GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN ALBINO RATS TREATED WITH AQUEOUS EXTRACT OF FRESH LEAVES OF MORINDA LUCIDA.

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ABSTRACT

In Eastern Nigeria, different parts of Morinda lucida have been successfully used by traditional medicine practitioners in management and treatment of several diseases such as malaria, typhoid fever, dysentery and diabetes. The present research was carried out to investigate the effect of aqueous extract of fresh leaves of Morinda lucida on serum activity of glucose-6-phosphate dehydrogenase in adult male albino rats. A total of twenty five adult male albino rats, used in this research, were distributed into five groups, A, B, C, D and E. Groups B, C, D and E were administered 200, 400, 600 and 800 mg/kg body weight of the extract respectively for seven consecutive days. Group A (the control) was given distilled water only. There was a decrease in physical activities, rates of feed and water intake and body weights of the animals in the treated groups, while the control group did not show any significant change. The activity of glucose-6-phosphate dehydrogenase recorded in the serum of the tests groups was significantly lower (P<0.05) than that of the control. The concentration of total protein of the treated groups did not differ significantly (P>0.05) from the control. These effects were observed to be dose-dependent. The findings of this research indicate that fresh leaves of aqueous extract of Morinda lucida possess chemical compounds which can inhibit the activity of glucose-6-phosphate dehydrogenase. This decrease in the activity of glucose-6-phosphate dehydrogenase may be partly responsible for the use of the plant leaves in the treatment and management of malaria.

Keywords: Glucose-6-phosphate dehydrogenase, Albino rats, Aqueous extract and Morinda lucida.
INTRODUCTION

Treatment with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature dates from the far past. A medicinal plant is a plant which has similar properties as synthetic pharmaceutical drugs. Plants have been used from ancient times to attempt cures for diseases and to relieve physical suffering. Ancient peoples all had acquired some knowledge of medicinal plants. Oftentimes these primitive attempts at medicine were based on superstition and speculation. The progress of medicine has often been guided by the earlier observations and beliefs[1]. These plants include: *Astragalus propinquus* used to strengthen the immune system, *Barberis vulgaris* used in treatment of gastrointestinal ailments, Bitter gourd (*Momordica charantia*) used as an agent to reduce blood glucose level, Bitter leaf (*Vernonia amygdalina*) used to cure dysentery, Black cohosh (*Actaea racemosa*) used in treatment of arthritis and conditions related to menopause cum menstruation, Blessed thistle (*Cnicus benedictus*) used to reverse loss of appetite, Blueberries used as antioxidants in treatment of urinary tract ailments, Chamomille (*Matricaria recutita*) used as a therapeutic agent against sleeplessness, Clove (*Syzygium aromaticum*) used topically to treat a toothache. *Morinda lucida* used as an antimalarial agent [2]. As early as 5,000 B.C. many drugs were in use in China. Sanskrit writings testify to methods of gathering and preparing drugs in these early times [3]. Pharmacology and pharmacognosy owe their beginnings to the earlier beliefs and knowledge about medicinal plants. The interest in medicinal plants was especially pronounced among the early botanists who were often physicians [4].

*Morinda lucida* is a tropical West Africa rainforest tree also called Brimstone tree. The plant can be grown majorly in the most temperate regions of Africa. They grow in a dense forest. It’s very common in grassland, wetland and coastal regions and riparian zones. The plant is a medium-sized tree with a dense crown of slender, cracked branches. It can grow from 2.4 – 18 meters tall, 8cm long bearing at base a stalked cup-shaped gland. Flowers bisexual, heterostylous calyx, 1.5cm long greenish lobes. The branches are often crooked or gnarled. The leaves are broad having deep greenish colour with a bitter taste (Appendix I). It is tolerant of saline soils, drought conditions and therefore, is capable of growing in a wide variety of halafats. *Morinda lucida* is mainly used as an antimalarial drug and anti-diabetic agent and seldomly used in cancer management [5].

