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In vivo Antioxidant Activity of *Sida cordifolia* Linn. in $K_2Cr_2O_7$ Induced Oxidative Stress by Measurement of Reactive Oxygen Species Levels in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author MG designed the study and the experimental protocol, analyzed the obtained data and wrote the first draft of the manuscript. Author SP supervised the animal experimentation. Authors NK and ST evaluated the phytochemical parameters and in vivo antioxidant activities. Author SC performed the statistical analysis and validation of experimental data. All authors read and approved the final manuscript.

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ABSTRACT

Ayurvedic textbooks ascribe many therapeutic properties such as anti-inflammatory, antimicrobial and antioxidant actions to *Sida cordifolia* Linn. which contains many polyphenols in its roots and seeds. Evaluation of *in vivo* antioxidant action in rats of the aqueous extract of *Sida cordifolia* Linn. roots has been done by introducing potassium dichromate for creating acute and chronic oxidative stress. The Folin- Ciocalteu reagent was utilized for assessing the phenol content which was determined as 27.375 mg Gallic acid/gm. During the acute toxicity tests using mice, no significant adverse signs or symptoms of toxicity were noticed up to the dose of 1500 mg/kg and no mortality was reported until this dosage. The Phosphomolybdate Assay using 3, 6 and 9 mg/ml of research drug concentrations resulted in determination of the Total Antioxidant Capacity that was found to be 15.40 ±9.13, 19.00 ±7.56 and 21.02 ±8.42 gm equivalents of Ascorbic acid. The results of the d-

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ROMs test which assessed the hydroperoxide levels clearly suggested that introduction of research drug could significantly counter the negative effects of both acute and chronic oxidative stress. Broadly similar findings were obtained by using ELISA kit for assessing ROS values where concurrent administration of the research drug resulted in very significant amelioration in respect of oxidative stress conditions.

Keywords: Sida cordifolia; antioxidant; reactive oxygen species; $K_2Cr_2O_7$; oxidative stress.

1. INTRODUCTION

All plants possess the ability to biosynthesize a variety of non-enzymatic antioxidants that can ameliorate ROS- induced oxidative damage. The antioxidant activity of many plants has been exhibited during the *in vitro* studies. At the same time, in order to translate and validate this therapeutic effect of medicinal plants in the form of *in vivo* efficacy, the plant antioxidants have to be subjected to many more pharmacological evaluations. However, as a primary estimation, the findings obtained during *in vitro* evaluations are frequently utilized as a benchmark in place of standardized *in vivo* antioxidant studies [1].

The present research effort undertakes *in vivo* experimental evaluation of the antioxidant potential of aqueous extract of *Sida cordifolia* Linn. using rats. Chromium (Cr) metal has two valence states – three and six. Out of these two, the hexavalent Cr (VI) has been found in many studies to be quite harmful for animals since it tends to get reduced to lower valence state as Cr (V), Cr (IV) and then Cr (III) by various reducing agents present inside the cells such as enzymes [2]. During these processes of Chromium getting reduced to lower valence states, many reactive oxygen species (ROS) are produced in large amounts which have a deleterious effect upon the vital cellular structures such as proteins, lipids, and even DNA. This overall adverse effect upon the essential cell components which causes their damage and destruction is termed as oxidative stress [3,4,5]. During several laboratory experiments, Potassium Di-chromate ($K_2Cr_2O_7$) which contains Cr (VI), has been shown to produce very high amounts of reactive oxygen radicals. Therefore, potassium dichromate has been used as the oxidative stress agent in this study using two different techniques. In order to generate acute oxidative stress, $K_2Cr_2O_7$ is administered as a single dose, and to produce chronic stress conditions, it is given daily to the experimental animals for 26 days continuously. Some ill-effects of such oxidative stress can be countered by the antioxidants present inside the cells themselves such as cellular enzymes or vitamins. However, external compounds such as phenols and other phyto-chemical derivatives present in many plant extracts when introduced into animal subjects have been shown to neutralize such adverse impacts of oxidative stress quite efficiently during experimentation [6,7,8].

