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Antioxidant activity of *cissus quadrangularis* on sodium perchlorate-induced oxidative damage in rats

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ABSTRACT

The present study was carried out to evaluate the antioxidant activity of flavonoid rich fraction from *Cissus quadrangularis(C.Q)* Linn on sodium perchlorate induced oxidative stress in rats. Animals were divided into four groups of six animals each. Male Albino rats were fed with 0.2% sodium perchlorate to induce oxidative stress. The flavonoid rich fraction of the plant (1mg/100gm, 2mg/100gm) was administered orally along with sodium perchlorate two groups of animals for 30 days. Animals showed increased antioxidant levels in serum, heart, liver, kidney compared with sodium perchlorate treated group. The results of this study suggest that a flavonoid rich fraction of *C.Q* has potent antioxidant property. Hence, It could be used as a potential antioxidant agent in the treatment of various diseases.

KEYWORDS: *Cissus quadrangularis,* flavonoid fraction, oxidative stress, sodium perchlorate

INTRODUCTION

Free radicals or oxidative stress plays an important role in cell death associated with many diseases. Oxygen free radicals can initiate peroxidation of lipids, which in turn stimulate glycation of proteins, inactivation of enzymes and alternation in the structure and function of collagen basement membrane, which can lead to long term complications of diabetes. Reactive oxygen species can damage DNA, cause the dysplastic cellular appearance, deregulated cell growth and finally leads to cancer [1].Moreover, they can also cause ageing, coronary heart diseases, cataract, neurological disorders and gastrointestinal diseases. Before the onset of synthetic era, the man was completely depend on medicinal herbs for

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prevention and treatment of diseases. Dr. SzentGyorgyi, a pioneer and Nobel laureate in biochemistry discovered flavonoid in 1935 from lemon juice [2]. This poly-phenolic compounds such as flavonoides synthesized by a combination of shikimic acid and acyl polymalonate pathway [3]. The concept of antioxidants is firstly catching up and latest research has shown that a number of herbal derivatives have excellent antioxidant activity. *Cissus quadrangularis* (*C.Q*) is a perennial plant of the grape family. It's commonly known as veldt grape or Devils Backbone. It has widely used in the Ayurvedic system of medicine as an antioxidant, anti-osteoprotic, antiulcer, anabolic and androgenic, anti-inflammatory, antihemorrhoidal and parasympathommitic. The plant extract showed increase fracture repair process [4]. Methanolic extract of this plant showed antiulcerogenic activity [5] and also this plant used in the management of weight loss and metabolic syndrome [6]. A survey of the literature revealed that effect of *C.Q* on sodium perchlorate induced oxidative has not been studied. So, it was decided to investigate the antioxidant property of this plant.

MATERIALS AND METHODS

Plant material and extract preparation

The whole plant material was collected from Thiruvanthapuram District, Kerala, India and authenticated by an expert Dr. Hariharan [Herbarim: Tropical Botanical Garden and Research Institute, Palode, Thiruvanthapuram. Herbarium voucher No:1615 (TBGRI)]. Plant material was thoroughly cleaned and dried under shade. Then the dried plant material (1.5 kg) was pulverized to a fine powder and shifted. 80% methanol was added [7] to coarsely powdered plant material and refluxed in a water bath for 2 days at 60^0C. Alcohol was evaporated from extract by using a rotor evaporator. The extract contains other plant materials like fats, terpenes, chlorophyll and xanthophylls were removed by repeated extractions with petroleum, ether ($60\text{-}80^0$C), benzene and ethyl acetate respectively. Ethyl acetate extract contained bulk of flavonoides and this extract was evaporated in vacuum and dried. The eyelid of extract is 68g and the flavonoid content was determined by [8] using $TiCl_4$ reagent and Quercetin as a standard. The flavonoid fraction was dissolved in distilled water used for the present study.

Experimental animals

The male Wister strain of Albino rats weighing 100-120g were used for the study. Rats were housed under controlled conditions of temperature 23±2^{o}C, humidity 50±5% and 10-14 hours light and dark cycle respectively. The animals were housed individually in polypropylene cages containing sterile paddy husk as bedding . Animals were maintained on a normal diet (Amrut lab animal feed, Pranav agro industries Ltd., Sangli, Maharashtra, India.) and water *ad libitum*. The study was undertaken after obtaining approbation from approval of the Institutional Animal Ethics Committee (SMIMS/08/06/2011).

Chemicals

All chemicals were purchased from Amrut lab animal feed, Pranav agro industries Ltd., Sangli, Maharashtra, India.

