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LIPID PEROXIDATION AND *IN VIVO* ANTIOXIDANT EFFECT OF WHOLE PLANT OF *SACCHARUM SPONTANEUM* (LINN.) IN RATS FED WITH ATHEROGENIC DIET

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ABSTRACT

Background: The objective of the present investigation was to evaluate the *in vivo* antioxidant and lipid peroxidation effect of various extracts from whole plant of *Saccharum spontaneum (Linn.)*in rat fed with atherogenic diet.

Methods: A total number of 30 rats were divided into five groups of six rats each. One group was kept as control (normal) group, fed on standard rabbit diet and other 4 groups were fed on Atherogenic diet (AD). Out of four AD groups one group was kept as control (HCD) and other two groups were treated with different doses (200 and 400mg/kg/day) of *S. spontaneum* for 9 weeks and the tissue (aorta, heart and liver) samples were collected at the end of experimental period. The enzymatic and non enzymatic antioxidant and lipid peroxidation studies have been done.

Results: Atherogenic diet rats showed significantly (P<0.001) reduced the levels of tissues enzymatic antioxidant and non enzymatic antioxidant (Glutathione). The level of thiobarbituric acid reactive substances (TBARS) are elevated in AD rats (group II) when compared with control rats (group I). After administration of ethanolic extract of *Saccharum spontaneum* in atherogenic diet rats were showed significantly (P<0.001) increased the levels of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and increased the level of non enzymatic antioxidant glutathione (GSH) when compared with AD rats (Group II). The ethanolic extract of *Saccharum spontaneum* in atherogenic diet rats were found reduced the concentration of TBARS than that of AD rats (group II).

Conclusion: Based on the results, we concluded that the ethanolic extract of *Saccharum spontaneum* is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: *Saccharum spontaneum*, *in vivo* antioxidant, atherogenic diet, rats.

INTRODUCTION

It is plainly established that long-term utilization of an atherogenic diet quickens the advancement of Coronary Heart Disease (CHD). Dietary cholesterol can expand the level of serum cholesterol to levels which can put a person at increased risk for the improvement or exacerbation of atherosclerosis [1,2]. Therapeutic agents are control the levels of serum cholesterol have ended up being powerful in the treatment of CHD [3,4]. The high levels of free radicals in living systems can oxidize bio molecules, prompting tissue damage, cell demise or different diseases, for example, malignancy, cardiovascular infections, arteriosclerosis, neural disorders, skin irritations and inflammations [5,6]. Antioxidant compounds can deactivate and scavenge the free radicals. Antioxidants can repress the impact of oxidants by donating hydrogen atom or chelating metals [7-9].

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Synthetic antioxidants, for example, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are utilized as additives in foods to prevent oxidation of lipids [10-12]. In addition, BHA and BHT are confined by legislative principles on account of questions over their harmful and cancer-causing impacts. Along these lines, there is a growing request and enthusiasm on natural and more safer antioxidants in food applications, and a developing trend in buyer inclinations for natural antioxidants [13,14]. Natural antioxidants are concentrated widely for their ability to protect organisms and cells from harm incited by oxidative stress, the last being viewed as a reason for maturing and degenerative diseases [15]. Recently, examination of new sources of natural antioxidants turned out to be vital for human health. Natural antioxidants normally exist on plants which contain polyphenolic compounds [16-18].

Saccharum spontaneum (Linn.); Synonyms, Ahlek, loa, wild cane, wild sugarcane, Family: Poaceae. In India, it is considered as valuable aromatic plant in traditional systems of medicine. It is popular folk medication. The whole plant used to treat diseases such as vomiting, mental diseases, abdominal

disorders, dyspnoea, anaemia, and obesity. The rural public use the fresh juice of the stem of *Saccharum spontaneum* plant for the treatment of mental illness and mental disturbances. The stems are also useful for renal and vesicol calculi dyspepsia, haemorrhoids, menorrhagia dysentery, agalactia phthisis and general debility. The roots are sweet, astringent, emollient, refrigerant, diuretic, lithontriptic, purgative, tonic, aphrodisiac and useful in the treatment of dyspepsia, burning sensation, piles, sexual weakness, gynaecological troubles, respiratory troubles etc [19]. Leaves are employed for cathartic and diuretics [20]. However, the plant is reported to possess the activities like anti-diarrhoeal [21], CNS depressant [22] and antiurolithiatic activity [23]. Literature survey revealed that there is a no earlier scientific reports regarding *in vivo* antioxidant and lipid peroxidation effect of this plant. Therefore, objective of the present investigation was to study the *in vivo* antioxidant and lipid peroxidation effect of ethanolic extract of whole plant of *Saccharum spontaneum* (Linn.) in rat fed with atherogenic diet in rats.

