ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(1):157-161

ORIGINAL RESEARCH ARTICLE

Amelioration of *in-vivo* Antioxidant Activity by Banana Extracts

Ashish Baldi*[1], Manish K Pandit[2] and Payal Ranka[2]

[1]*Mandsaur Institute of Pharmacy ,Rewas-Dewda Road, Mandsaur-458001(M.P), India*
[2]*Dept of Life Science, Mandsaur Institute of Science and Technology, Mandsaur-458001(M.P), India*

Received 05 Oct 2011; Revised 11 Dec 2011; Accepted 18 Dec 2011

ABSTRACT

Antioxidants have attracted immense interest of researchers because of their implied role in the protection of biological system. Antioxidants are produced in body and can be sequestered from fruits, vegetables or other natural sources. Phytochemicals such as polyphenols and carotenoids are gaining importance because of their contribution to human health and their multiple biological effects such as antioxidant, antimutagenic, anticarcinogenic, and cytoprotective activities and their therapeutic properties. Banana peel is a major by-product in pulp industry and it contains various bioactive compounds like polyphenols, carotenoids, and others. The present study was undertaken to observe the free radical scavenging property and antioxidant potential *in-vivo* of two different parts of banana. Results of the study revealed free radical scavenging activity of alcoholic extract of banana as marked by significant decline in levels of lipid peroxidation (LPO). Present study indicates promising antioxidant potentials of alcoholic extracts of banana as manifested by elevation of reduced glutathione (GSH) content. The findings of present investigation suggest that the unripe banana extracts had higher antioxidant potency than ripe one.

Key words: Antioxidants, Glutathione, Lipid peroxidation, Phytochemicals

INTRODUCTION

Oxygen is an essential element for life to perform biological functions such as catabolism of proteins and carbohydrates in order to generate energy for growth and other activities. However a parallel role of oxygen as a toxic agent for living tissues has also been discovered. Oxygen, though not dangerous by itself, is involved in generation of various kinds of "Reactive Oxygen Species" (ROS). ROS, formed during metabolism or trough the action of ionizing radiation can interact with biomolecules and ultimately lead to an onset of degenerative disease such as cancers, cardiovascular disease (CVD) and other illness.

To protect against the destruction caused by free radicals, nature has created an antioxidant defense system composed of group of compounds and enzyme potent enough to remove free radicals before they cause tissue damage. Some antioxidants are produced in the body, while others must be sequestered from the diet or through supplementation. Most citrus and dried fruits, cruciferous vegetables, garlic, onions, carrots, tomatoes, sweet potatoes, sesame and olive oil are rich source of antioxidants. There are several naturally occurring and synthetic antioxidants known. These antioxidants belong to different classes of compounds, such as carotenoids, polyphenolics, polyamines, gallic acid derivatives, tannins and catechins. Examples include phytic acid, lipoic acid, bilirubin, melatonin, quercetin, carnosol, carnosic acid, hydroxytyrosol, rutin, butylated hydroxyanisole, and butylated hydroxy toluene. Vitamins E and C are among the most effective antioxidants with preventive effect against heart disease and cancers[14]. Flavonoids, widespread in plant tissues are classical antioxidants in treatment of various disorders including diabetes and neurodegenerative disorders. Grape seed extract is used in treatment of diabetic kidney disease. Garlic and high dose of vitamin E have been suggested to be beneficial for lowering cholesterol and atherosclerosis. Reports indicate strong antioxidant activity in gallocatechin isolated from commercial banana Musa Cavendish [12]. Antioxidant property of methanolic crude extracts of some commonly used medicinal plants for their free radical scavenging properties using ascorbic acid as standard antioxidant [5]. There is significant evidence that plant antioxidant might reduce the symptoms of oxidative stress mediated tissue

***Corresponding Author:** Dr.Ashish Baldi**, Email:** baldiashish@gmail.com

injury. Therefore, the present study was designed to evaluate the *in-vivo* antioxidant property of banana extract.

