

**EFFECT OF AFRICAN POTATO (*Hypoxis hemerocallidea*) EXTRACT ON
OXIDATIVE STRESS INDUCED BY CHLOROQUINE IN ALBINO RATS****Chaturvedi P^{*1} and MP Mwape¹****Padmaja Chaturvedi**

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ABSTRACT

This study evaluated the antioxidant and protective effects of *Hypoxis hemerocallidea* Fisch. & C. A. Mey. (Hypoxidaceae) Corm ('African potato') water extract (HH) against chloroquine induced oxidative stress in albino rats. Chloroquine has been known to generate oxidative stress when used for different types of treatment like malaria, prophylactic treatment and treatment of rheumatoid arthritis. There is also biomedical evidence that African potato corm extracts possess anti-inflammatory, anti-nociceptive, antioxidant and anti-diabetic properties in vivo and in vitro. Twenty five albino rats were used for this study and were divided into five groups; chloroquine control group (CQ) received chloroquine (25 mg/kg every day), normal control group (NC) received distilled water every day, normal experimental group (HYP) received water decoction of corm (10 ml/kg), two experimental groups (E1 and E2) received chloroquine (25 mg/kg) plus 10 ml and 20 ml of extract kg⁻¹ body weight respectively. The experiment was conducted for a period of 30 days after which the rats were killed under ether anaesthesia, blood collected and liver removed for the measurement of thiobarbituric acid reactive substance, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, reduced glutathione, superoxide dismutase, glutathione per-oxidase and tocopherol. The result showed that the water extract maintained the normal non enzymatic and enzymatic anti oxidants status and thus protected the rats in two experimental groups from oxidative stress generated due to chloroquine in dose dependant manner ($P \leq 0.001$). The extract also increased the levels of reduced glutathione and tocopherol in HYP group ($P \leq 0.0001$). It appears that under normal condition, *H. hemerocallidea* acts as booster plant to antioxidant system increasing the levels of GSH and tocopherol. Thus from this study it can be concluded that *H. hemerocallidea* has potentials to protect the organism from oxidative stress generated by chloroquine and also to strengthen the antioxidant system under normal conditions.

Key words: antioxidant, chloroquine, glutathione, superoxide dismutase

INTRODUCTION

Chloroquine is a synthetic aminoquinoline and is widely used in the treatment of malaria [1] and also as a prophylactic drug in the prevention of malaria [2]. It possesses diverse pharmacological activity. During prophylactic treatment, chloroquine affects the enzymatic and non enzymatic antioxidant defense indices. Chloroquine also induces oxidative stress in cells such as, kidney cells and astroglial cells having high metabolic activities [3, 4]. Since it also possesses immuno suppressive potency [5], it is also used for the treatment of inflammation and rheumatoid arthritis [6]. Treatment with chloroquine results in several side effects for example treatment of rheumatoid arthritis with chloroquine, inhibits lymphocyte proliferation, releases enzymes from lysosomes and reactive oxygen species from macrophages [7]. Thus it is clear that chloroquine exerts its toxic effects on the vital organs. Therefore there is a need to supplement the chloroquine treatment with antioxidants that could prevent/inhibit its toxic effect by reducing the oxidative stress. Plants such as *Hypoxis hemerocallidea* are the good options because of their richness in antioxidants [8].

African potato (*Hypoxis hemerocallidea*) is a native plant of South Africa (grass land of Cape, Kwazulu-Natal, Mpumalanga and Gauteng) and also found in Botswana, Swaziland and Lesotho. It is an annual plant and overcome winter condition in the form of an underground root stock called corm. Corms are hard, fleshy, and mucilaginous. In spring, a new set of leaves grows from the apex of the corm. Leaves are arranged one above the other in three rows that radiate outwards. Flowers are bright yellow and star shaped. The African potato corm has been used by traditional healers for various ailments in southern Africa including Botswana in Kumakwane district [9]. In the recent years; it has become a subject of scientific study and is potentially an important breakthrough in the medicinal field [10]. In vitro, antioxidant activity of *H. hemerocallidea* extract was investigated by Nair et al. [9]. Cardio depressant, antihypertensive, hypoglycemic, anti nociceptive, antineoplastic and anti inflammatory effects of *H. hemerocallidea* have been reported by Ojewole et al. [11, 12, 13]. Amongst the chemical constituents present in African potato, medically important constituents are hypoxidiae, sterols, B-sitosterol, stigmasterol and stanol [14].

