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Full Length Research Paper

Effect of methanolic extract of *Tulbaghia violacea* rhizomes on antioxidant enzymes and lipid profile in normal rats.

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Most human diseases such as atherosclerosis, aging, arthritis, cancer and diabetics are believed to be associated with increased free radicals generation and oxidative stress. *Tulbaghia violacea* is one of the commonly used plants in the management of free radicals related diseases. To investigate the effects of daily intake of methanolic extract of rhizomes of *T. violacea* on antioxidant enzymes and lipid profiles, 24 rats were randomly divided into four groups and administered with three doses (125, 250 and 500 mg/kg body weight) of the extracts with saline as a control. After 28 days, plasma concentration of catalase, superoxide dismutase, glutathione peroxidase, thiobarbituric acid reactive substance (TBARS), triglyceride (TG), cholesterol, low density lipoprotein (LDL)-cholesterol and HDL-cholesterol were measured. A significant ($p < 0.05$) dose dependent increase in superoxide dismutase (SOD), catalase and glutathione peroxidase activities were observed in extract treated groups when compared with the control. TBARS, a marker of lipid peroxidation, was significantly lower in extract treated groups compared with the control. The extract also demonstrates a dose dependents hypolipidemic activity as it reduced plasma cholesterol, triglyceride, LDL-C; very low density lipoprotein (VLDL) and atherogenic index in normal rats. The results of this study suggested that oral intake of methanolic extract of rhizomes of *T. violacea* may enhance the status of antioxidant defence enzymes, HDL-cholesterol and decrease serum concentration of malondialdehyde, Hence, the extract may reduce the risk of oxidative induce diseases.

Key words: *Tulbaghia violacea*, rhizomes, antioxidant enzymes, free radicals, lipid.

INTRODUCTION

Free radical stress has been implicated in the etiopathology of many human diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging (Satlisha et al., 2011). Free radicals are highly reactive molecules derived from the metabolism of oxygen (Temraz and EL-Tantawy, 2008). Some of them play a positive role in biochemical (energy production), immunological (phagocytosis) and physiological (regulation of cell growth and intercellular signalling) processes. However, when they are produced in excess and cannot be destroyed, their accumulation in the body generates a phenomenon called oxidative stress (Pham-Huy et al., 2008). Inability to destroy or removed excess free radicals has been attributed to many reasons such as decreases in antioxidant endogenous enzymes (superoxide dismutase, glutathione peroxidase and catalase) synthesis or activities and reduction in non-enzymatic protection (α-tocopherol, ascorbic acid, β-carotene, and uric acid) (Lien et al., 2008). Excessive free radicals that are generated are capable of reacting with unsaturated lipids thereby initiating self-perpetuating chain reactions of lipid peroxidation in the membranes (Salvemini and Cuzzocrea, 2003). Free radicals also known as reacting oxygen species can also cause oxidation of sulphydryl groups inproteins and strand scission in nucleic acids (Kaul et al., 1993).

Research evidence has showed that a potent scavenger of these free radical species may serve as a possible preventive intervention for free radical mediated

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diseases (Alluri et al., 2009).

Recent studies revealed that a number of plant extracts possess potent antioxidant activity (Krishnamoorthy et al., 2007). Rhizome of T*ulbaghia violacea* Harv. is a valuable medicinal plant used in Eastern Cape of South Africa for the treatment of heart diseases and several human ailments (Olorunnisola et al., 2011; Bungu et al., 2006). Aside, pharmacological activities such as anthelmintic and anticancer properties (Bungu et al., 2006; Duncan et al., 1999; McGaw et al., 2000). Olorunnisola et al. (2011) reported that dry and fresh extract of rhizomes of *T. violacea* possess a strong *in vitro* antioxidant properties which is comparable to garlic extract. This property then suggested that extract of rhizomes of *T. violacea* may be employed in the treatment of free radical mediated diseases. The objective of this study is to assess the effect of the extract of rhizomes of *T. violacea* on antioxidant enzymes, lipid profile and serum electrolytes in normal albino Wistar rats.