MATERIALS AND METHODS

a) Preparation of extracts:

Ripe and Unripe banana fruit were collected from local fruit market of Mandsaur (M.P.) during the month of February 2011. Peel and pulp of ripe and unripe banana were dried in shade and powdered followed by extraction with soxhlet apparatus. Extraction was done with alcohol at 40^{o}C for 15 hrs and % yield was calculated for each extracts after drying under water bath. Qualitative chemical evaluation of the obtained extract was done to detect various phytochemicals [6].

b) Dose preparation:

The amount of 250 µg/ml of alcoholic extracts of both ripe and unripe peel and pulp of banana extracts were given orally to experimental animal,

c) Group assignments:

To observe the response of plants extract in prevention of oxidative stress in streptozotocin induced mice, animals were divide in six groups. The first group of animals was treated with streptozotocin (50mg/kg bw). Second group of animals was treated with streptozotocin and alcoholic extract of unripe banana peel (250 µg/ml). Third group of animals was treated with was treated with streptozotocin and alcoholic extract of ripe banana peel (250 µg/ml). Fourth and fifth groups of animals were treated with streptozotocin and alcoholic extract of unripe banana pulp and ripe banana pulp (250 µg/ml) respectively. One group was kept as control and treated with normal saline.

d) Tissue preparation:

Animals were anesthetized and blood was collected from various routes. Serum was separated from blood for measuring oxidative stress and antioxidant activity.

e) Hydrogen peroxide scavenging method

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch (1989). A solution of hydrogen peroxide was prepared in phosphate buffer. The concentration of hydrogen peroxide was determined by absorption at 230 nm. Extracts in distilled water were added to a hydrogen peroxide solution. The absorbance of hydrogen peroxide at 230nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds were calculated.

f) Phosphomolybdenum Method:

Antioxidant activity of both extracts of peel and pulp were performed using phosphomolybdenum method. The antioxidant activity is based on the reduction of Mo(IV) to Mo(V) by the test sample and the subsequent formation of green phosphate/Mo(V) complex at acidic pH. An aliquot of 0.1ml sample was mixed with 1 ml of reagent solution. The tubes were incubated at 95^{o}C for 90 minutes and cooled at 27±2°C. The absorbance was measured at 695nm. The antioxidant activity of samples was expressed as mM of ascorbic acid equivalent/gm of sample.

g) *In-vivo* study:

In-vivo antioxidant status was estimated by measuring reduced glutathione content in serum and oxidative stress was measured by serum malonaldehyde (MDA) content.

Measurement of lipid peroxidation was done by measuring the levels of thiobarbaturic acid reactive substance (TBARS) by the method of Ohkawa et al., (1979). Reduced glutathione content (GSH) was estimated by the method of Jollow et al., (1974).

RESULTS

a) Hydrogen Peroxide Scavenging Method

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with $Fe^{2+,}$ and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. The antioxidant activity of banana extract as hydrogen peroxide scavenger is summarized as (**Table 1**).

Table 1: Hydrogen peroxide scavenging activity of banana extract

S. No.	Sample	Scavenging capacity (%) at concentration				
		50 µg /ml	100 µg /ml	150 µg /ml	200 µg /ml	250 µg /ml
1	Unripe peel extract	16.92	23.75	32.82	45.27	51.25
2	Unripe pulp extract	21.25	30.15	42.65	47.20	58.50
3	Ripe peel extract	2.82	11.27	22.11	31.15	42.15
4	Ripe pulp extract	16.87	25.37	38.10	48.42	53.20
5	Standard (Ascorbic Acid)	14.20	25.20	38.20	51.20	59.2

As shown in Table 1, alcoholic extract of unripe pulp had shown almost equal scavenging capacity to that of standard used in the study i.e. ascorbic acid. The antioxidant capacity of unripe pulp extract was found highest in comparison of other extracts at all concentration tested. Scavenging of

hydrogen peroxide by plant extract may be attributed to their phenolic content, as determined by phytochemical screening, which could donate electrons to hydrogen peroxide, thus neutralizing it to water.

b)Total Antioxidant Capacity by Phosphomolybdenum Method

The antioxidant capacity of the fraction was measured spectrophotometrically through phosphomolybdenum method, which was based on the reduction of Mo(4) to Mo(5) by the mole analyte and the subsequent formation of green phosphate compounds with the maximum absorbance at 695 nm. Antioxidant capacity of extract was found in the following order unripe pulp > ripe pulp > unripe peel > ripe peel. The results are given in (**Table 2**)