Experimental protocol

The animals were divided into four groups of six each as.

Study Design

Group I: Control (Normal lab diet 100gm/ day)

Group II: Normal lab diet + Sodium perchlorate (0.2% in 100gm diet/ day)

Group III: Normal lab diet + Sodium perchlorate (0.2% in 100gm diet/ day) + Flavonoid rich fraction of *Cissus quadrangularis* (1mg/ kg body weight/ day)

GroupIV: Normal lab diet + Sodium perchlorate (0.2% in 100gm diet/ day) + Flavonoid rich fraction of *Cissus quadrangularis* (2mg/ kg body weight/ day)

Sodium perchlorate (0.2% in 100g diet) was mixed with normal lab diet and plant extract were administered orally by gastric intubation to the animals daily for 30 days, except to the control group. Every two weeks body weight of the rats recorded. At the end of 30 day period, rats were deprived of food overnight and subjected to the ethauanasia [9]. The blood, liver, kidney and heart tissues were collected for various biochemical estimations.

Biochemical estimations

The levels of Malondialdehyde[10], Hydroperoxidase [11], conjugated dienes[12], Catalase[13],glutathione reductase[14], glutathione peroxidase[15],glutathionetranferase[16],Superoxie disumatase[17] were estimated in the liver, kidney and heart muscle homogenate by according to the standard procedures.

Statistical analysis

Data was analysed by one-way analysis of variance (ANOVA) followed by Dunnet's test using SPSS 16.0. Results were expressed as Mean ± SEM and significance was set at $p < 0.05$.

RESULTS

Flavonoid rich fraction of *Cissus Quadrangularis* Linn in graded concentration was tested for antioxidant activity in four different groups. It was observed that no significant difference in body and organ weights in between four groups (Table 1).Decreased the malondialdehyde, hydroperoxidase and conjugated dienes levels in plant treated group compared with sodium perchlorate treated group (Table-2). Antioxidant levels decreased in sodium perchlorate treated group but significant increase was observed plant treated group (Table 3&4).

DISCUSSION

Administration of sodium perchlorate at the dose of (0.2% in 100gm diet) for 30 days induced hyperlipidemia and lipid peroxidation in albino rats. Peroxidation was also increased in this group as assessed by the highly significant rise in the concentration of malondialdehyde, hydroperoxidase and conjugated diene. Antioxidant activity was also defective to meet the increased demand as evidenced by decreased activity of SOD, Catalase, glutathione peroxidase and glutathione reductase. Administration of a flavonoid rich fraction of *Cissus quadrangularis* at the dose of (1mg and 2mg /100gm body weight) compensated for the increased antioxidant enzymes and decreased the lipid peroxidation and lipid profile in the groups. The plant dose 2mg/100gm body weight showed significant activity in all the observations than 1mg/100gm body weight. The above study provided valuable information on the potential medicinal property of flavonoid compounds from *Cissus quadrangularis*. Further purification and characterization and essential to gain a complete understanding of the potential action of active compound present in it. Intensive studies using various experiential models may provide better scientific evidence that may lead to the development of new effective drugs. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful drugs to treat various ailments like cardiovascular diseases, cancer, diabetes and other diseases.

Table1: Effect of C.Q extract and sodium perchlorate on body weight and organ weights

Various stages of experiment

Groups	Drug, Dose & Route of administration	Initial Weight (gm)	After 4 weeks (gm)	Liver (gm)	Kidney (mg)	Heart (mg)
Group I Control	Normal lab diet (100mg/day)	116.83±0.66	145.54±0.45	2.74±0.67	437.33±0.56	440.83±0.12
Group II Sodium perchlorate	Normal lab diet+ Sodium perchlorate (0.2 % in 100g diet/ per day/orally)	114.33±0.78	146.76±0.56	2.57±0.24	426.50±0.65	424.66±0.56
Group III	Normal lab diet + Sodium perchlorate (0.2% in 100gm diet/per day/ orally) +*C.Q* (1mg/100g body weight/ orally)	118.89±0.56	143.83±0.67	2.60±0.13	429.83±0.17	429.63±0.46
Group IV	Normal lab diet + Sodium perchlorate (0.2% in 100gm diet/ per day/ orally) + *C.Q* (2mg/100g body weight/ orally)	119.16±0.45	147.33±0.56	2.64±0.34	427.36±0.24	443.34±0.86

Values expressed in MEAN±SEM of six animals; no significant difference observed when compared in between all groups.