However, in vivo antioxidant study of *H. hemerocallidea* extract against chloroquine induced oxidative stress has not been yet reported. This study therefore, evaluated the effect African potato (*Hypoxis hemerocallidea*) on oxidative stress induced by chloroquine in albino rats.

MATERIALS AND METHODS

Chemicals

Chloroquine was purchased in Gaborone from Pharmaplus Chemist in syrup form. Diagnostic kits to measure the activities of Serum Glutamate Oxaloacetate Transaminase and Serum Glutamate Pyruvate Transaminase were purchased from

Span Diagnostics, India and other chemicals were bought from Sigma-Aldrich (St. Louis, USA).

Collection of plant material and preparation of African potato extract (HH)

African Potato was purchased from local traditional healers in Gaborone. Authenticity of the plant corm was confirmed from the Botany section of the Biology department, University of Botswana. Water decoction was prepared as described by Shobha Hiremath [15]. The fresh corms were crushed to pulp-like texture and were boiled with water. The ratio of pulp to water was 1: 4. Boiling of the pulp and water mixture was stopped when the volume of water reduced to 1/4th of the original water volume.

Experimental rats

Male albino rats each weighing approximately 250 g were used for all the experiments. All rats were housed in a temperature-controlled environment (25 ± 2 °C) with an alternating cycle of 12 h light and dark. They were fed with a standard rat pelleted diet under the trade name Nola Food, South Africa. They had free access to water and food *ad libitum*. The experiment adhered to the internationally accepted principles for laboratory animal care as observed by Department of Biological Sciences, University of Botswana, Gaborone.

Experimental design

Twenty five rats were used for this experiment and were divided into five groups of five each consisting of normal control group (NC) which were given distilled water; chloroquine control group (CQ) that received chloroquine (25mg/kg body weight); experimental group one (E1) whose members received chloroquine (25mg/kg body weight) and HH (10 ml/kg body weight); experimental group two (E2) that were administered with chloroquine (25mg/kg body weight) and HH (20 ml / kg body weight) and hypoxis control group (HYP) that received only HH (10 ml/kg body weight). All the treatments were given orally.

The experiments were conducted over a period of 30 days after which the rats were treated with ether anesthesia and dissected. Their blood was collected from brachial artery; plasma was separated and frozen. The liver was removed and washed with cold normal saline before freezing. Parameters assayed were thiobarbituric acid reactive substance (TBARS), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), reduced glutathione (GSH), ascorbic acid, tocopherol, super oxide dismutase and glutathione peroxidase in red blood cells (RBC) and in liver.

Biochemical determinations

Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation in plasma was estimated in terms of thiobarbituric acid reactive substance as described by Shushmakumari [16] with little modification. Original method describes the measurement of TBARS in tissue and it **was** adopted for plasma. Plasma or liver homogenate (0.1ml) was treated with 2 ml of Tricarboxylic acid (TCA)-Thiobarbituric acid (TBA) –Hydrochloric acid (HCL) (TBA 0.37%,

0.25N HCl and 15% TCA) reagent (1:1:1) and incubated in boiling water bath for 10 minutes, the mixture was cooled and mixed with 2ml of freshly prepared 1N NaOH and absorbance measured at 535 nm.

Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate oxaloacetate pyruvate transaminase (SGPT)

Diagnostic kits for SGOT and SGPT were bought from Span Diagnostics, India and manufacturer's guidelines were strictly followed.

Reduced glutathione (GSH)

Reduced glutathione was measured by the method described by Ellman [17]. Red blood cell lysate precipitated with metaphosphoric acid (0.25 ml of lysate/liver homogenate) was mixed with 0.5ml of precipitating buffer (1.67 g Metaphosphoric acid, 0.2g EDTA, 30 g sodium chloride dissolved in 100 ml of double distilled water) and centrifuged. Supernatant was collected and mixed with 2.5ml of 0.3 M phosphate buffer. Colour was developed by adding 100 μ l of 0.01% 5, 5-dithiobis 2-nitrobenzoic acid (DTNB) and read at 412 nm.