Table 4: Antioxidant activity of banana extracts by phosphomolybdenum method

S.No	Sample @ 250 µg/ml concentration	Total antioxidant capacity (mg of ascorbic acid/gm of extract)
1	Ripe peel extract	530
2	Ripe pulp extract	625
3	Unripe peel extract	533
4	Unripe pulp extract	680

***In-vivo* studies:**

Treatments with streptozotocin and banana extracts were found to alter lipid peroxidation (LPO) levels in serum. Administration of streptozotocin had resulted in marked increase in lipid peroxidation, observed as malonaldehyde content. Significant decline in LPO was seen upon treatment with alcoholic extracts of banana irrespective of plant part used however degree of reduction was highest when extract of unripe peel was administered at a dose of 250 µg/ml. The results are summarized as (**Fig 1**).

Treatment of streptozotocin also resulted in significant decline of glutathione content (GSH) in comparison to control animals. GSH content was found to be restored upon administration of banana extracts. Amelioration of invivo antioxidant activity by banana extracts was extablished by significant ($p<0.05$) increase in GSH content. The results are given in (**Fig 2**).

Results of present study indicated promising effect of banana extract in improving antioxidant status against streptozotocin induced oxidative stress.

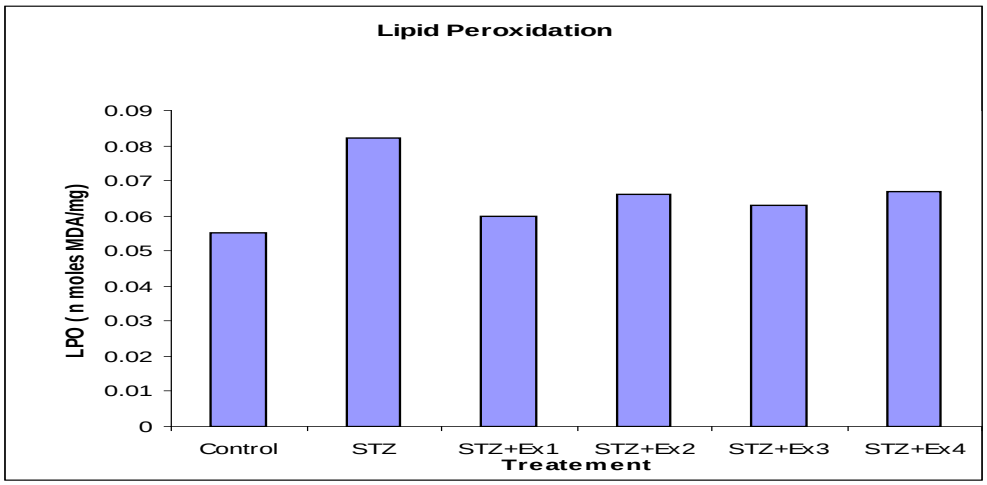

Fig 1: Lipid peroxidation (LPO) levels on treatment with streptozotocin (STZ) and banana extracts (Ex). Ex1 = alcoholic extract of unripe peel and Ex2 = alcoholic extract of ripe peel. Ex3 = alcoholic extract of unripe pulp and Ex4 = alcoholic extract of ripe pulp.

