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Evaluation of hepatoprotective and antioxidant activity of *Stereospermum suaveolens* against Aluminium fluoride induced hepatotoxicity.

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Abstract

To evaluate the hepatoprotective activity of methanol stem bark extract of *Stereospermum suaveolens* DC (Bignoniaceae) against aluminium fluoride ($AlF_4É$)-induced hepatic damage in albino mice. The animals were divided into six groups of seven animals each. Group I served as normal; group II served as control, receives $AlF_4É$ through their drinking water; group III served as standard, receives 500 mg/kg of Liv-52. The groups IV, V and VI were treated with 125, 250 and 500 mg/kg doses of methanol extract of *Stereospermum suaveolens* orally. After the 24 h of the last dose of $AlF_4É$, serum biochemical and liver enzymes were assessed along with histopathological studies of liver sections. The $AlF_4É$-treated group showed a significant (p<0.05) increase in serum biochemical markers like SGOT, SGPT, ALP, total bilirubin, LDL-cholesterol and lipid peroxidation levels in liver homogenate along with significant reduced levels of GSH, total thiols, CAT and SOD. The pretreatment groups with different doses of extract and standard Liv-52 were significantly (p<0.05) reversed the above parameters as compared to control group. From the results it can be concluded that hepatoprotective and antioxidant activity of methanol extract may be due to presence of flavonoids and tannins.

Key words : Antioxidants, free radicals, hepatoprotective, *Stereospermum suaveolens*.

1. Introduction

Liver plays a major role in detoxification and excretion of endogenous and exogenous compounds. Any injury to it or impairment of its functions may lead to many implications on one's health[1]. Free radicals and other reactive oxygen species (ROS) are formed constantly in human body and are removed by enzymatic and non-enzymatic oxidative defense systems[2]. Aluminium and fluoride are both known to be potential environmental hazards which may cause liver damage at higher doses[3]. In addition human beings consume a lot of synthetic drugs

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during diseased conditions which are alien to body, organs, may produce a variety of toxic manifestations. Management of liver diseases is still a challenging to the modern medicine. Conventional drugs used in the treatment of liver diseases are often inadequate. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace the currently used drugs of doubtful efficacy and safety[4]. Ayurveda the ancient system of Indian medicine, has cited various herbs were for the management of liver disorders. Some studies conducted on hepatoprotective plants revealed that the activity is because of inhibition of free radical-mediated damage to cells[5]. Many herbal formulations like Liv-52[6], *Picrorrhiza kurroa*[7] and *Ocimum sanctum*[8] (Labiatae) are available for treating liver disorders.

The *Stereospermum suaveolens* DC (Bignoniaceae) commonly known as 'Patala' is widely available in India. It mainly contains lapachol, dinatin, β-sitosterol, saponin and palmitic, stearic and oleic acids[9]. Traditionally it is mainly used as analgesic, wound healing, antidyspeptic, astringent and liver stimulant[10]. Thus, based on above literature and as ethnic medicines have safe and multitherapeutic applications, traditionally and in folk medicine claim *Stereospermum suaveolens* was evaluated for hepatoprotective and antioxidant activity in experimental animal model.

2. Materials and Methods

2.1 Chemicals and reagents

2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Sigma-Aldrich Corporation, St. Louis, MO, USA and standard ranitidine tablets were obtained from Dr. Reddy's laboratory, Hyderabad, India. Serum GOT GPT, ALP, Total Bilirubin and LDL-cholesterol enzyme test kits were obtained from Erba Diagnostic, Germany. Aluminium fluoride was obtained from HiMedia Lab. Pvt. Ltd, Mumbai. All other reagents were of analytical grade. Refrigerated centrifuge MPW-350R MED instruments, Poland. UV-Spectrophotometer UV-1601 Shimadzu Corporation, Kyoto, Japan.

2.2 Plant material and preparation of extract

Stereospermum suaveolens is widely available in India. The fresh stem barks were collected from Kolhapur district of Maharashtra. The specimen was further identified and authenticated in Department of Botany, Basaveshwar Science College, Bagalkot, Karnataka (Voucher specimen: BSC/Pharmacy/2008/1/11). The bark was subjected to coarse powdered (#: 44) to obtain uniform texture. The sieved powder was subjected hot continuous Soxhlet extraction with petroleum ether (60-80°) and methanol for 24 h cycle at 60-65°. Excessive solvent was removed by solvent distillation apparatus and residue was concentrated by using Lyotrap dryer. Suspension of extract was prepared using Tween-80 and subjected for hepatoprotective and antioxidant activity.

2.3 Phytochemical screening

The extract was subjected to qualitative chemical tests to determine the presence of phytoconstituents[11].

