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RESEARCH ARTICLE

IN VIVO SIMULATED *IN VITRO* MODEL OF *SOLANUM VILLOSUM* (MILL) USING MAMMALIAN LIVER SLICE TECHNIQUE

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ABSTRACT

To evaluate the antioxidant status of ethanolic extract of Solanum villosum leaves (EESVL) using mammalian liver slice technique in in vivo simulated in vitro model. Antioxidant activity of Solanum villosum was studied against H2O2 induced free radicals in goat liver. Administration of H2O2 showed significant decline in the levels of antioxidant enzymes in liver homogenate. Pretreatment with Solanum villosum leaf extract had significant protection in those levels within normal range. Also the plant normalized the lipid peroxidation which evidently showed that the ethanol extract of Solanum villosum had a potent antilipid peroxidative effect. The present study suggests that Solanum villosum had a potent antioxidant effect and it can be used to treat various diseases caused by free radicals.

INTRODUCTION

The present study may open up several promising avenues of possible research in *in viro* studies. The plant, *Solanum villosum* (Mill) belongs to solanaceae family. *Solanum villosum* and related species are widely used as leafy herbs and vegetables, as a source of fruit and for various medicinal purposes. The *Solanum villosum* plant has been used in many ayurvedic medicines. In spite of known uses in traditional medicines, no documented evidence is available on their free radical scavenging effect. So the free radical scavenging effect of *Solanum villosum* is evaluated in a systematic manner to provide information for treating and preventing free radicals and other diseases. Free radicals are atoms or molecules with singlet, i.e. unpaired electron which makes them highly reactive. Oxidative free radicals are generated by metabolic reactions create a chain reaction leading to membrane and other lipid peroxidation, DNA damage, etc., Liver is also under constant threat of oxidants and some of the free radical especially H_2O_2 (Rahmat, 2012; Langeswaran *et al*., 2012). When such defense mechanisms become unbalanced by antioxidant supplement can be used to reduce the oxidative damage.

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The antioxidants mainly enzymic and non – enzymic antioxidants. Repairing such damages by naturally occurring substances mainly by supplementation of food having antioxidant property is becoming one of the most acceptable modes of modern therapy. The liver, a highly specialized organ regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions. It also performs important digestive and excretory functions, stores and processes nutrients, synthesizes new molecules, detoxifies harmful chemicals (Greek, 2008; Aashish *et al*., 2012). So, if the liver is affected by some abnormal changes, the homeostasis of the physiological system will be disturbed.

MATERIALS AND METHODS

Preparation of the extracts

The plant, *Solanum villosum* (Mill) were collected from Thadagam hills at Coimbatore district, Tamilnadu, India. The specimen sample was identified and authenticated by Dr.G.V.S. Murthy, Joint Director, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification No. BSI/SRC/5/23/2014-15/Tech/255. The *Solanum villosum* leaves were washed, shade dried and powdered using mixer grinder.

The powdered material (10 g) was extracted with 100 ml of ethanol using soxhlet apparatus and filtered through Whatmann No 1 filter paper. The filtrate was concentrated and dried under reduced pressure and controlled temperature. The concentrated extracts of the leaves were stored in small vials at -20º C and used for further analysis.

Preparation of mammalian liver slices

The goat liver was selected as the mammalian tissue to determine the antioxidant effect of ethanol extract in the presence and absence of the standard oxidizing compound (H_2O_2). The dose of H_2O_2 used was the same as the level used *in vivo* studies by intraperitonial administration (2ml/kg) tissue). The fresh liver was collected from local slaughter house immediately after the sacrifice of the animal.

The tissue was quickly plunged into cold sterile Hanks balanced salt solution (HBSS) buffer and maintained at 40˚ C. very thin (⁼ 1mm) slices of the tissues were cut by using the sterile scalpel and tissue (250 mg) was taken in 1.0 ml of sterile HBBS, in broad, flat bottomed flasks. The necessary compounds (H_2O_2 and ethanol extract) were added and incubated at 37˚ C for one hour with mild shaking. Appropriate control groups were also set up. The standard oxidant H_2O_2 was used at a concentration of 2 ml/kg tissue. After the incubation period, the tissues were homogenized in the same aliquot of the HBSS buffer using a Teflon homogenizer and centrifuged to remove the debris. The supernatant was then used for the estimation of various parameters to assess the antioxidant potential.