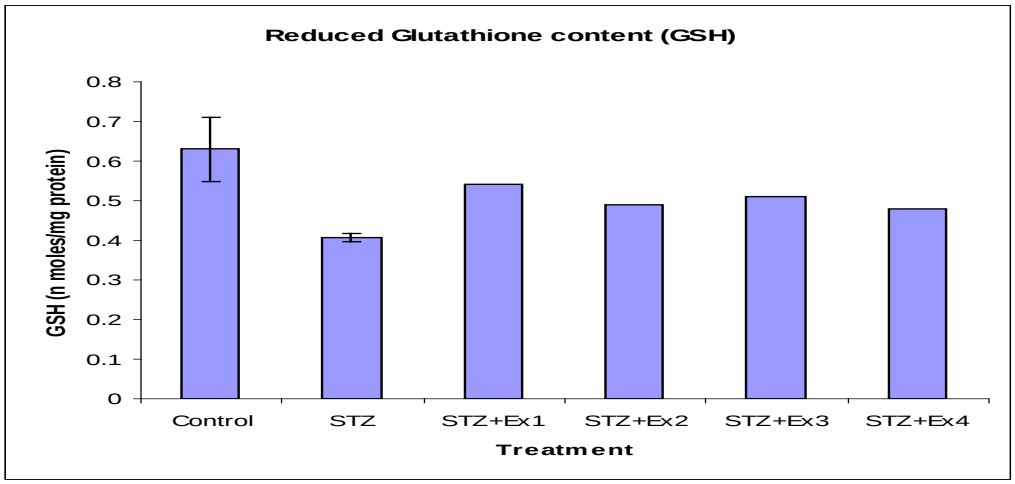

Fig 2: Showing Reduced Glutathione content (GSH) on treatment with streptozotocin (STZ) and banana extracts (Ex). Ex1 = alcoholic extract of unripe peel and Ex2 = alcoholic extract of ripe peel. Ex3 = alcoholic extract of unripe pulp and Ex4 = alcoholic extract of ripe pulp.

DISCUSSION

Oxidation is one of the most important chemical reactions involved in various metabolic activities in living organisms. However during these biological processes, oxygen centered free radicals and ROS are continuously produced, which may cause tissue damage and even cell death. Oxidative stress appears to be the basis of a wide array of physiological aberrations in mammals including carcinogenesis, ischemic reperfusion injury, inflammation etc[5]. There is substantial evidence that free radicals induced oxidative damage play a significant role in the progression of various diseases. Oxidative stress can produce major interrelated derangements of cell metabolism, including DNA strand breakage, rise in intracellular free Ca^{2+}, damage to membrane ion transport and other specific proteins and peroxidations of lipids [4].

To protect cell and organs from the oxidative stress induced by ROS, living organisms have evolved with an extremely efficient and highly sophisticated protective system, the so called "antioxidant defensive system". It involves a variety of components, both endogenous and exogenous in origin. These components function interactively and synergistically to neutralize free radicals. It is also reported that dietary intake of antioxidant – rich food/herbs decreases the incidence of a number of human disorders. Hence research on evaluation and establishment of antioxidant potential has increased in recent times. Furthermore many methods for estimation of this bioactivity had reported. Studies have indicated antioxidant property of banana flower [8] . Studies with plantain banana (*Musa sapientum* var. paradisiaca) have indicated its ulcer protective and healing activities through its predominant effect on various mucosal defensive factors[11].In present study hydrogen peroxide scavenging activity and total antioxidant activity assay were used for establishment of *in-vitro* antioxidant potential of banana in comparison to ascorbic acid as standard compound. Also *in-vivo* antioxidant activity of extracts of unripe and ripe banana peel and pulp was measured.

Oxidative stress has been reported to be the major cause of pathogenesis in various diseases. Oxidative stress causes tissue damage and injury to all molecular targets in DNA, proteins and lipids, thus causing cell death. In biological systems LPO has been known to be responsible for various normal and pathological phenomenon. Biological membranes are particularly susceptible to LPO owing to their fatty acid. The aim of the present study was to observe the effect of extracts of different part of banana against strptozotocin induced oxidative stress. The present study shows increased levels of LPO after treatment with streptozotocin. The increased level of LPO is due to oxidative stress produced as a result for formation of reactive oxygen species. Production of ROS also effected the antioxidant status by reducing reduced glutathione (GSH) content.