2.4 Animals

Swiss albino mice of either sex (25-30 g) were used for the study. Animals were procured from National Institute of Nutrition, Hyderabad and were kept in quarantine for 10 days under standard husbandry conditions (Temp 22-28°C; Relative humidity 65 ± 10%) for 12 h dark and light cycle respectively and are given standard pellet food (Hindustan lever) and water *ad libitum* throughout the experimental period. The study received approval from the Institutional Animal Ethical Committee (IAEC-

Clearance: 1-8/2007) H. S. K College of Pharmacy and Research Centre, Bagalkot, Karnataka, India.

2.5 Acute toxicity study

Mice were divided into eight groups of six animals each. The control group received normal saline (2 ml/kg p.o). The other groups received 50, 100, 200, 400, 800, 1000, 2000 and 5000 mg/kg of extracts respectively. Immediately after dosing the animals were observed for their behavior continuously for the first four hours. They were kept under observation up to 14 days after extract administration to find out the mortality and body weight was observed[12]. At 5000 mg/kg body weight no mortality was observed and is considered as the cut off point. Therefore 1/10th of this dose i.e. 500 mg/kg was taken as experimental dose for subsequent hepatoprotective and antioxidant study. The extract was administered at doses of 125, 250 and 500 mg/kg body weight.

2.6 Aluminium fluoride induced hepatotoxicity in mice

The animals were divided into six groups of eight animals each. Group I served as normal, received normal saline (10 ml/kg b.wt); group II served as control, received Aluminium fluoride at the dose of 600 ppm for seven days through their drinking water; group III served as standard, received 500 mg/kg of Liv-52[13]. The groups IV, V and VI were treated with 125, 250 and 500 mg/kg doses of methanol extract of *Stereospermum suaveolens* orally for 10 days prior to Aluminium fluoride treatment through their drinking water[14].

2.7 Biochemical estimation in blood serum

After the 24 h of the last dose of AlF_4^-, blood was collected by retro-orbital plexus and centrifuged (3000 rpm for 10 min) to separate serum for the estimation of Serum GOT, GPT[15], ALP[16], total bilirubin[17] and serum LDL-cholesterol[18].

2.8 Antioxidant enzyme estimation

Each mice of respective group was autopsied under light ether anesthesia, livers were isolated and perfused with 0.9% ice cold normal saline and 10% w/v liver homogenate were prepared with 0.1M phosphate buffer (pH 7.4) and centrifuged at 3000 rpm for 10 min at 4°C. Immediately supernatant was stored at -20°C for the further estimation of Total protein[19], LPO[20,21], GSH[22], Total thiol[23], SOD[24] and CAT[25] levels in liver tissue.

2.9 Statistical analysis

The datas of biochemical parameters were expressed as Mean ± SEM. Results were analyzed statistically using one way analysis of variance (ANOVA) followed by multiple Dunnet *t*-test. The minimum level of significance was fixed at $p<0.05$. The effects of different treated groups were compared with that of control group.

3. Results

At the end of assigned treatment, blood samples of AlF_4É- treated group animals showed significant increase in the levels of serum biochemical markers as compared to normal group animals where as animals treated with 125, 250, 500 mg/kg of methanol extract showed significant ($p<0.05$) decrease in the levels of serum GOT, GPT, ALP, total bilirubin and LDL-cholesterol when compared with control group except dose 125 mg/kg not shows any significant effect on LDL-cholesterol

(Table 1). Similarly, The significant reduced activities of CAT, SOD, GSH and total thiols and increased levels of LPO were observed in control group animals when compared with normal group where as the different doses of extract and standard Liv-52 treated group animals showed significant (p<0.05) rise in these antioxidant enzymes with decreased levels of NO and LPO when compared with control group (Table 2).

Table 1: Effect of different doses of methanol extract of *Stereospermum suaveolens* on serum biochemical enzymes against Aluminium fluoride induced liver damage in mice.

Group	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TB (mg/dl)	LDL-cholesterol (mg/dl)
Normal	44.95±1.14	57.56±1.14	36.81±1.55	0.35±0.01	32.82±0.45
AlF_4É-treated	179.6±4.34[a]	160.1±10.2[a]	168.2±6.64[a]	1.33±0.05[a]	85.94±1.18[a]
StandardLiv-52	53.29±3.28[*]	63.14±2.90[*]	41.08±2.00[*]	0.42±0.03[*]	34.50±0.61[*]
MES125 mg/kg	98.25±2.17[*]	93.19±2.77[*]	65.11±4.50[*]	0.66±0.03[*]	69.47±1.39
MES250 mg/kg	77.28±2.03[*]	77.23±3.00[*]	61.62±4.25[*]	0.55±0.02[*]	56.86±1.12[*]
MES500 mg/kg	96.98±2.45[*]	91.17±2.17[*]	71.26±3.42[*]	0.60±0.02[*]	61.18±0.92[*]

Values are mean ± SEM, n=7. Data was analyzed by one-way ANOVA followed by Dennett's test.
[*]p<0.05 when compared with control group and [a]p<0.05 when compared with normal group.
(MES - Methanol extract of *Stereospermum suaveolens*)

Table 2: Effect of different doses of methanol extract of *Stereospermum suaveolens* on liver antioxidant enzyme levels against Aluminium fluoride induced liver damage in albino mice.