The following groups were set up for assay of antioxidants.

Experimental Design

- Group IControl
- Group IIH_2O_2 induced (2 ml / kg tissue)
- Group IIIH_2O_2 induced + Treatment with EESVL at 20 mg (20 µl) per ml of HBSS
- Group IVH_2O_2 induced + Treatment with Rutin at 70 mg / kg tissue
- Group VTreatment with EESVL alone at 20 mg (20 µl) per ml of HBSS.

Analysis of antioxidant status

The homogenized liver tissues were used for the analysis of antioxidant enzymes such as superoxide dismutase (SOD) (Das *et al*., 2000), catalase (CAT) (Sinha, 1972), glutathione peroxidase (GPx) (Rotruck *et al*., 1973), glutathione transferase (GST) (Mannervik, 1985), glucose -6 – phosphate dehydrogenase (G-6-PD) (Balinsky and Bernstein, 1963), reduced glutathione (GSH) (Moron *et al*., 1979), vitamin C (Omaye *et al*., 1979), vitamin E (Rosenberg, 1992), lipid perooxidation (LPO) (Niehius and Samuelsson, 1968) and protein (Lowry *et al*., 1957).

Statistical Analysis

Values were expressed as mean ± SD. Statistical difference in mean was analyzed using one way analyzed using one way ANOVA and followed by least significance difference comparison tests (LSD). P< 0.05 was considered statistically significant.

RESULTS

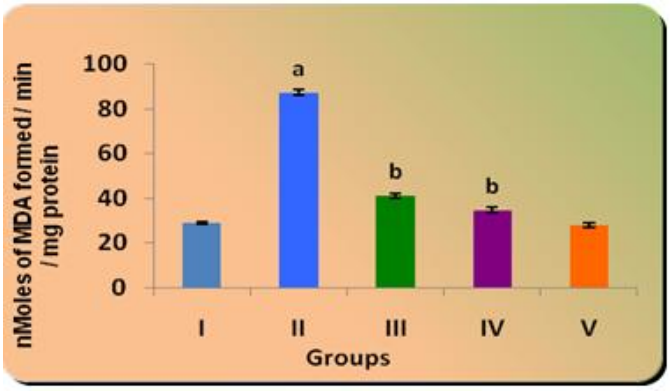
Enzymic and non-enzymic antioxidants

The activities of enzymic and non- enzymic antioxidants in the liver slices exposed to H_2O_2 and treated with *Solanum villosum* leaf extract are represented in Table 1 and Table 2.

The results of the present study showed that, exposure to H_2O_2 showed a significant ($p < 0.05$) depletion of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-s-transferase (GST) and glucose 6-phosphate dehydrogenase (G6PD) activity. Treatment with ethanolic leaf extract of *Solanum villosum* exerted a significant increase in all the enzymic antioxidants when compared to H_2O_2 induced group.

Lipid Peroxidation

The level of LPO on H_2O_2 induced tissue homogenate is represented in Figure 1.



Values are expressed as mean ± SD (n=3)
Comparison between the groups
a: statistically significant ($p < 0.05$) compared with control
b: statistically significant ($p < 0.05$) compared with H_2O_2 induced group.

Figure 1. Effect of ethanolic leaf extract of *Solanum villosum* on lipid peroxidation in liver slices of control and experimental groups

Significantly elevated levels of lipid peroxidation were observed in H_2O_2 assaulted goat liver slices. Exposure of ethanol leaf extract of *Solanum villosum* maintained the level of MDA formed. The ethanol extract of the *Piper trioicum* Roxb. and *Physalis minima* L. has been shown to suppress lipid peroxidation in goat liver homogenate reported by Dinakaran *et al*., (2011). This supportive study is in accordance with our reports indicating the protective effect of *Solanum villosum* leaves extract against H_2O_2 induced oxidative stress. The results showed that the ethanol leaf extract of *Solanum villosum* possesses inhibition of lipid peroxidation and potent antioxidant activity. It improves the antioxidant status in the goat liver slices exposed to oxidative stress. Significant antioxidant potential observed in this study may be due to the increased availability of antioxidants which may be rendered by the secondary metabolites present in *Solanum villosum* leaves.