MATERIALS AND METHODS

Plant materials:

The whole plant of *Saccharum spontaneum* (Linn.), were collected from Cheranmahadevi, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Saccharum spontaneum* (Linn.), were dried under shade, segregated, pulverized by a mechanical processor and passed through a 40 mesh sieve. The powdered materials were stored in a polythene bag.

Preparation of Extracts:

The above powdered materials were successively extracted with ethanol (60-80ºC) by hot continuous percolation method in Soxhlet apparatus [24] for 24 hours. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Animals:

Thirty adult male healthy Wistar rats, weighing approximately 150-180g were obtained from Central Animal House, Rajah Muthiah Medical College, Annamalai University. The animals were kept in cages, 2 per cage, with relative humidity (55%) in a 12 hour light/dark cycle at
25º±2ºC. They were offered access to water and a standard chow pellet *ad libitum*. The experiment were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC), Annamalai University (Approved number: 160/1999/CPCSEA/1083).

Animal diet: The compositions of the two diets were used as follows [25]:

Control diet: Wheat flour 22.5%, roasted bengal gram powder 60%, skimmed milk powder 5%, casein 4%, refined oil 4%, salt blend with starch 4% and vitamin & choline blend 0.5%.

Atherogenic diet: Wheat flour 20.5%, roasted bengal gram 52.6%, skimmed milk powder 5%, casein 4%, refined oil 4%, coconut oil 9%, salt blend with starch 4% and vitamin & choline blend 0.5%, cholesterol 0.4%.

Experimental Design:

A total number of 30 rats were divided into five groups of six rats each:

Group I : Standard chow pellet (Control).
Group II : Atherogenic diet (AD).
Group III : AD + Ethanolic extract of *Saccharum spontaneum* (200mg/kg b.wt)
Group IV : AD + Ethanolic extract of *Saccharum spontaneum* (400mg/kg b.wt)
Group V : AD + standard drug atorvastatin (1.2 mg/kg body weight)

Testing of in vivo antioxidant and lipid peroxidation:

Rats of group III, IV and V were orally fed with the various extracts of *Saccharum spontaneum* (200mg/kg body weight) and rats of group VI were fed with standard drug atorvastatin (1.2 mg/kg body weight). The dose was fixed as per the OECD guidelines. All the three extracts as well as standard drug atorvastatin were suspended in 2% tween 80 [26] separately and fed to the respective rats by oral intubation. At the end of 9 weeks all the rats were sacrificed by cervical dislocation after overnight fasting. Aorta, heart and liver were cleared of adhering fat, weighed accurately and used for the preparation of homogenate. Animals were given enough care as per the Animal Ethical Committee's recommendations. Portions of the tissues from liver, heart and aorta were blotted, weighed and homogenized with methanol (3 volumes). The lipid extract obtained by the method of Folch *et al* [27] . It was used for the estimation of thiobarbituric acid reactive substances [28] (TBARS) and conjugated diene. Another portion of the tissues was homogenized with phosphate buffer saline and used for the estimation of reduced Glutathione [29](GSH), Superoxide dismutase [30](SOD), Catalase [31](CAT), Glutathione peroxidase [32](GPx), Glutathione reductase [33](GR) and Glutathione-s-transferase (GST).

Statistical Analysis:

Data obtained from experiment animals were expressed as mean ± standard error (± SEM).

Statistical differences between the control and experimental groups were evaluated by one-way ANOVA and Duncan multiple comparison tests. A difference in the mean values of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Adverse effect of AD on the health of humans and animal species has previously been emphasized by several researchers [34,35]. It has been reported that high levels of fat increase fat-mediated oxidative stress and decrease antioxidative enzyme activity [36] therefore, many chronic health problems that are attributed to AD are known to be the consequences of oxidative damage [37]. On this account, there are various reports indicating the beneficial effects of antioxidant supplementation in preventing dyslipidemia and cardiovascular disease [38-40]. Increase lipid peroxidation refers an imbalance between intracellular free radical production, cellular defence mechanisms and melandialdehyde as one of the most important lipid peroxidation markers [41]. As shown in Table 1, AD rats significantly increased TBARS levels in aorta, heart and liver compared to control group. However, the ethanolic extract of *Saccharum spontaneum* at the dose of 400 mg/kg significantly ($p<0.001$) decreased tissues (Liver, heart and aorta) TBARS levels in ethanolic extract plus AD group compared to AD rats (group II). The similar result was not found in other ethanolic extract at the dose 200mg/kg treatment groups. This result indicates that ethanolic extract of *Saccharum spontaneum* decreases lipid peroxidation and eventually may have a role in reducing the hazardous effects of atherogenic diet.