Sida cordifolia, also known by its Hindi name of *Bala*, is a small and erect downy shrub. It belongs to the Malvaceae family and is widely quoted in Ayurvedic medical literature as a very important medicinal plant. Its fruits contain a pair of awns on each carpel while the structure of its leaves is broadly ovate-oblong and chordate-oblong. The root structure consists of a cluster of tap roots which are 5-15 cm long having very few lateral branches. Roots are grayish yellow in color, having bitter taste and no odor. While the seeds and roots of *Sida cordifolia* have been known to possess many important chemicals such as sitosterol, stigmasterol, vasicinol, vasicinone, β- and N-methyl tryptophan, its leaves contain ephedrine and pseudoephedrine. The important pharmacological properties that this plant possesses include anti-microbial, antioxidant, anti-inflammatory, analgesic, hepato-protective and wound healing. In the traditional Ayurvedic system of medicine, this herb has been used for centuries for treatment of ailments such as fever, wounds, skin diseases, joint pain, swelling, inflammation, cardiac diseases, paralysis, lack of sexual desire, etc., [9,10,11,12, 13,14,15].

Oxidative stress is produced when vital cell structures are seriously adversely affected on account of excess generation of Reactive Oxygen Species (ROS), chemically active compounds that contain Oxygen. Typical examples of ROS include hydroxyl groups, different peroxides and superoxides. Under normal conditions, the production of ROS is a normal feature of cell metabolism and does not cause any adverse impact, but external stimulation or changes in the surroundings as well as deliberate introduction of certain foreign elements can cause surge in ROS generation leading to production of harmful intermediates after reacting with water present in the body

through radiolysis. During this process, water is sequentially converted into hydroxyl radical, hydrogen peroxide, superoxide radical and ultimately oxygen. Among these compounds, the hydroxyl radical is very reactive in nature and its interaction with other compounds lead to further formation of free radicals and increase in oxidative stress levels [9].

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The procurement of the primary raw material, namely the *Sida cordifolia* Linn. roots, was done from the authorized herbal drug supplier of the Institute. The identification and authentication of the plant samples was done at the Botanical Survey of India, Howrah, India vide their reference number CNH/Tech II/2016/46 dated 7.9.2016 and a sample of the plant specimen has been preserved in the herbarium at the Dravyaguna departmental museum of the Institute.

2.2 Chemicals

Ferrous Sulphate (ID: 064963) was procured from Sisco Research Laboratories Pvt. Ltd, Mumbai while N, N-diethyl-p-phenylenediamine (ID: 24855816) and Sodium Acetate (ID: 24899538) were purchased from Sigma-Aldrich, Mumbai. Glacial acetic acid (ID: CD3C630227) was obtained from Merck Specialties Pvt. Ltd., Mumbai, while ROS Elisa kit has been purchased from Elabscience (Lot No: AK0016FEB18047).

2.3 Experimental Animals

All animals used for experiments were procured from a Government approved small animal breeder, M/s Satyacharan Ghosh, Kolkata. The in-*vivo* evaluation was done using Swiss albino mice of both sexes weighing around 20-30 gm, and Wistar albino rats of either sex, weighing about 120-130 gm. All animals were housed in standard polypropylene cages and provided standard pellet food and water *ad libitum*. They were acclimatized for 14 days prior to performing any experiments by keeping them under standard environmental conditions with fixed 12 hour light/dark cycles and a temperature of approximately 25℃ in the animal house of IPGAE&R. The Institutional Animal Ethics Committee of the Institute approved the various protocols of animal experimentation before the study vide their memo no. SVP/PG/370/2013 dated 22.3.2013.

2.4 Preparation of Plant Extracts

Before commencing the experiments, *Sida cordifolia* Linn. roots were thoroughly cleaned using potable water, dried under sun and crushed into 40 mesh particle size. The obtained coarse powder was extracted sequentially with chloroform, acetone, petroleum ether (60℃-80℃), ethanol and water using the soxhlet apparatus. A Buckner funnel and Whatman No. 1 filter paper were used to filter these extracts at room temperature. The obtained extract was further concentrated using rotary evaporator at reduced temperature and pressure. The final extracts were carefully stored for subsequent experiments in refrigerator below 10℃. For the purpose of this study, the aqueous extract of *Sida cordifolia* roots was finally used.