Table 1. Effect of ethanolic extract of *Solanum villosum* leaves on enzymic antioxidants in liver slices of control and experimental groups

Groups	SOD	CAT	GPx	GST	G-6-PD
Group I	6.38±0.02	31.36±0.47	7.30±0.03	2.66±0.07	1.01±0.01
Group II	3.67±0.32 [a]	21.01±0.41 [a]	4.56±0.48 [a]	1.68±0.10 [a]	0.57±0.02 [a]
Group III	5.01±0.28 [b]	27.21±0.47 [b]	6.11±0.47 [b]	2.32±0.21 [b]	0.85±0.02 [b]
Group IV	7.43±0.50 [b]	28.25±0.16 [b]	6.73±0.39 [b]	2.33±0.08 [b]	0.92±0.02 [b]
Group V	6.39±0.30	31.10±1.72	7.63±0.10	2.61±0.09	0.99±0.01

Values are expressed as mean ± SD (n=3)
Comparison between the groups
a: statistically significant ($p < 0.05$) compared with control
b: statistically significant ($p < 0.05$) compared with H_2O_2 induced group
Units: SOD - 50 % inhibition of nitrite formation / min / mg protein; CAT - µmoles of H_2O_2 d ecomposed / min / mg protein; GPx - µg of glutathione utilized / min / mg protein; GST – µmoles of CDNB conjucated / min / mg protein; G6PD - 0.01 OD change / min / mg protein.

Table 2. Effect of ethanolic leaf extract of *Solanum villosum* on non-enzymic antioxidants in liver slices of control and experimental groups

Groups	GSH	Vitamin - C	Vitamin - E
Group I	38.93 ± 0.69	3.12 ± 0.04	17.22 ± 0.39
GroupII	26.41 ± 0.69 [a]	2.42 ± 0.06 [a]	12.84 ± 0.54 [a]
Group III	36.61 ± 1.06 [b]	3.01 ± 0.04 [b]	15.48 ± 0.57 [b]
Group IV	37.54 ± 0.69 [b]	3.03 ± 0.04 [b]	16.47 ± 0.10 [b]
Group V	38.01 ± 1.74	3.09 ± 0.09	16.94 ± 0.15

Values are expressed as mean ± SD (n=3)
Comparison between the groups
a: statistically significant ($p < 0.05$) compared with control
b**:** statistically significant ($p < 0.05$) compared with H_2O_2 induced group
Units: GSH-µg / g tissue; Vitamin - C- µg / g tissue; Vitamin - E- µg / g tissue.

The above finding strongly warrants closer attention to this plant for the development of drugs to treat various complications initiated by free radicals.

DISCUSSION

Superoxide dismutase, as an antioxidant, help to protect against cell destruction by superoxide radical anion (O_2^-) and keeps the concentration of superoxide radicals at low levels and therefore play an important role in the defense against oxidative stress (Manju *et al*., 2002). The level of SOD was found to be increased in the treatment group when compared with H_2O_2 induced group. In the present investigation, the reduction in SOD activity in the liver slices is suggestive of the increased oxidative stress. Additionally, the significant increase in the activity of the leaf extract implies that they can effectively alleviate the oxidative stress. Catalase is a heme containing enzyme, which is present in most cells and catalyses the decomposition of hydrogen peroxide to water and oxygen.

Cytosolic catalase is found to be important in the inactivation of many environmental mutagens. Catalase is responsible for the breakdown of H_2O_2, an important ROS (McCord, 2000; Ramanathan *et al*., 2002). The catalase activity in the liver slices was significantly reduced, whereas, treatment with the *Solanum villosum* leaf extract, the enzyme activity was increased compared to the oxidative stress induced group. Glutathione Peroxidase is a well-known first line of defense against oxidative stress, which in turn requires glutathione as cofactor. It catalyses the oxidation of GSH to GSSG at the expense of H_2O_2. It is found in both cytosol (70 %) and mitochondria (30 %) of various tissues (Lindholm *et al*., 2010). The level of this enzyme was decreased in toxic groups compared to control. From the Table 1 and 2, it was observed that the levels of enzymatic and non-enzymatic antioxidants were increased in the *Solanum villosum* leaf extract treated group compared with the oxidative stress induced group.