Super oxide dismutase

Super oxide dismutase was assayed by the method described by Tripathi [18]. Lysate was treated with a mixture of chloroform and ethanol. The reaction mixture consisted of 150 μ l EDTA (0.5 mmol), 600 μ l L-methionine (130 mmol) and 300 μ l NBT (750 μ mol). The volume of reaction mixture was made up 2.8 μ l with SOD buffer. Then 200 μ l of sample (lysate / liver homogenate) was added except in the control. Finally 200 μ l of riboflavin was added to start the reaction. All the test tubes were placed under fluorescent lamp except the blank. Absorbance was recorded at 540nm after for 4 minutes. One unit of enzyme activity was calculated as the activity that was required to inhibit the reduction of NBT by 50%.

Tocopherol (Vitamin-E)

Tocopherol was estimated using the method of Martinek [19]. The sample (0.5 ml), distilled water as blank (0.5ml) and standard solution (0.5ml) were taken in three centrifuge tubes. To all three tubes 0.5ml xylene was added. The tubes were stoppered, mixed and centrifuged. The xylene layer (containing the precipitated tocopherol) was then carefully pipetted into clean tubes and mixed with 0.35 ml ∞ - ∞ ' Dipyrindyl reagent. The extinction of the test and standard against blank were read at 460nm.

In turn, beginning with the blank tube, 0.33ml of ferric chloride solution was added to all the tubes. Optical density of the test and the standard against blank were read at 520nm after 1.5minutes.

Amount of tocopherol present in mg/100 ml was calculated using the formula:
$$\text{mg/100 ml} = \frac{(\text{Reading unknown at 520nm} - \text{Reading unknown at 460nm}) * 0.29}{\text{Reading of standard at 520nm}}$$

Glutathione Peroxidase

Activity of glutathione peroxidase was measured by the method described by Paglia [20]. Into 1 ml of phosphate buffer were added: 200µl EDTA, 200µl GSH, 200µl of sodium azide, 200µl of H₂O₂, 200µl of NADPH, one unit of glutathione reductase and an appropriate amount of the sample (100µl). The decrease in absorbance due to NADPH oxidation was monitored at 340nm for 3 minutes. Glutathione Peroxidase activity was calculated using the extinction coefficient of NADPH ($6.22 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$) and the results were expressed as nmol of NADPH oxidized /min/mg Hb.

Haemoglobin

Haemoglobin content of blood was measured with Sahli haemometer and method described with the instrument was followed. Blood (0.1 ml) was added to N/10 HCl that converts Hb into acid haematin. Brown color of the haematin was matched with brown color of the comparator and calculation was done as described in the method.

Estimation of protein

Protein was measured by the method of Lowry using Folin-Ciocalteu reagent. [21]. One ml of sample was mixed with 3 ml of Lowry's reagent (prepared by mixing 98 ml of 2% sodium bicarbonate solution, 1 ml of 1% copper sulfate solution, and 1 ml of sodium potassium tartrate) and incubated for 10 minutes at room temperature. After incubation, 0.3 ml of Folin-Ciocalteu reagent (diluted with equal volume of water) was added. The color formed was read against blank (1 ml of distilled water) at 670 nm after 30 minutes.

Statistical analysis

Sigma Stat (3.1 version) programme was used to analyse data. Data was subjected to descriptive statistics. Differences among the groups were analyzed using two way ANOVA followed by Holm- Sidak test for comparisons. Data that failed the equal variance test were analyzed by Tukey's test.

RESULTS

Effects of HH on marker parameters of liver damage and lipid peroxidation are presented in Table 1. The levels of SGOT and SGPT were significantly ($P \leq 0.001$) higher in CQ as compared to the levels in HYP, NC and two experimental groups after 30 days of chloroquine administration. The levels of these two transaminases in HYP were also lower than the levels in NC. The results also showed significant ($P \leq 0.001$) increases in the levels of TBARS in liver and RBC as compared to the levels in HYP, NC and E1 & E2.

Effects of HH on enzymatic and non-enzymatic antioxidant in rats subjected to oxidative stress induced by chloroquine are presented in Table 2. Activity of both liver and RBC glutathione peroxidase and superoxide dismutase were significantly ($P \leq 0.001$) reduced in CHQ group as compared to NC, two experimental groups and HYP groups. Non enzymatic antioxidants like reduced glutathione and tocopherol of RBC and liver showed similar response. Close analysis showed that HH also

possesses drug dose response. For example, the levels of plasma GSH in E1 was significantly lower ($P \leq 0.004$) from the levels in E2. Liver GSH did not show significant statistical differences but values for GSH were higher in E2 than E1 (elevated by 8.7 %). Similarly levels of all antioxidants, both enzymatic and non enzymatic were higher in HYP and in some cases it differed significantly ($P \leq 0.001$) for values in experimental and control groups. Red blood cell GSH levels in HYP were significantly higher ($P \leq 0.001$) than levels in E1 and NC. Similarly RBC tocopherol in E1 were significantly ($P \leq 0.001$) lower than levels in HYP. The levels of tocopherol of RBC also showed elevated levels in HYP than in NC (13%).