In living system, varieties of antioxidant mechanisms play an important role in combating ROS. Few of the mechanisms are free radical scavenging, complexation of pro-oxidant metals, reduction and quenching of singlet oxygen formation. The antioxidants may also act by up-regulating the expressions of genes encoding the enzymes such as superoxide dismutase (SOD), catalase or glutathione peroxidase and thus increasing the levels of endogenous defenses. Studies have shown that free radicals scavengers are useful in protecting the tissue against ROS. The availability of ROS to initiate lipid peroxidation is largely dependent upon cellular antioxidant. Reduced glutathione and enzymes associated with its metabolism provide a major defense against ROS induced cellular damage [2]. Glutathione (GSH) is the most abundant intracellular non protein thiol that functions as antioxidants to detoxify reactive oxygen metabolites of endogenous or exogenous origins. GSH is present in high concentrations in tissues [11] and normal cellular homeostasis is maintained via de novo synthesis from sulfur containing precursor amino acid cystein and methionine and regeneration of glutathione disulphide (GSSH). The involvement of ROS in cellular regulatory and cytotoxic process implies that the cellular redox environment plays a major role in their action. The outcome depend on the concentration of ROS and availability of reducing equivalent, both intra and extra cellular.

The present study demonstrated that GSH content was declined significantly with the treatment of streptozotocin, which is due to induction of free radical induced oxidative stress. However supplementation of alcoholic extract of ripe and unripe banana peel and pulp produced and increase in GSH level of serum. The results suggested that the extracts may be helpful in maintaining the reducing environment in serum and may prevent from oxidative damage.

CONCLUSION

The results of this study revealed that banana extracts had shown comparable antioxidant and free radical scavenging capacity with ascorbic acid. The plant extracts were effective in reducing the lipid peroxidation and elevating the GSH in mice induced by streptozoticin *in-vivo*. The present investigation reveals beneficial effect of different part of banana in reducing oxidative stress and improving antioxidant levels. Hence, regular use of banana in the diet may provide beneficial effects associated with antioxidants in the body.

REFERENCES

1. Archana Borah, R. N. S. Yadav and B. G. Unni. *In Vitro* Antioxidant And Free Radical Scavenging Activity Of *Alternanthera sessilis*. *International Journal of Pharmaceutical Science and Research*. Vol. 2(6): 1502-1506. 2011
2. Cardozo-Pelaez F, Brooks PJ Stedeford T, Song S and Sanchez-Ramos **J**. DNA damage, repair, and antioxidant systems in brain regions: a correlative study. *Free Radic Biol Med.*, 28(5): 779-85, 2000 *Experimental Biology*, 1, 57-61. 2005
3. Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? *The Lancet*, 344: 721-724. 1994
4. Jollow DJ, Mitchell JR, Zampaglione N and Gillete JR. Bromobenzene-induced liver necrosis, Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*, 11: 151-169. 1974
5. Kappus, H.,. Overview of enzyme systems involved in bio-reduction of drugs and in redox cycling. Biochem. Pharmacol., 35: 1-6. 1986
6. Khalaf NA, Shakya AK, Al-othman A, Ahbar Z, Farah H. Antioxdidant activity of some common plants, Turk. J. Biol., 31: 1-5. 2007
7. Kokate, C. K., A. P. Purohit and S. B. Gokhale.,. Pharmacognosy, 3rd edition, Nirali Prakashan, Pune. 1995
8. Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*., 95: 351-358, 1979
9. Pari, L., Umamaheshwari, J.,. Antihyperglycemic activity of *Musa sapientum* flowers: effect on lipid peroxidation in alloxan diabetic rats. Phytotherapy Research 14, 136-138. 2000
10. R. J. Ruch, S. J. Cheng, and J. E. Klaunig. "Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea," *Carcinogenesis*, vol. 10, no. 6, pp. 1003–1008, 1989.
11. R.K. Goel, K. Sairam. Anti-ulcer Drugs from Indigenous Sources with Emphasis on *Musa sapientum, Tamrabhasma, Asparagus racemosus and zingiber officinale. Indian Journal of Pharmacology*, 34: 100-110. 2002
12. Shan, X., Aw, T. Y. & Jones, D. P. Glutathione-dependent protection against oxidative injury. *Pharmacology and Therapeutics 41, 6* 1-7 1. 1990
13. Someya, S. Antioxidant compounds from bananas (Musa Cavendish). Food Chemistry *79*, 351-354. 2002
14. Tapondjou, L. A., Makhmoor T., Choudhary M. I., Lontsi, D., Sondengam, L. B., Atta-ur-Rhman, Bancoensone, a new antioxidant caffeoyl derivative from *Cussonia bancoensis*, *Cameroon Journal of Experimental Biology*, 1, 57-61. 2005