MATERIALS AND METHODS

Plant collection

Whole fresh rhizomes of *T. violacea* Harv. was collected from Alice, Eastern Cape, South Africa. They were collected in April, 2011 and authenticated by Professor D. S. Grierson of Botany Department, University of Fort Hare and was deposited (Sin 2010/2) at the Giffen Herbarium (Olorunnisola et al., 2011).

Preparation and extraction of plant materials

327.4 g of chopped *T. violacea* rhizome was homogenized in a blender with 1.6 L of 100% methanol at 4°C. The crude extracts were incubated at 37°C for 15 min, followed by centrifugation at 1500 × g for 10 min at 4°C (Mohammad and Woodward, 1986). The supernatant was filtered using Whatman No. 1 filter paper and was concentrated under *vacuo* at 65°C using rotary evaporator. Since the rhizomes are fresh; the extract did not dry completely (Olorunnisola et al., 2011).

Animals

Twenty- four female albino Wistar rats 6 weeks Old (135 to 155)g were maintained under a constant 12-h dark/light cycle at an environmental temperature of 22 ± 2°C at University of Fort Hare , Central Animal house, housed in stainless-steel cages and fed a standard laboratory diet with water provided *ad libitum*. The body weights were measured once a week during the 28 days study period. All procedures used in the present study followed the “Principles of Laboratory Animal Care” from NIH Publication No.85-23 and were approved by the Animal Ethics Committee of University of Fort Hare.

Experimental design

A total of 24 rats were divided into four of six rats per group. Group 1 receive 125 mg/kg/body weight, group 2 receive 250 mg/kg/body weight and group 3 was given 500 mg/kg per body weight. All rats were treated once daily by intragastric tube. After 28 days blood was collected into EDTA tubes and the plasma separated by centrifuging at 1000 g for 15 min. Superoxide dismutase, catalase, malondialdehyde and glutathione peroxidase were determine in plasma.

Estimation of superoxide dismutase (SOD) activity

The activity of SOD was assayed following the method originally developed by Nishikimi et al. (1972) and then modified by Kakkar et al. (1984). The sample containing 5 µg proteins was mixed with sodium pyrophosphate buffer, phenazinemethosulphate (PMT) and nitrobluetetrazolium (NBT). The reaction was started by the addition of NADH. Reaction mixture was then incubated at 30°C for 90 s and stopped by the addition of 1 ml of glacial acetic acid. The absorbance of the chromogen formed was measured at 560 nm. One unit of SOD activity is defined, as the enzyme concentration required inhibiting chromogen production by 50% in 1 min under the assay condition.

Estimation of catalyse activity

Catalase was assayed colorimetrically at 620 nm and expressed as moles of H_2O_2 consumed/ min/ mg protein as described by Sinha, (1972). The reaction mixture 1.5 ml contained 1.0 ml of 0.01 M pH 7.0 phosphate buffer, 0.1 ml of serum and 0.4 ml of 2 M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acid were mixed in 1:3 ratio).

Estimation of glutathione peroxidase

Glutathione peroxidase was measured by the method described by Rotruck et al. (1973). To 0.2 ml Tris buffer, 0.2 ml of ethylene diaminetetraacetic acid (EDTA), 0.1 ml of sodium azide and 0.5 ml of serum (Tris buffer 0.4 M, pH 7.0) were added. To the mixture, 0.2 ml of glutathione (GSH) followed by 0.1 ml of H_2O_2 was added. The contents were mixed well and incubated at 37°C for 10 min, along with a control containing all reagents except serum. After 10 min, the reaction was arrested by the addition of 0.5 ml of 10% trichloroaceticacid (TCA) and centrifuged. The activity was expressed as mg of GSH consumed/ min/ mg protein.

Estimation of thiobarbituric acid reacting substances (TBARS)

The level of TBARS in plasma were estimated by measuring malondialdehyde and TBARS reactivity with thiobarbituric acid (TBA) to generate a pink colourchromophere, which was read at 535 nm. The transmissions were measured by calorimeter and expressed in terms of mM/ 100 g wet tissue.