Table- 1: Effect of ethanolic extract of *Saccharum spontaneum* on tissues TBARS in rats fed AD

Groups	TBARS (n mol of MDA formed/g tissue)		
	Aorta	**Heart**	**Liver**
Group I	17.42±0.15 ^{b*}	41.58±0.18 ^{b*}	24.33±0.22 ^{b*}
Group II	68.56±0.22 ^{a*}	83.52±0.26 ^{a**}	74.55±0.27 ^{a*}
Group III	28.08±0.20 ^{a**,b*}	52.16±0.24 ^{a**,b**}	30.26±0.25 ^{a**,b*}
Group IV	23.14±0.21 ^{a*,b*}	45.90±0.23 ^{a*,b*}	27.11±0.21 ^{a*,b*}
Group V	17.06±0.21 ^{a*,b*}	41.80±0.21 ^{a*,b*}	24.98±0.24 ^{a*,b*}

Values are expressed as mean ± SE (n=6 rats),*P* values : *<0.001, **<0.05,NS: Non-significant

a →group I compared with groups II, III, IV, V.

b →group II compared with groups III, IV, V.

Group I : Standard chow pellet (Control).

Group II : Atherogenic diet (AD).

Group III : AD + Ethanolic extract of *Saccharum spontaneum* (200mg/kg b.wt)

Group IV : AD + Ethanolic extract of *Saccharum spontaneum* (400mg/kg b.wt)

Group V : AD + Standard drug atorvastatin (1.2 mg/kg b.wt)

As shown in Table 2, Atherogenic diet rats significantly decreased conjugated diene levels in aorta, heart and liver compared to control group. However, the ethanolic extract of *Saccharum spontaneum* at the dose of 400 mg/kg significantly ($p<0.001$) decreased tissues (Aorta, heart and liver) conjugated diene levels in ethanolic extract plus AD group compared to AD rats (group II). The similar result was not found in low dose ethanolic extract at 200mg/kg treatment groups. This result indicates that ethanolic extract of *Saccharum spontaneum* decreases lipid peroxidation and eventually may have a role in reducing the hazardous effects of atherogenic diet.

Table-2: Effect of ethanolic extract of *Saccharum spontaneum* on tissues conjugated diene in rats fed AD

Groups	Conjugated diene (µ moles/g tissue)		
	Aorta	**Heart**	**Liver**
Group I	170.43±0.62 ^{b*}	152.22±0.43 ^{b*}	170.78±0.58 ^{b*}
Group II	750.86±1.45 ^{a*}	242.10±0.40 ^{a**}	254.33±0.60 ^{a*}
Group III	427.79±1.14 ^{a*,b*}	175.95±0.68 ^{a**,b*}	198.20±0.024 ^{a**,b*}
Group IV	397.56±0.33 ^{a*,b**}	165.88±0.03 ^{a*,b*}	187.26±0.04 ^{a*,b*}
Group V	393.88±0.35 ^{a*,b*}	162.20±0.04 ^{a*,b*}	182.52±0.04 ^{a*,b*}

Values are expressed as mean ± SE (n=6 rats), *P* values : *< 0.001, **< 0.05, NS: Non Significant

Particulars of group I-V are same as 1st Table.

Glutathione (GSH) is essential for the cellular antioxidant defence response and acts as an essential cofactor for antioxidant enzymes [42]. As shown in Table 3. A significant (p<0.001) decrease in aorta, heart and liver GSH levels in AD rats compared to control rats (group I),while increase in aorta, heart and liver GSH levels in ethanolic extract plus AD group compared to AD (group II) and other extracts treatment groups (III &IV). Under the oxidative stress conditions, GSH is consumed by the GSH related enzymes to detoxify peroxides produced due to increased lipid peroxidation [43]. In AD group, significant raise in lipid peroxidation and concomitant GSH activity may be a consequence of depleted glutathione stores.