Glucose–6-phosphate dehydrogenase (G6PD) is the key enzyme of pentose phosphate pathway that catalyses the conversion of glucose-6-phosphate to 6-phosphogluconate in the presence of NADP⁺ producing reducing power to all cells inform of NADPH. NADPH
enables cells to counterbalance oxidative stress that can be triggered by free radicals. Deficiency leads to haemolytic anaemia and neonatal jaundice which result from the deprivation on the normal level which is about 70-99mg/dl (3.9-5.5mmol/L) [6]. Meanwhile, some factors may affect this range. These factors are: glutathione level, glucose level, disease condition such as malaria and genetic factors [7].

Aim and objective

The research was carried out to investigate the effect of aqueous extract of fresh leaves of Morinda lucida on serum concentration of glucose-6-phosphate dehydrogenase in adult male albino rats.

MATERIALS AND METHODS

METHODS

Collection of Animals and plant leaves.

Twenty-five (25) adult male albino rats were obtained from zoology department, university of Nigeria Nsukka Enugu State, and transported to the animal house, Department of Biochemistry Ebonyi State University, Abakaliki. Leaves of Morindalucida were collected from Abakaliki in Ebonyi State, Nigeria.

Preparation

The leaves, 120g, was weighed and pounded to pulp/paste. The pulp was soaked in 300ml of distilled water for one hour, which was followed by careful decantation.

ANIMAL (RATS) HANDLING AND TREATMENT

Animal Grouping

The 25 adult male albino rats were placed in five cages (A, B, C, D and E). Each containing five rats. All the animals were allowed free access to water and feed (Growers mash) and finally acclimatised for 7 days before administration of sample was commenced.

Measurement of weights

Each animal was weighed consecutively for 7 days and the weight differences monitored.
Administration of Samples to Animals

Doses of 200, 400, 600 and 800mg/kg weight were administered by oral intubation to groups, B, C, D and E respectively for 7 days consecutively.

Collection of Blood from Animals

After seven days of drug administration to the albino rats, the rats were starved for 24 hours and sacrificed under mild anaesthesia using chloroform. Blood samples were collected from the albino rats by cardiac puncture into sterile bottle void of anticoagulant and properly labeled.

Preparation of serum

The blood sample collected from the animals was allowed to clot after which they were centrifuged for ten minutes at 3000rpm and left to stand. The supernatant was collected with the aid of micropipette.

Measurement of Parameters

Determination of Total Protein Concentration

This was achieved by using the lowry’s method [8].

Determination of Glucose–6-phosphate Dehydrogenase Activity

The activity of G-6-PD was measured by the method of [2].

RESULTS

Physical observation

Within the seven days of treatment, there was an obvious decrease in physical activities of the animals as the administration of the aqueous extract of fresh leaves of *Morinda lucida* proceeded. There was also a reduction in feed and water intake by rats in the treated groups while the control does not show an easily noticeable change.
Changes in average Weight of Rats during Seven (7) days of Administration of the Aqueous Extract

The average body weights of the groups are shown in table 1. The result showed that the mean values of the test group reduced compared to that of the control. I.e. There was a significant ($P<0.05$) decrease in average body weights of the albino rats in the groups given the extracts relative to the control. This change in body weights of the test groups varied linearly with the doses.

Table 1: AVERAGE BODY WEIGHTS OF THE RATS (g)