Estimation of lipid profile

The plasma samples were analysed for total cholesterol, low density lipoprotein (LDL)- cholesterol, (high density lipoprotein) HDL-cholesterol and triglycerides using sigmadiagnostic assay kit.

Data analysis statistical analysis

Data were expressed as mean ± SD of six replicates and were subjected to one way analysis of variance (ANOVA) followed by Duncan multiple range tests to determine significant differences in all the parameters. Values were considered statistically significant at $p < 0.05$.

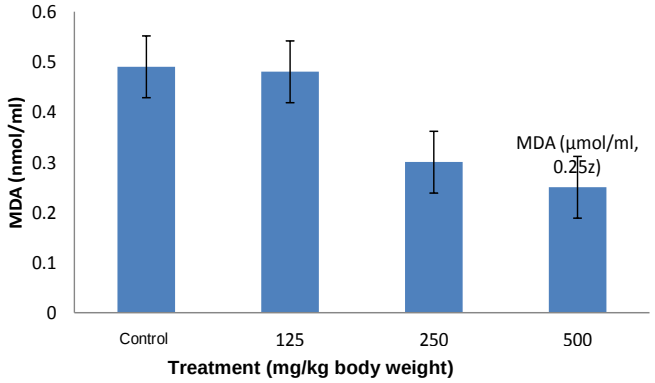

Figure 1. Malondialdehyde (MDA) levels in the plasma of rats 28 days after administration of methanolic extract of rhizomes of *T. violacea*. The data are expressed as the mean ± D of 6 animals.

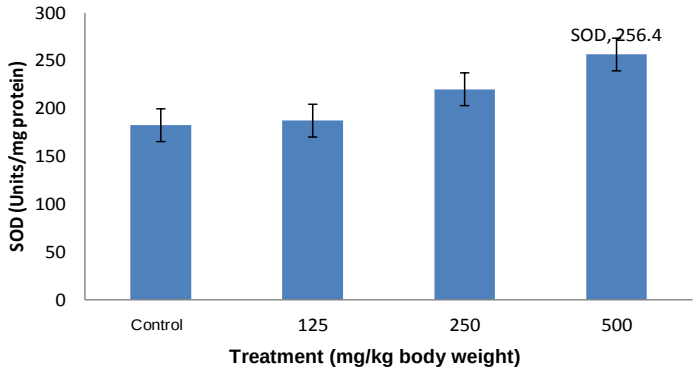

Figure 2. Superoxide dismutase (SOD) levels in the plasma of rats 28 days after administration of methanolic extract of rhizomes of *T. violacea*. The data are expressed as the mean ± D of 6 animals.

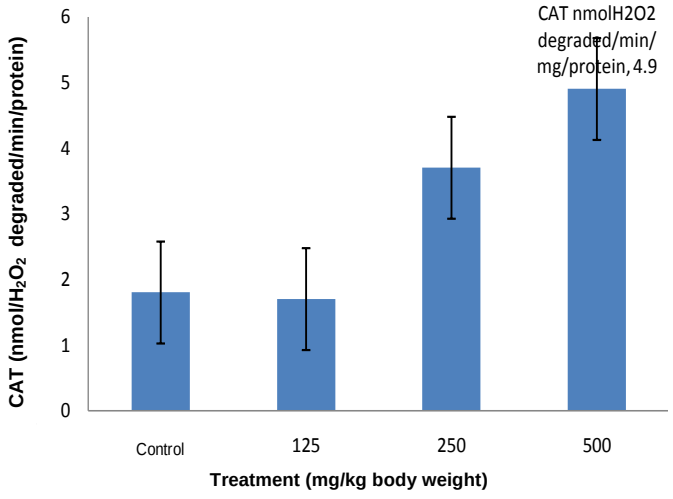

Figure 3. Catalase (CAT) levels in the plasma of rats 28 days after administration of methanolic extract of rhizomes of *T. violacea*. The data are expressed as the mean ± D of 6 animals.