DISCUSSION

Liver is the main centre for all metabolic activities and it also takes active part in detoxification. Due to this property, it is the most vulnerable organ exposed to toxins, antibiotics, antimalarial drug, analgesics as well as different metabolites which are harmful to the body. Chloroquine is one of the antimalarial drugs. Plasmacidal activity of chloroquine is due to augmentation of oxidative stress in the *Plasmodium* sp. Metabolic activities of this malaria parasite generates ferriprotoporphyrin IX. This compound is detoxified by many glutathione dependent pathways. Chloroquine reduces the availability of reduced glutathione to pathways involved in detoxification process. This leads to auto oxidation and ultimately death of the parasite. Chloroquine also reacts with ferriprotoporphyrin IX and produces highly reactive radicals that generate oxidative stress in hosts. Thus, chloroquine is well known to induce oxidative stress by inducing lipid peroxidation [3, 4]. This is well supported from the present study which showed that the levels of TBARS are significantly elevated in CQ group as compared to other group. Since TBARS are important markers of lipid peroxidation [22], it is evident from this study that the animals of CQ groups were perhaps under oxidative stress because of chloroquine. On the other hand levels of TBARS were significantly ($P \leq 0.001$) low in E1, E2 and in HYP as compared to CQ. This indicates that HH reduced the lipid peroxidation. Values in E1 and E2 were matching closely with NC and they do not differ significantly ($P \leq 0.001$). It is therefore logical to state that HH had relieved and protected E1 and E2 animals from oxidative stress and thus rendered them the normal physiological status in terms of lipid peroxidation.

Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) enzymes are marker parameters of hepatic cell damage. These enzymes are located in hepatic cells and are released after cell damage [23] and hence their high levels in CQ group indicates hepatic cell damage in this group while their low levels in experimental groups indicates that HH maintained the integrity of cell membranes by preventing lipid peroxidation. This is also supported by the fact that the values of TBARS in E1 and E2 were corresponding to those in NC and there was no significant difference between experimental groups (E1 and E2) and NC.

Reduced glutathione, tocopherol and ascorbic acid are non enzymatic antioxidants. Reduced glutathione is a cellular antioxidant and has a central role in reducing

reactive oxygen species. It scavenges free radicals and thus maintains the structural integrity of cell membrane and membrane organelles. It also maintains the levels of ascorbic acid and tocopherol [23, 24, and 25] Vitamin E works synergistically with Vitamin C and they have greater effect when they work together than their individual effects on the antioxidant system. These vitamins reinforce and extend each other's antioxidant activity. Vitamin C attacks free radicals in the cell fluid while vitamin E scavenges dangerous free radicals of the cell membrane [26]. The results showed the depletion of both GSH and tocopherol in CQ group. Depletion in the levels of GSH is possibly due to its over usage in scavenging large amount of free radicals generated due to chloroquine. Low levels of tocopherol might also be due to the same reason. Comparison of results shows that levels of GSH in RBC is significantly ($P \leq 0.05$) high in HYP than NC and E1. Tocopherol levels were also elevated in HYP and E2 as compared to E1 and NC. Elevation of tocopherol might be due to its presence in the plant extract that acts as supplement to the antioxidant system. Thus, these results show that HH is participating well in strengthening the components like GSH and tocopherol of the anti oxidant system.

Super oxide dismutase catalyzes the dismutation of super oxide into oxygen and hydrogen peroxide. Hydrogen peroxide is not reactive to cause chain of lipid peroxidation reaction, but its combination with super oxide radical produces hydroxyl radical which is highly reactive and thus initiates lipid oxidation reactions. Glutathione peroxidase (GPx) catalyses the conversion of hydrogen peroxide to water and non-reactive oxygen species by glutathione and hence protects cells from oxidative damage. Activities of both liver and lysate SOD and GPx were significantly low in CQ indicating oxidative stress by chloroquine. The activity of SOD and GPx of RBC and liver is normal in E1 and E2. Thus it is logical to say that HH has maintained the normal activities of enzymatic anti oxidants like SOD and GPx in experimental groups although the animals were exposed to chloroquine stress.