Table-3: Effect of ethanolic extract of *Saccharum spontaneum* on tissues glutathione (GSH) in rats fed AD

Groups	Glutathione (mg/g tissue)		
	Aorta	Heart	Liver
Group I	6.05±0.30 ^{b*}	7.54±0.42 ^{b*}	4.55±0.35 ^{b*}
Group II	3.20±0.18 ^{a*}	3.90±0.25 ^{a**}	1.58±0.30 ^{a*}
Group III	4.64±0.22 ^{a**,b*}	6.20±0.24 ^{a**,b*}	2.98±0.28 ^{a**,b**}
Group IV	5.38±0.14 ^{a*,b*}	6.64±0.19 ^{a*,b*}	3.80±0.23 ^{a*,b*}
Group V	5.70±0.28 ^{a*,b*}	7.26±0.24 ^{a*,b*}	4.55±0.22 ^{a*,b*}

Values are expressed as mean ± SE (n=6 rats), *P* values : *< 0.001, ** < 0.05, NS: Non Significant

Particulars of group I-V are same as 1st Table.

A cholesterol-rich diet brings about remarkable modifications in antioxidant defence mechanisms. In addition to, recently report shown that hypercholesterolemia diminishes the antioxidant defence system and decreases the activities of superoxide dismutase (SOD) and catalase (CAT), elevating the lipid peroxide content [44]. As shown in Table 4,5. The activities of SOD and CAT in the tissue like aorta, heart and liver were significantly (P<0.001) lowered in rats fed with atherogenic diet (group II) than control group animals. Atherogenic diet can cause the formation of toxic intermediates that can inhibit the activity of antioxidant enzymes [45] and the accumulation of O2- and H2O2 which in turn forms hydroxyl radicals [46]. After administration of ethanolic extract of *Saccharum spontaneum* along with AD significantly increases the activities of SOD and CAT in tissues of rats when compared with atherogenic diet rats(group II).

Table- 4: Effect of ethanolic extract of *Saccharum spontaneum* on tissue superoxide dismutase (SOD) in rats fed AD

Groups	SOD (unit min/mg protein)		
	Aorta	Heart	Liver
Group I	2.86±0.18 ^{b*}	1.75±0.13 ^{b*}	3.68±0.24 ^{b*}
Group II	1.51±0.10 ^{a*}	0.86±0.07 ^{a**}	1.78±0.22 ^{a*}
Group III	2.42±0.14 ^{a**,b**}	1.54±0.09 ^{a*,b*}	2.88±0.19 ^{a**,b*}
Group IV	2.64±0.11 ^{a*,b*}	1.70±0.10 ^{a*,b*}	3.20±0.14 ^{a*,b*}
Group V	2.86±0.14 ^{a*,b*}	1.73±0.18 ^{a*,b*}	3.68±0.16 ^{a*,b*}

Values are expressed as mean ± SE (n=6 rats), *P* values : *< 0.001, ** < 0.05, NS: Non Significant

Particulars of group I-V are same as 1st Table.

Table -5: Effect of ethanolic extract of *Saccharum spontaneum* on tissue catalase (CAT) in rats fed AD

Groups	CAT (µ moles of H_2O_2, consumed min/mg protein)		
	Aorta	Heart	Liver
Group I	30.88±2.14 ^{b*}	48.38±3.65 ^{b*}	28.45±1.09 ^{b*}
Group II	21.58±2.10 ^{a*}	30.90±1.72 ^{a**}	16.85±1.60 ^{a*}
Group III	26.74±2.34 ^{a**,b*}	43.84±3.60 ^{a**,b*}	23.98±2.42 ^{a**,b*}
Group IV	28.46±2.16 ^{a*,b*}	45.36±2.98 ^{a*,b*}	26.08±2.05 ^{a*,b*}
Group V	31.14±2.54 ^{a*,b*}	48.08±2.92 ^{a*,b*}	29.08±2.86 ^{a*,b*}

Values are expressed as mean ± SE (n=6 rats), *P* values : $^{*} < 0.001$, $^{**} < 0.05$, NS: Non Significant
Particulars of group I-V are same as 1st Table.

