E = 0.2ml distilled water.

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