<table>
<thead>
<tr>
<th>No of days of administration</th>
<th>A(g)</th>
<th>B(g)</th>
<th>C(g)</th>
<th>D(g)</th>
<th>E(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>96.40±9.82</td>
<td>88.12±9.42</td>
<td>124.11±9.42</td>
<td>140.06±11.83</td>
<td>112.12±10.61</td>
</tr>
<tr>
<td>2</td>
<td>96.21±9.82</td>
<td>86.24±9.68</td>
<td>119.14±05.46</td>
<td>128.23±11.33</td>
<td>102.16±10.20</td>
</tr>
<tr>
<td>3</td>
<td>98.32±10.99</td>
<td>78.10±10.99</td>
<td>132.08±11.57</td>
<td>116.42±3.28</td>
<td>96.23±10.90</td>
</tr>
<tr>
<td>4</td>
<td>88.11±9.39</td>
<td>75.37±3.70</td>
<td>98.80±10.01</td>
<td>116.16±10.81</td>
<td>83.45±13.46</td>
</tr>
<tr>
<td>5</td>
<td>88.20±5.54</td>
<td>71.14±10.93</td>
<td>88.41±11.46</td>
<td>105.18±12.01</td>
<td>82.50±6.07</td>
</tr>
<tr>
<td>6</td>
<td>86.33±9.92</td>
<td>67.11±4.22</td>
<td>72.44±15.91</td>
<td>97.15±7.50</td>
<td>77.30±12.54</td>
</tr>
<tr>
<td>7</td>
<td>82.25±3.61</td>
<td>93.00±9.67</td>
<td>67.07±6.04</td>
<td>83.11±11.55</td>
<td>64.42±5.78</td>
</tr>
</tbody>
</table>

All values are mean ± standard deviation; n=5
Average Enzyme activity of Glucose-6-phosphate Dehydrogenase and Total protein concentration after Seven (7) days of Administration of Extracts

The results obtained on the parameters are shown in table 2. The concentration of glucose-6-phosphate dehydrogenase and total protein in the serum of rats administered the extract were significantly lower (P<0.05) than those of the control group.

Table 2: Average Enzyme activity of Glucose-6-phosphate Dehydrogenase and Total protein concentration after Seven (7) days of Administration of Extracts

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose-6-phosphate dehydrogenase(U/L)</th>
<th>Protein concentration(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(Control)</td>
<td>9.57±0.97</td>
<td>0.76±0.06</td>
</tr>
<tr>
<td>B</td>
<td>5.70±0.24</td>
<td>0.70±0.05</td>
</tr>
<tr>
<td>C</td>
<td>4.90±0.20</td>
<td>0.74±0.01</td>
</tr>
<tr>
<td>D</td>
<td>3.27±0.24</td>
<td>0.50±0.01</td>
</tr>
<tr>
<td>E</td>
<td>1.87±0.15</td>
<td>0.42±0.02</td>
</tr>
</tbody>
</table>

All values are mean ± standard deviation; n=5.

DISCUSSION AND CONCLUSION

Discussion

During the seven days of administration of the aqueous extract of fresh leaves of *Morinda lucida*. There was an obvious decrease in physical activities of the animals as compared to that of the control. 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. Saponins have the unique ability to stimulate the cell-mediated immune system, as well as to enhance antibody production [9]. The average body weight of groups given the extract decreased throughout the period of administration relative to the control (Table 1). This decrease in body weight may be due to the observed decrease in feed and water intake. Similar observations have been reported by [10].

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 extract at the doses
administered may not have a significant effect on the rate of protein biosynthesis and degradation.

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. 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 decrement in the enzyme activity. This action of the extract may be a contributing factor to the utilisation of leaves by herbal medicine practitioners in treatment/management of malaria and several other diseases. In vitro studies show that the malaria parasite, *Plasmodium falciparum* is inhibited in G6PD deficient erythrocytes. The parasite is very sensitive to oxidative damage and is killed in G6PD deficient erythrocytes. Hence, G6PD deficiency confers malaria resistance to G6PD deficient individuals. Similar results of oxidative stress that is tolerable to a G6PD deficient human host were documented by previous workers on pathological effects on body organs caused by oral administration of artemether and some other anti-malarial agents.

**Conclusion**

From the findings of this research, aqueous extract of fresh leaves of *Morinda lucida* possess chemical constituents that lower the activity of G6PD, therefore can be used in the management/treatment of malaria and related diseases. However, investigation of antimalarial action of the leaves is still in progress in our laboratory.

**REFERENCES**

2. Bishop, C. (2006). Assay of glucose-6 phosphate dehydrogenase (EC 1.1.1.49) and Glucose-6-phosphate dehydrogenase (EC 1.1.1.44) in red cells. *J Lab Clin Med* 68:149,