Group	SOD (U/mg of protein)	CAT (U/mg of protein)	GSH (nM/mg of Protein)	Total thiols (µM/mg of Protein)	LPO (nM/mg of protein)
Normal	258.3±12.2	0.45±0.011	13.36±0.44	18.18±0.68	24.96±1.71
AlF_4É- treated	97.27±5.34[a]	0.19±0.008[a]	6.13±0.30[a]	9.80±0.35[a]	48.15±2.15[a]
StandardLiv-52	231.7±8.10[*]	0.43±0.013[*]	11.61±0.20[*]	17.57±0.36[*]	14.12±0.51[*]
MES125 mg/kg	219.7±7.86[*]	0.36±0.015[*]	9.74±0.09[*]	12.98±0.15[*]	27.65±0.89[*]
MES250 mg/kg	246.2±5.18[*]	0.40±0.005[*]	11.06±0.48[*]	16.93±0.59[*]	27.16±0.62[*]
MES500 mg/kg	229.7±5.65[*]	0.39±0.007[*]	10.45±0.27[*]	14.46±0.54[*]	31.98±0.64[*]

Values are mean ± SEM, n=7. Data was analyzed by one-way ANOVA followed by Dennett's test.
[*]p<0.05 when compared with control group and [a]p<0.05 when compared with normal group.
(MES - Methanol extract of *Stereospermum suaveolens*)

4. Discussion

Reactive oxygen species (ROS) are the intermediate products resulting from univalent reduction of molecular oxygen[22] and these differ significantly in their interactions and can cause extensive cellular damage such as nucleic acid strand scission[26], modification of polypeptides[27] and lipid peroxidation[28]. Many of these free radicals have been also implicated

in the pathology of various human diseases[29]. However antioxidant enzymes like superoxide dismutase[30], catalase[31], and glutathione peroxides[32] (GPX) as well as smaller molecules such as Vitamin E[33] are mainly responsible for the primary defense mechanism against oxidative damage. Several studies have demonstrated that plants produce potent antioxidants and represent an important source of natural antioxidants.

Fluorides are widely distributed throughout the environment in various anthropogenic and natural forms. Apparently, even if F is essential, the dietary requirement is so small that it is easily met by even highly purified diets[34,35]. There was suggested that, drinking high-fluoride water over a long period can damage the liver. Fluoride induced hepatotoxicity due to the formation of free radicals and decreased activity of the antioxidant system and enhanced lipid peroxidation, which involves polyunsaturated fatty acids in hepatocytes of animals and humans have been reported. Oxidative stress produced by free radicals and hydrogen peroxide is greater if fluoride impairs the production of free radical scavengers such as GSH, GSH-Px, SOD, and ascorbic acid[36]. Fluoride exposure also induces histopathological changes in liver involving focal necrosis, infiltration of leucocytes, swelling of Kupffer cells, extensive vacuolisation, hemorrhagic areas, ultrastructural alterations in hepatocytes, and increased apoptosis in animals and humans[37].

The data obtained from our present study showed that, the AlF_4^--treated group animals shows the increased levels of serum biochemical enzymes and reduced activities of antioxidants and increased levels of lipid peroxidation in liver homogenate. The extract pretreatment groups significantly decrease the serum biochemical markers, ameliorate the aluminium fluoride induced changes in mice by increasing the antioxidant enzyme activity and showed lipid protection and neutralize the free radical induced damage. The significant increased levels of superoxide dismutase, catalase and decreased lipid peroxidation in extract treated groups may be due to antioxidant activity of the extract and the increase in hepatic glutathione and total thiols levels may be due to de novo glutathione and total thiols synthesis or increased circulation.

Phytoconstituents like flavonoids, triterpinoids, saponins and alkaloids are known to possess hepatoprotective activity[38]. Phytochemical investigations of methanol extract of stem bark of *Stereospermum suaveolens* revealed the presence of alkaloids, phenols, saponins, flavonoids and tannins. These antioxidant constituents present in methanol extract might be responsible for the free radical scavenging activity, anti-lipid peroxidation. Further work is necessary to isolate active principles and elucidate the actual mechanism involved in the hepatoprotective and antioxidant activity of this plant.

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