2.5 Phenol Content

The Folin- Ciocalteu reagent was used for determining the Total phenol content of the extracts. An amount of 2.5 ml Folin- Ciocalteu's reagent (10%) and 2 ml of 7.5% of sodium carbonate were added to 0.5 ml aliquot of research drug aqueous extract. After 30 minutes of incubation period, the absorbance was read at room temperature at 760 nm colorimetrically. A standard calibration plot was generated at 760 nm using 100, 200, 300, 400, and 500 µg/ml concentrations of Gallic acid and the phenol concentrations in the test samples were estimated from this calibration plot. The Total phenolic content was expressed as mg Gallic Acid Equivalents (GAE). All experiments were repeated thrice and results were expressed as mg Gallic acid equivalents per gm sample extract [9,16,17].

2.6 Total Antioxidant Capacity by Phosphomolybdate Assay

The method of Prieto et al. (1999) was used to evaluate the total antioxidant capacity. The basic principle here is the reduction of Mo (VI)–Mo (V) by the research drug extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. An amount of 0.3 ml of *Sida cordifolia* Linn. aqueous extract in different concentrations (300, 600 & 900 µg/ml) was added with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes

containing the reaction solution were incubated for 90 minutes at 95ºC temperature and thereafter the absorbance was measured after cooling the solution to room temperature using a UV-VIS spectrophotometer (Shimadzu UV 2450) at 695 nm against blank. Alcohol (0.3 ml) in place of the extract was used as the blank. The total antioxidant activity is expressed as the number of gm equivalents of ascorbic acid. The calibration curve was prepared by mixing ascorbic acid (7.5, 15, 30, 60 and 150 µg/ml) with Alcohol [9,18].

2.7 Acute Toxicity Study

OECD guidelines were used to assess the acute toxicity using healthy Swiss *albino* mice. The animals of both sexes were selected by random sampling technique and divided into 5 groups of 6 animals each. A single oral dose of the extract was administered at the level of 300, 600, 900, 1200 and 1500 mg/kg body weight respectively. All the animals were kept under close observation for appearance of toxic symptoms including muscle spasm, tremors, locomotion, convulsions, loss of righting reflex, behavioral changes and mortality for 1, 2, 4, 8 and 24 hours. Prolonged supervision was thereafter continued for a period of 14 days for observing any occurrence of toxic symptoms and mortality [19].

2.8 *In-vivo* Antioxidant Analysis

The assessment of in-vivo antioxidant capacity of aqueous extract of the research drug was done in rats after inducing oxidative stress using potassium dichromate as the oxidative stress agent in two different ways. While acute oxidative stress was produced by administration of a single dose in the animals, chronic oxidative stress was caused through administration of the dose daily up to 26 days following the standard methods [20,21]. The standard methods for experimental determination of Oxygen Reactive Metabolites and Oxygen Reactive Species were used for determining the antioxidant level in the blood serum of animals which was collected from the tail of each animal. Each experimental method was repeated thrice and readings were analyzed in comparison to the control group readings.

The grouping of animals was done in the following manner:

Group A: Control group (administered 10 ml/kg bw, p.o distilled water).

Group B: Administered Potassium Dichromate ($K_2Cr_2O_7$) at 0.4 mg/kg bw, i.p. for 26 days.

Group C: Administered 600 mg/kg bw, p.o Research Drug along with $K_2Cr_2O_7$ at 0.4 mg/kg bw, i.p. for 26 days.

Group D: Administered 600 mg/kg bw, p.o. Research Drug for 26 days.

Group E: Administered 600 mg/kg bw, p.o. Research Drug for 26 days and thereafter Single Dose of $K_2Cr_2O_7$ at 15 mg/kg bw, i.p on 26th day.

Group F: Administered Single Dose of 15 mg/kg bw, i.p $K_2Cr_2O_7$ on 26th day.

Each research animal was closely observed throughout the study and its important parameters such as behavioral symptoms, weight, food intake, mortality, etc., were closely monitored. Blood sample was collected from the rat tails at room temperature on 27th day. From these samples, the blood plasma was separated and stored at 4℃, which was later used for Reactive Oxygen metabolite (DROM) and Reactive Oxygen Species (ROS) assessment [9].

Using the abovementioned methods, chronic oxidative stress was introduced for animals in Groups B and C, and comparison of data between these two groups indicated the efficacy of the research drug in tackling the effect of chronic oxidative stress. Similarly, Groups E and F were subjected to acute oxidative stress and data comparison was made between these two groups. All experimental groups were also compared to the control group to assess the normative oxidative stress levels.