Glutathione peroxidase (GPx) is more important than catalase for detoxification of hydrogen peroxide in brain, because the brain contains small amounts of catalase and GPx can also interact directly with lipid peroxides [47,48]. As shown in Table 3. Tissues glutathione peroxidase and reductase levels were significantly (p<0.001) decreased in rats fed with AD (group II) as compared to the control rats (group I). Atherogenic diet decreased the ratio of oxidized glutathione/reduced glutathione in tissue [49]. Administration of ethanolic extract of *Saccharum spontaneum* along with the AD significantly (p<0.001) enhanced the levels of glutathione peroxidase and glutathione reductase in all the tissues as compared with AD rats. A standard drug atorvastatin administered rats also showed elevated level of glutathione peroxidase and glutathione reductase.

Table- 6: Effect of ethanolic extract of *Saccharum spontaneum* on tissue glutathione peroxidase (GPx) in rats fed AD

Groups	GPx (mg of GSH consumed/min/mg protein)		
	Aorta	Heart	Liver
Group I	$14.70±1.25^{b*}$	$41.58±1.53^{b*}$	$24.33±0.03^{b*}$
Group II	$7.44±0.50^{a*}$	$83.52±0.42^{a**}$	$74.55±0.57^{a*}$
Group III	$11.69±0.54^{a**,b*}$	$52.16±0.36^{a**,b*}$	$30.26±0.04^{a**,b*}$
Group IV	$13.06±0.61^{a**,b*}$	$45.90±0.64^{a*,b*}$	$27.11±0.58^{a*,b*}$
Group V	$14.15±0.60^{a*,b*}$	$41.80±0.40^{a*,b*}$	$24.98±0.54^{a*,b*}$

Values are expressed as mean ± SE (n=6 rats), *P* values : $^{*} < 0.001$, $^{**} < 0.05$, NS: Non Significant
Particulars of group I-V are same as 1st Table.

Table- 7: Effect of ethanolic extract of *Saccharum spontaneum* on tissue glutathione reductase (GR) in rats fed AD

Groups	GR (mg of GSH consumed/min/mg protein)		
	Aorta	Heart	Liver
Group I	$1.73±0.15^{b*}$	$2.71±0.13^{b*}$	$1.44±0.17^{b*}$
Group II	$0.80±0.10^{a*}$	$1.34±0.07^{a**}$	$0.72±0.08^{a*}$
Group III	$1.37±0.06^{a**,b*}$	$2.28±0.04^{a**,b*}$	$1.13±0.05^{a**,b*}$
Group IV	$1.60±0.08^{a*,b*}$	$2.47±0.06^{a*,b*}$	$1.30±0.04^{a**,b*}$
Group V	$1.75±0.14^{a*,b*}$	$2.72±0.09^{a*,b*}$	$1.42±0.11^{a**,b*}$

Values are expressed as mean ± SE (n=6 rats), *P* values : $^{*} < 0.001$, $^{**} < 0.05$, NS: Non Significant
Particulars of group I-V are same as 1st Table.

Table- 8: Effect of ethanolic extract of *Saccharum spontaneum* on tissue glutathione-s-transferase(GST) in rats fed AD

Groups	Glutathione-s-transferase(GST) (µ mole of CDNB-GSH-conjugate/min/mg protein)		
	Aorta	Heart	Liver
Group I	$16.20±0.12^{b*}$	$19.78±0.03^{b*}$	$26.14±0.03^{b*}$
Group II	$8.33±0.10^{a*}$	$8.08±0.07^{a**}$	$10.25±0.08^{a*}$
Group III	$10.78±0.08^{a*,b**}$	$13.80±0.05^{a**,b*}$	$19.88±0.06^{a*,b*}$
Group IV	$11.70±0.04^{a*,b*}$	$15.12±0.03^{a*,b*}$	$22.08±0.04^{a*,b*}$
Group V	$12.80±0.08^{a*,b*}$	$16.60±0.04^{a*,b*}$	$23.66±0.06^{a*,b*}$

Values are expressed as mean ± SE (n=6 rats), *P* values : $^{*} < 0.001$, $^{**} < 0.05$,NS: Non Significant
Particulars of group I-V are same as 1st Table.

CONCLUSION

On the basis of the results obtained in the present study, we conclude that the ethanolic extract of whole plant *Saccharum spontaneum* had significant *in vivo* antioxidant and lipid peroxidation activity. These *in vivo* antioxidant study indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further studies are required to again more insight in to the possible mechanism of action.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Amutha Iswarya Devi was the principle investigator who performed the field trial, preparation of the manuscript, the pathological analysis, and statistical analysis, Kottai Muthu was conceived the idea and prepared the research proposal and helped in the preparation of the manuscript. All authors read and approved the final manuscript.

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