RESULTS AND DISCUSSION

Effect of the extract on lipid peroxidation

Diminished antioxidants system occasioned by increased free radicals generation during normal metabolic functions such as respiration, digestion, immune response and growthor introduced from the environment (Nagavani et al., 2010) have been reported to play an important role in the induction of oxidative stress (Prasanna and Purnima, 2011) and development of various human diseases such as ischemia, anaemia, asthma, arthritis, inflammation, heart diseases, Parkinson's diseases, mongolism, ageing process and perhapsdementias (Prasanna and Purnima, 2011). Hence, the use of antioxidant as supplement was recommended as a possible remedy in the control of the aforementioned diseases (Ayoola et al., 2011). In the present study, the effect of extract of *T. violacea* rhizome on lipid peroxidation in normal rats was analysed and the results revealed that it reduces TBARS generation. Daily administration of *T. violacea* rhizome extract significantly ($p < 0.05$) reduced plasma TBARS generation in dose dependant manner in treated groups compared to untreated normal group (Figure 1). Similar observation as also been reported for garlic extract (Noori et al., 2012). Increased TBARS is frequently used as index of lipid peroxidation (Yassa et al., 2008; Prasanna and Purnima, 2011) and is considered a valuable indicator of oxidative damage of cellular components (Morales et al., 2004). The significant reduction in the level of TBARS in the plasma of extract treated groups might be due to polyphenolic compoundspresent in the extract (Olorunnisola et al., 2011) and/or elevation of antioxidant defense enzymes activity. The implication of this is that plant extract could reduce the generation of free radicals or increase free radical scavenging mechanisms.

Effect of the extract on antioxidant enzymes

Figures 2 to 4 showed the effect of the plant extract on antioxidant enzymes SOD, CAT and GPx, respectively. The results revealed significantly low enzyme activities in saline treated group (control) when compared to the extract treated groups. Superoxide dismutase (SOD) and Catalase (CAT) are the major enzymes involved with detoxification of reactive oxygen species in most cells (Sivaraj et al., 2011). SOD catalyses the conversion of superoxide anions into hydrogen peroxide while catalase and glutathione peroxidase (GPx) detoxify H_20_2 and lipid peroxide to non-toxic alcohol (Anbazhagan and Chellappan, 2009). The decreased levels of antioxidant activities may be due, in part, to oxidative modification of the enzymatic proteins by excessive ROS generation or may stem from decrease in their rate of synthesis (Sivaraj et al., 2011).

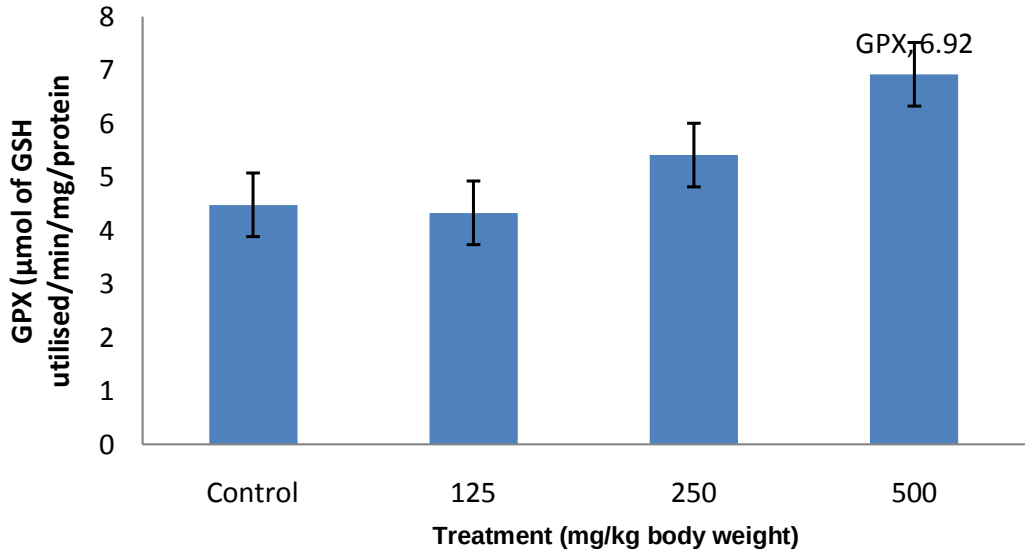

Figure 4. Glutathione peroxidase (GP_X) levels in the plasma of rats 28 days after administration of methanolic extract of rhizomes of *T. violacea*. The data are expressed as the mean ± D of 6 animals.