2.8.1 Determination of reactive oxygen metabolites (d-ROMs test)

The d-ROMs test detects the derivatives of reactive oxygen metabolites (d-ROMs) which is used to assess the level of hydroperoxides, which are markers and amplifiers of oxidative damage, produced by the attack of free radicals. The principle of Fenton's reaction is used in the d-ROMs test. Hydroperoxides are converted into radicals that oxidize N,N-diethyl-para-phenylendiamine which can be detected through spectrophotometric procedures as U.CARR. (Carratelli units). In this test, Chromogen (N, N-diethyl-paraphenylendiamine) is used which indicates the oxidative reaction by changing color and intensity of color is measured photometrically at 505 nm. An amount of 20 µl of Serum with 20 µl of FeSO4 and 20 µl of

D.E.P.P.D were added and thereafter 1.94 ml of 0.1 M of Acetate buffer was added, incubated for 1 min and vortexed. The absorbance of the all samples was taken at 505 nm wavelength in the spectrophotometric methods using the Shimadzu no. 2450 instrument which indicates the level of hydro - peroxides available in the sample. The results of the d-ROMs test are expressed in arbitrary units, called Carratelli Units (CARR U), where 1 CARR U is the equivalent of 0.08 mg/ 100 ml of H_2O_2 [22].

2.8.2 Determination of reactive oxygen species by ELISA kit

Reactive Oxygen Species (ROS) are being constantly produced during cellular metabolism and eliminated in the biological system and are required to drive regulatory pathways. Under normal physiological conditions, cells control ROS levels by balancing the generation of ROS with their elimination by scavenging system. However, under oxidative stress conditions, excessive ROS can damage cellular proteins, lipids and DNA, leading to fatal lesions in cells that contribute to carcinogenesis. In particular, one major contributor to oxidative damage is hydrogen peroxide (H_2O_2) which is converted from superoxide that leaks from the mitochondria. Catalase and superoxide dismutase ameliorate the damaging effects of hydrogen peroxide and superoxide by converting these compounds into oxygen and hydrogen peroxide (which is later converted to water), resulting in the production of benign molecules [23].

The ROS level in blood serum of rats was determined using Sandwich-ELISA ROS ELISA kit (Elabscience, Lot No: AK0016FEB18047). The micro ELISA plate is pre-coated with an antibody specific to ROS. Standards or samples are added to the micro ELISA plate and combined with the specific antibody. Then the biotinylated detection antibody specific for ROS and Avid in- Horseradish Peroxidase conjugate is added to each micro plate well successively and incubated. Free components are washed away and the substrate solution is added to each well. Only those wells that contain ROS, biotinylated detection antibody and Avid in-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of Sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectometrically at a wavelength of 450 nm. Since OD value is proportional to the concentration of ROS in the sample, it is calculated from the standard curve [9].

All the reagents were brought to room temperature and mixed thoroughly by gently swirling before pipetting. The assay process has been followed as per instructions contained in the literature kit of the provider.

2.9 Statistical Analysis

The data generated for each group was presented as Mean ± S.E.M. and comparison of each group was done with the control group. Statistical evaluation of data was done following Students' t-test and by ANOVA. The level of significance was fixed between $p < 0.05$ and $p < 0.01$.

3. RESULTS

3.1 Total Phenol Content (TPC)

The standard curve of Gallic acid was used to assess the total phenol concentration in terms of mg Gallic acid equivalent of phenol/gm of *Sida cordifolia*. Using the Folin- Ciocalteu reagent, the Phenol content of *Sida cordifolia* aqueous extract was experimentally evaluated to be 27.375 mg Gallic acid/gm of dry weight of research sample (Gallic acid equivalents or GAE).

3.2 Total Antioxidant Capacity by Phosphomolybdate Assay

Total antioxidant capacity (TAC) was assessed using the Phosphomolybdate Assay as per procedure described above and expressed in terms of ascorbic acid equivalents. The TAC of different concentrations of research drug extract was estimated using the Standard curve for Ascorbic acid as the reference level. The antioxidant capacity was assessed to be 15.40 ±9.13, 19.00 ±7.56 and 21.02 ±8.42 Ascorbic Acid gm equivalents in respect of 3, 6 and 9 mg/ml concentration levels of *Sida cordifolia* aqueous extract (Table 1).