Glutathione S-transferases (GSTs) are a family of enzymes that catalyze the addition of the tripeptide glutathione to endogenous and xenobiotic substrates which have electrophilic functional groups (Ji *et al*., 1992). GST offers protection against LPO by promoting the conjugation of toxic electrophiles with GSH (Jakoby, 1988). GSH, apart from being a strong antioxidant by itself also acts as a substrate for antioxidant enzymes like GPx and GST. Treated with *Solanum villlosum* leaf extracts in the present study, improved the GST activities from the effect of the oxidant assault. This observation shows that the leaf extracts are effective in ensuring GSH homeostasis in the cell, as the GR replenishes GSH (reduced) from GSSG (oxidized). Glucose 6-phosphate dehydrogenase (G6PD) is an important enzyme for the generation of NADPH, which is utilized for the regeneration of various antioxidant molecules. Exposure to H_2O_2 showed a significant depletion of G6PD activity. This effect was counteracted by the *Solanum villosum* leaf extract. Reduced glutathione constitutes the first line of defense against free radicals (Raja *et al*., 2007). It acts directly as a free radical scavenger by donating a hydrogen atom and thereby neutralizing the hydroxyl radical.

It also reduces peroxides and maintains protein thiols in the reduced state (Sies and Groot, 1992). GSH is the major cytosolic thiol compound and is required to maintain the normal reduced state of the cells and to counteract ROS,

thereby reducing the oxidative stress. GSH also preserves the cellular levels of active forms of vitamin C and vitamin E (Sivalokanathan *et al*., 2006). GSH level was drastically decreased on exposure to H_2O_2. Treated with *Solanum villosum* leaf extract and standard antioxidant rutin was significantly increased the GSH levels compared with oxidative stress induced group.

Vitamin C or L-ascorbate is an essential nutrient for a large number of higher primate species, a small number of other mammalian species, a few species of birds and some fish. The presence of ascorbate is required for a range of essential metabolic reactions in all animals and plants. In living organism ascorbate is an antioxidant, since it protects the body against oxidative stress and it is a cofactor in several vital enzymatic reactions (Kumar and Hemalatha, 2011). The recovery towards normalization of these enzymes caused by plant treatment was almost similar to that caused by rutin. Treatment of goat liver slices with H_2O_2 significantly depleted levels of Vitamin C which was effectively counteracted by the leaf extract. It showed a significant increase in the levels of vitamin C.

Vitamin E is chain breaking antioxidant present in the cell membrane. It provides protection against superoxides as well as H_2O_2 (Gupta *et al.*, 2010). Vitamin E is the major lipid soluble peroxyl radical scavenger, which can limit LPO by terminating chain reactions initiated in the membrane lipids (Wiseman and Halliwell, 1993). Vitamin E acts as a chain breaking antioxidant by denoting its labile hydrogen atom from phenolic hydroxyl groups to propagate lipid peroxyl and alkoxyl radical intermediates of LPO, thus terminating the chain reaction (Stocker *et al*., 1991). The vitamin E levels significantly reduced in liver slices when challenged with H_2O_2. While supplementation with *Solanum villosum* leaf extract elevated the levels of vitamin E. LPO is a free radical mediated process leading to oxidative deterioration of polyunsaturated lipids. LPO is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation. It has been found to be connected with various disease processes, such as carcinogenesis, atherosclerosis and hypertension. To control LPO, there is a defensive system consisting of antioxidant enzymes that play an important role in scavenging reactive oxygen species (Mahboob *et al*., 2003).

Conclusion

In conclusion, antioxidants are given importance due to a large number of lifestyle diseases like aging, cancer, diabetes, cardiovascular and other degenerative diseases etc. Owing to our sedentary way of life and stressful existence. Exposure of H_2O_2 caused a significant decrease in all the antioxidant contents and an elevation in lipid peroxidation. Co-treatment with the extracts, improved the antioxidant status in goat liver slices challenged with oxidative stress. The above findings showed that the ethanolic extract of *Solanum villosum* leaves possesses the significant antioxidant potential, which may be rendered by the primary and secondary metabolites present in the leaf extract.

Conflict of interest

The authors report no conflict of interest.

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