Table 1. Concentrations of lipids in plasma.

	Plasma concentration (mg/dl)					
Treatment	**TCHOL**	**TG**	**LDL-cholesterol**	**VLDL**	**HDL- cholesterol**	**TG/HDL**
Control	110.1 ± 0.25	98.21 ± 0.81	23.21 ± 0.62	20.51 ± 0.32	47.56 ± 0.23	1.6
125	108.1 ± 0.42	97.65 ± 0.12	22.51 ± 0.52	19.55 ± 0.51	52.76 ± 0.10*	1.5
250	100.4 ± 0.33	95.82 ± 0.49	19.78 ± 0.66*	18.93 ± 0.49*	58.18 ± 0.97*	1.5
500	95.9 ± 0.13*	84.14 ± 0.91*	15.05 ± 0.17*	17.89 ± 0.75*	63.51± 0.7 1*	1.5

TCHOL, total cholesterol; TG, triglyceride. Results are mean ± SD *, $p < 0.05$ significantly difference from control.

While the observed dose dependent increased in the antioxidant enzymes status in the extract treated groups may be due to enhancement of antioxidant enzyme synthesis by acting on the antioxidant response elements in the enhancer region at the promoter site of the gene that codes for the enzymes (Ayoola et al., 2011).

Effect of the *T. violacea* on lipid profile

Derangements in metabolism and oxidation of lipid molecules have been implicated in the etiopathogenesis and progression of human diseases (Juan et al., 2007). Raised serum lipid levels, particularly of cholesterol along with generation of reactive oxygen species (ROS) play a key role in the development of coronary artery disease and atherosclerosis (Ratheesh et al., 2011). Yakubu and Afolayan (2009) reported that elevated levels of all lipids except the HDL-C are associated with increased risk of atherosclerosis. Also, several studies have reported that oxidation of LDL promote vascular dysfunction, enhance the production of inflammatory mediators and contribute to initiation and progression of heart disease (Ginter and Simko, 2010). There is now overwhelming evidence that herbal drugs may be helpful in the treatment and control of hyperlipidemia and this may translate directly or indirectly to the management of cardiovascular diseases. In the present study, oral administration of methanol extract of rhizomes of *T. violacea* (125, 250 and 500 mg/kg body weight) showed dose dependents hypolipidemic activity. It reduced plasma cholesterol, triglyceride, LDL-C, very low density lipoprotein (VLDL) and atherogenic index in normal rats (Table 1). This observation is consistence with hypolipidemic activities of other member of Alliaceae family such as *Allium sativum* and *Allium tuberosum* (Raghuveer, 2008). The mechanism(s) of hypolipidemic activity of *T. violacea* is unknown but it may be due to direct activating effect on Lipoprotein lipase a vital enzyme in the metabolism of triglyceride or prevention of production of cholesterol in the liver by blocking HMG-CoA reductase or increase level of HDL. It could also be due to presence of

phytochemicals such as organosulphur compounds (Kubec et al., 1999; Olorunnisola et al., 2011). In addition, to hypolipidemic activity, *T. violacea* also cause a significant ($p < 0.05$) dose dependent increased in levels of high density lipoproteins (HDL). High density lipoprotein (HDL) commonly refer to as 'good cholesterol' possess the ability to reverse cholesterol transport and also protect LDL from oxidation, thereby minimizing the deleterious consequences of LDL oxidation (Bonnefont-Rousselot et al., 1999). The results imply that *T. violacea* extract may possess beneficial effect (s) by reducing plasma lipid profiles.

In conclusion, this results suggested that extract of *T. violacea* rhizome extract may be useful in protection against oxidative stress induce diseases. Further research work is currently going on in our laboratory to investigate the effect of *T. violacea* in diet induced hypercholesterolemic rats.

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