Table 1. Total antioxidant capacity of aqueous extract of *Sida cordifolia*

Concentration (mg/ml)	Anti-oxidant content (µg/mg) equivalent to ascorbic acid
3.0	15.40 ± 9.13
6.0	19.00 ± 7.56
9.0	21.02 ± 8.42

3.3 Acute Toxicity

During the acute toxicity tests, experimental animals showed no noticeable toxic symptoms such as convulsion, irritation, diarrhea, sedation, etc. and no signs of behavioral changes in the aqueous extract up to 1500 mg/kg dose. No mortality was reported during the first 24 hours and during the subsequent 14 days at 1500 mg/kg dose. The therapeutic dosage for the experiments was selected as 600 mg/kg on the basis of occurrence of symptoms and mortality because some physical changes were observed in a few animals between the doses of 600 mg/kg and 1500 mg/kg, but these temporary symptoms subsided within one to two hours and no mortality ultimately occurred.

3.4 *In-vivo* Determination of Reactive Oxygen Metabolites (d-ROM) Anti-oxidant Assay

The d-ROMs test evaluates the oxidative stress in terms of the hydroperoxide concentration expressed in units of CARR as detailed above. The obtained CARR values in case of different groups are shown in Table 2 as well as Fig. 1. The Control Group (Group A) is used as the benchmark for evaluating the oxidative stress level of all other groups under different experimental situations.

It is observed that in case of Group D, which receives only the research drug continuously for 26 days, the CARR values (80 ±3.51) are quite close to those of Group A (78 ±4.81), suggesting that even the regular continuous administration of the research drug does not lead to any appreciable adverse impact upon the oxidative stress condition.

Groups B and C are used to assess and evaluate the impact of chronic form of oxidative stress. Data obtained for Group B indicates that its CARR values (162 ±10.73†) are more than double those in case of Group A, signifying a very high surge in oxidative stress due to continuous administration of $K_2Cr_2O_7$ in animals. At the same time, the CARR values of Group C (83 ±1.47‡) are not very different from those in case of Groups A & D, indicating that the presence of *Sida cordifolia* aqueous extract in animals was able to counteract the adverse effects of potassium dichromate under chronic stress conditions.

The CARR values of Groups E and F were used to assess and compare the effect of acute oxidative stress upon animals on account of administration of $K_2Cr_2O_7$ on the 26th day. It was observed that oxidative stress levels increased drastically due to abovementioned introduction of potassium dichromate on a single day as evidenced from the CARR values in case of Group F (121 ±2.58). However, the low CARR values of Group E (89 ±1.47) imply that the same administration of $K_2Cr_2O_7$ does not lead to any significant enhancement in the oxidative stress condition in this group on account of simultaneous administration of *Sida cordifolia* aqueous extract in the experimental animals.

In brief, the d-ROMs test suggested that the administration of research drug was able to significantly neutralize and ameliorate the adverse effects of $K_2Cr_2O_7$ in both acute and chronic oxidative stress cases. However, the efficacy of *Sida cordifolia* aqueous extract was higher in case of chronic stress as compared to acute stress condition.

3.5 ROS Anti-oxidant Assay (Determination of Reactive Oxygen Species)

The sandwich-ELISA ROS kit was used to determine the optical density (OD) which is

Table 2. CARR values in different group of animals

Group	Treatment protocol	CARR 1 U. CARR = 0.08 mg H_2O_2/dl
A.	Control (distilled water 10 ml/kg bw)	78 ±4.81
B.	$K_2Cr_2O_7$ treated group	162 ±10.73†
C.	Research drug & $K_2Cr_2O_7$ treated group for 26 days	83 ±1.47‡
D.	Research drug treated group for 26 days	80 ±3.51
E.	Research drug for 26 days and single dose of $K_2Cr_2O_7$ treated group on 26^{th} day	89 ±1.47†
F.	Single dose of $K_2Cr_2O_7$ treated group on 26^{th} day	121 ±2.58