It appears that the protective effects of HH might be due to its antioxidant properties which are consistent with previous findings reported by others [27, 28]. A study carried out by Steenkamp [29] demonstrated that both water and ethanol extracts can scavenge hydroxyl radicals. Anti oxidant property of African potato is due to hypoxidase which is converted to rooperol in vivo [8]. It appears that combined effects of hypoxidase together with other constituents like stigmaterols help combat the oxidative stress generated by chloroquine by inhibiting lipid per oxidation [30].

CONCLUSION AND RECOMMENDATIONS

African potato protected albino rats from oxidative stress generated by chloroquine and also strengthened the anti-oxidant system by providing supplementation of non enzymatic anti-oxidants. Further study is required to trace the mechanism of action by the components of African potato extract.

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Table 1: Effects of African potato extract (HH) on marker parameters of liver damage against chloroquine induced hepatotoxicity in albino rats

Treatment Groups	CQ	HYP	NC	E1	E2
Parameters					
SGOT (U/L)	56.41 ± 3.67 ^a	18.02 ± 2.34	25.69 ± 3.91	37.13 ± 3.63	31.37 ± 3.29
SGPT (U/L)	81.81 ± 2.22 ^a	40.43 ± 2.97	47.59 ± 1.14	54.21 ± 1.59	44.23 ± 1.49
SerumTBARS (µmol/dl)	1.37 ± 0.02 ^a	0.49 ± 0.01	0.55 ± 0.03	0.71 ± 0.03	0.58 ± 0.01
Liver TBARS (µmol/g of wet Liver tissue)	2.05 ± 0.08 ^a	0.51 ± 0.01	0.59 ± 0.04	0.63 ± 0.03	0.52 ± 0.08

CQ: Chloroquine Control

NC: Normal control

E1: Experimental group 1

E2: Experimental group 2

HYP Normal experimental group

N= 5 in all groups

^a is significant ($P \leq 0.001$) when compared with HYP, NC, E1 & E2

Table 2: Effects of African potato extract (HH) on liver and RBC antioxidants in rats with chloroquine induced hepatotoxicity

Parameters	Treatment Groups				
	CQ	HYP	NC	E1	E2
GSH - Liver (mg/ g of wet tissue)	10.20 ± 0.96 ^a	32.66 ± 2.86	32.66 ± 2.86	23.80 ± 2.91	26.08 ± 4.27
GSH - RBC (mg/ g of Hb)	51.23 ± 2.72 ^a	136.01 ± 2.75	120.13 ± 1.64 ^c	110.11 ± 2.91 ^c	127.62 ± 2.60
SOD - Liver (U/mg of protein)	19.58 ± 1.44 ^a	38.08 ± 1.44	30.07 ± 1.44	28.79 ± 1.53	33.91 ± 3.16
SOD - RBC (U/mg of Hb)	38.17 ± 1.81 ^a	96.57 ± 4.21	94.19 ± 1.87	84.21 ± 2.66	93.62 ± 2.06
GPX - Liver (U/mg of protein)	1.01 ± 0.54 ^a	11.34 ± 0.62	10.10 ± 0.84	9.47 ± 0.67	10.45 ± 1.30
GPX - RBC (U/mg of Hb)	4.74 ± 0.26 ^a	23.46 ± 0.92	18.16 ± 0.48	17.68 ± 0.48	21.34 ± 0.79
Tocopherols - RBC mg/ g of Hb	0.80 ± 0.03 ^a	1.83 ± 0.11	1.59 ± 0.05	1.45 ± 0.36	1.67 ± 0.07
Tocopherols - Liver mg/ g of wet tissue	1.22 ± 0.07 ^a	2.19 ± 0.08 ^a	1.87 ± 0.06	1.75 ± 0.05 ^b	2.05 ± 0.11

CQ: Chloroquine Control

NC: Normal control

E1: Experimental group 1

E2: Experimental group 2

HYP Normal experimental group

N= 5 in all groups

^a is significant ($P \leq 0.001$) when compared with HYP, NC, E1 & E2

^a is significant ($P \leq 0.0001$) when CHQ compared with NC, E1 & E2, HYP and HYP compared with NC

^b is significant ($P = 0.001$) when E2 compared with E1

^c is significant ($P = 0.001$) when HYP compared with NC and E1

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