Table 2: Effect ofC.Q and sodium perchlorate on Malondialdehyde, Hydroperoxidase, conjugated dienes levels in tissue homogenate

Groups	**Malondialdehyde (mM/100g wet tissue)**			**Hydroperoxidase (mM/100g wet tissue)**			**Conjugated dienes (mM/100g wet tissue)**		
	Liver	Kidney	Heart	Liver	Kidney	Heart	Liver	Kidney	Heart
Group I Control	0.57± 0.11	1.37± 0.30	0.23± 0.10	7.72± 0.20	1.89± 0.40	41.84± 1.05	75.40± 1.81	15.24± 0.36	13.10± 0.31
Group II Sodium perchlorate	1.42± 0.35 *	1.66± 0.50	0.94± 0.24 *	20.45± 0.42 *	6.87± 0.15 *	114.65± 2.74 *	113.02± 2.78 *	19.84± 0.48 *	67.52± 2.76 *
Group III (Test 1)	0.75± 0.23 †	1.45± 0.50	0.51± 0.31 †	15.29± 0.14 †	4.60± 0.60 *†	70.56± 1.58 *†	84.01± 2.10 *†	16.89± 0.38	35.05± 1.43 *†
Group IV (Test 2)	0.59± 0.15 †	1.29± 0.30	0.34± 0.80 *,†	12.29± 0.86 *†	2.63± 0.71 *	63.56± 0.71 *	79.89± 1.40 *	15.41± 0.38	21.96± 1.89 *

$P<0.05$ significant as compared with Group I, $^{\dagger}P<0.05$ significant as compared with Group II

Table 3: Effect of C.Q and sodium perchlorate on Catalase, Glutathione, Superoxide dismutase levels in tissue homogenate

Groups	Catalase (Values X 10^{-3} mg protein)			Glutathione (mg/100gm wet tissue)			Superoxide dismutase (Units/mg protein)		
	Liver	Kidney	Heart	Liver	Kidney	Heart	Liver	Kidney	Heart
Group I Control	68.83± 0.17	9.84± 0.25	27.50± 0.69	440.56± 11.0	89.56± 2.15	400.24± 9.67	5.12± 0.21	9.24± 0.31	8.34± 0.29
Group II Sodium perchlorate	25.63± 0.67 *	1.45± 0.40 *	14.80± 0.38 *	314.56± 8.04 *	51.14± 1.25 *	133.39± 3.26 *	1.09± 0.11 *	5.78± 0.26 *	3.19± 0.12 *
Group III (Test 1)	49.14± 1.59 *†	7.34± 0.34 *	14.96± 0.56 *	395.90± 6.73 * †	75.89± 2.04 *	295.47± 7.33 *†	3.94± 0.19 *	7.45± 0.31 *	5.90± 0.13 *
Group IV (Test 2)	61.18± 1.59 *	8.34± 0.58 *†	21.05± 0.14 *	410.56± 4.89 *	83.23± 1.56 *	367.87± 2.08 *†	4.67± 0.37 *	8.61± 0.45 *	6.93± 0.49 *

P<0.05 significant as compared with Group I, † P<0.05 significant as compared with Group II

Table 4: Effect of C.Q and sodium perchlorate on antioxidant levels in tissue homogenate

Groups	Glutathionereductase (Uni. Values X 10^{-3} µM/min/mg protein)			Glutathioneperoxidase (Uni. Values X 10^{-3} µM/min/mg protein)			Glutathione-S-transferase (µM/min/mg protein)		
	Liver	Kidney	Heart	Liver	Kidney	Heart	Liver	Kidney	Heart
Group I Control	5.52± 0.13	7.83± 0.19	4.53± 0.11	0.17± 0.04	0.18± 0.04	0.61± 0.03	2.85± 0.07	6.85± 0.17	4.40± 0.11
Group II Sodium perchlorate	3.72± 0.09 *	3.48± 0.13 *	3.15± 0.45 *	0.09± 0.02 *	0.05± 0.06 *	0.32± 0.09 *	1.95± 0.05 *	4.56± 0.14 *	2.95± 0.08 *
Group III (Test 1)	4.34± 0.24	6.57± 0.25 †	3.96± 0.54	0.12± 0.03	0.08± 0.05 *†	0.43± 0.03	2.16± 0.03	5.45± 0.14 *	3.24± 0.05 *
Group IV (Test 2)	4.67± 0.34 †	7.13± 0.56 †	4.13± 0.46 †	0.15± 0.05 †	0.15± 0.03 †	0.56± 0.02 †	2.67± 0.02 †	6.53± 0.18 †	4.22± 0.08 †

P<0.05 significant as compared with Group I, † P<0.05 significant as compared with Group II

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