Values are presented as Mean ±SEM. n = 6. † p < 0.001; ‡ p = 0.005

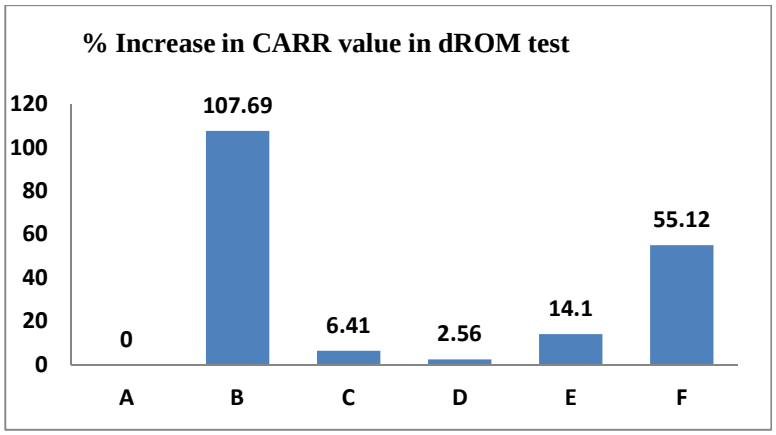

Fig 1. Percentage increase in CARR values in experimental groups

a linear relation of the Reactive Oxygen Species (ROS) concentration. The ROS are produced in experimental animals when $K_2Cr_2O_7$ is introduced to generate oxidative stress during this procedure. The observed OD values at different concentrations are shown in Table 3, which are compared with the standard curve for ROS shown as Fig. 2. Thereafter, the ROS values obtained in respect of the various groups are mentioned in Table 4 and the comparative position is illustrated in Fig. 3.

Daily administering of $K_2Cr_2O_7$ can cause chronic oxidative stress whose impact can be measured by comparing the values of Groups B and C [24]. It is observed that while ROS values increase by more than four times in case of Group B (0.35 ±0.044), they surge only marginally in Group C (0.11±0.021) as compared to the control group. These findings seem to suggest that when *Sida cordifolia* aqueous extract is provided concurrently with potassium dichromate to the experimental animals, the deleterious impact of this oxidative stress agent is eliminated to a very great extent.

Similar results are obtained when acute oxidative stress is created by one-time introduction of $K_2Cr_2O_7$ in research animals on the 26th day. The comparison of ROS values in case of Groups E (0.12 ±0.011) and F (0.30 ±0.047) clearly shows that the oxidative stress levels are around four times in case of Group F while they have increased only marginally in case of Group E. Therefore, the impact of acute oxidative stress seems to get mitigated to a great extent when there is concurrent use of research formulation. At the same time, standalone daily use of the research drug in case of Group D (ROS values 0.10 ±0.022) does not lead to any adverse impact from the oxidative stress point of view.

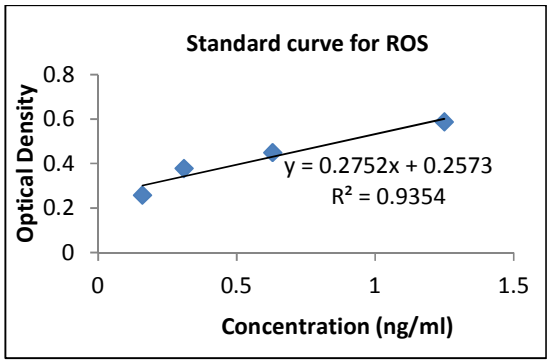

Fig. 2. Standard curve for ROS

Table 3. Absorbance of standard in different concentrations

Concentration (ng/ml)	Optical density
0.16	0.259
0.31	0.379
0.63	0.450
1.25	0.588

4. DISCUSSION

The traditional Ayurvedic medical system relies on use of medicinal plants and extracts of their various parts for treatment of human ailments and for preventive effect as a tonic and rejuvenator. In fact, most medicinal plants report high antioxidant properties which could be the reason for these beneficial effects associated with their usage. One such plant is *Sida cordifolia* which is well known for its utility in treatment of

Table 4. ROS values of different groups

Group	Treatment protocol	ROS equivalent to standard (in ng/ml)
Group A	Control (distilled water 10 ml/kg bw)	0.08 ±0.013
Group B	$K_2Cr_2O_7$ treated group	0.35 ±0.044¶
Group C	Research drug & $K_2Cr_2O_7$ treated group for 26 days	0.11 ±0.021
Group D	Research drug treated group for 26 days	0.10 ±0.022
Group E	Research drug for 26 days and single dose of $K_2Cr_2O_7$ treated group on 26th day	0.12 ±0.011¶
Group F	Single dose of $K_2Cr_2O_7$ treated group on 26th day	0.30 ±0.047

Values are shown mean ± SEM. n = 6. ¶: Values are statistically significant (p < 0.001)

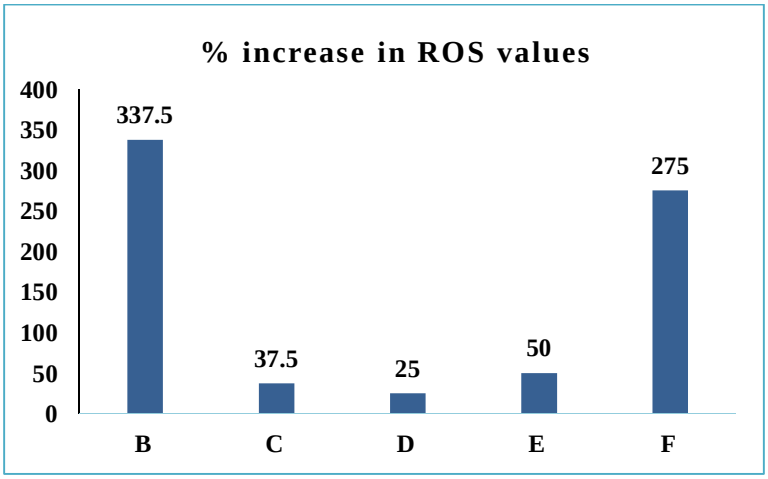

Fig. 3. Percentage of increase in ROS values in experimental groups

various ailments of blood, urinary system, respiratory system, throat, and reproductive system on account of presence of high levels of flavonoids in its roots [11].

Phytochemical and pharmacognostical analysis of *Sida cordifolia* roots revealed the presence of tannins, alkaloids and flavonoids, apart from a number of phenolic compounds. During acute toxicity tests, the administration of research drug did not result in any mortality up to 1500 mg/kg body weight dosage. Evaluation of the total antioxidant capacity in terms of gram equivalents of ascorbic acid was found to be 5.58 ±1.72, 17.83 ±8.38 and 21.92 ±11.72 respectively in respect of 3, 6 and 9 mg/ml concentrations of the research drug.

Since there were no scientific studies regarding the antioxidant efficacy of the research drug in animal experiments, the present study assesses the oxidative stress level by measuring two parameters in blood serum– the Reactive Oxygen Metabolites level (using the d-ROMs test) and the Reactive oxygen species (ROS) concentration. The outcome of the d-ROMs test indicates that Groups B & F where *Sida cordifolia* was not administered to the animals had very high induced oxidative stress in chronic as well as acute stress scenarios, as evidenced by the % increase in CARR from 107.6% in case of Group B to 55.12% in Group F. Similarly, these % CARR increase values were very nominal in case of Group E (14.10 %) and Group C (6.41 %), suggesting that oral daily administration of the research drug over a period of 26 days had mostly mitigated the adverse impact of potassium dichromate.

The antioxidant activity demonstrated during animal experimentation could be on account of the high phenolic concentration in the research drug since these two attributes have been found to be correlated. Most phenolic compounds exhibit strong antioxidant, anticancer and energy boosting properties. A good example of such polyphenolic compounds are flavonoids which are commonly found in fruits, vegetables, nuts, tea, coffee and red wine in abundance [1,16].

The findings of the ROS test indicate significant impact of research drug in neutralizing the malevolent effects of introducing oxidative stress conditions using potassium dichromate under both acute and chronic conditions. Thus, animals that were administered *Sida cordifolia* aqueous extract daily for 26 days exhibited very low increases in oxidative stress, varying from 25% in case of Group D to 37.5% in Group C and 50 % in Group E. On the other hand, group of animals which did not receive the research drug during the study demonstrated very high increases in stress levels ranging from 337.5% in Group B to 275.0% in Group F. Therefore, *Sida cordifolia* aqueous extract seems to play a protective role upon cell health parameters by reducing the production of reactive oxygen species [25].

5. CONCLUSION

The assessment of the *in vivo* antioxidant activity of *Sida cordifolia* aqueous extract using d-ROMs and ROS tests and by Phosphomolybdate methods suggests that it is an effective and significant antioxidant. When either acute or chronic stress is produced by introducing potassium dichromate, the research drug ameliorates its adverse effects to a substantial degree under experimental conditions possibly on account of the high levels of phenols and flavonoids in its extracts.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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