

Antioxidant Activity of Fruit Pulp Powder of *Cassia fistula*

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Abstract

Fruit pulp powder of *Cassia fistula* was investigated for its antioxidant activity both *in vitro* and *in vivo*. Preliminary phytochemical analysis showed high phenolic and flavone content (22 mg/kg and 4 mg/kg respectively) in pulp. A concentration dependent, increase in FRAP value obtained; suggest high antioxidant property of the extract. *In vivo* study in young adult mice fed fruit pulp powder extract (100 mg/ kg/BW single dose daily for 30 days) one hour prior to Combination of stresses (immobilization followed by swimming type) of 2 hrs duration daily up to 30 days, showed significant increase in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) levels in brain, gastronomies muscle, heart, kidney, lung and stomach of these mice as compared to mice given only stress. Levels of malondialdehyde (MDA) were significantly lowered after the drug treatment in all the tissues in stress group mice as compared to control. High antioxidant activity of *Cassia fistula* may be contributed to its high phenolic and flavonoid content.

Keywords: Antioxidant, *C. fistula*, free radicals, mice.

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INTRODUCTION

Cassia fistula Linn. (Leguminosae) is commonly known as Amaltas, Indian Laburnum or pudding pipe tree and Aragvadh. The plant is found in India, Pakistan, Bangladesh, Tropical Africa, South America and the West Indies. It is native of tropical climatic area and is present all over Asia. It is also present in the western tropical region of America. It grows freely all over India. It is a medium sized deciduous tree that reaches the height of about 25 to 30 feet. The bark is of reddish brown in color. Leaves are alternate, pinnate, 1 to 1.5 feet long and possess pairs of four to eight ovate leaflets about 7 to 15 centimeter long and nearly 2, 5 to 3 centimeter broad, This entire petiole is about 3 to 7 millimeter long. It bears yellow colored flowers. That droops down depending upon the length of the rachis. The fruit beared by the amallas tree is pendulous, cylindrical, brown and septate having a length of 25 to 45 centimeters and possess a

diameter of 1 to 3 centimeters. It has within it about 30 to 100 seeds, Seeds are lenticular with red-brownish texture. Tree sheds its leaves in March and April. Fruits arise by rainy season^[1]. Preparation of bark, leaves and flowers are commonly used as household medicine for various ailments.

Leaves of *Cassia fistula* shown to possess *antitussive* and wound healing properties^[2,3]. Antitumor property has also been shown by the seed extract^[4]. Bark of this plant is reported to possess anti-malarial^[5]. and Anti-inflammatory and antioxidant activities^[6]. Similarly antioxidant activity of its flowers has been reported in alloxan induced diabetic rats^[7]. Its antioxidant activity is suggested because of the presence of flavanoids^[8,9]. Rhein glycosides, sinoside A and B in pods are also isolated. A new bioactive flavone glycoside 5,3',4'-tri-hydroxy-6-methoxy-7-O-alpha-L-rhamnopyranosyl-(1 --> 2)-O-beta-D-galactopyranoside with antimicrobial activity was reported by Yadav and Verma^[9]. Four new compounds,

5-(2-hydroxyphenoxyethyl)furfural, (2'S)-7-hydroxy-5-hydroxymethyl-2-(2'-hydroxypropyl) chromone, benzyl 2-hydroxy-3,6-dimethoxybenzoate, and benzyl 2beta-O-D-glucopyranosyl-3,6-dimethoxybenzoate, together with four known compounds, 5-hydroxymethylfurfural, (2'S)-7-hydroxy-2-(2'-hydroxypropyl)-5-methylchromone, and two oxyanthraquinones, chrysophanol and chrysophanein, were also isolated from the seeds of *Cassia fistula* by Kuo et al.,^[10]. *Cassia fistula* is rich in tannin, anthraquinones, rhein I, emodin II, kaempferol and roanthocyanidin^[11,12].

Although various parts i.e., leaves, bark, seed etc., have been studied for their antioxidant property and thus of high medicinal value, but review of literature reveal that pod pulp of the *C. fistula* has not been studied in spite of its common use in Indian household medicines for various ailments. Oxidative stress is an important contributor to the patho-physiology of variety of diseases, including cardiovascular dysfunctions, cancer, diabetes, atherosclerosis, inflammatory diseases and neurodegenerative diseases etc.,^[13,14]. Human body has multiple mechanisms especially enzymatic and non-enzymatic antioxidant systems to protect cells and their constituents against reactive oxygen species (ROS) induced damage^[15]. As the innate defense is not enough against severe or continuous oxidative stress, exogenous antioxidants are required. Although many synthetic antioxidants are in use but many of them have side effects, hence compounds from natural resources need to be identified having potency to prevent ROS induced damage.

In present paper, we have reported the preliminary evidence of antioxidant activity of the pulp powder extract of *C. fistula*. FRAP assay (Ferric reducing antioxidant power assay) was carried in *in vitro*. Antioxidant profile i.e., Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Reduced glutathione (GSH), and Malondialdehyde (MDA) were studied in brain, liver, kidney, muscle, heart, intestine and lung of animals fed pulp powder extract.

MATERIAL AND METHOD

Chemicals:

Sodium acetate trihydrate, acetic acid, EDTA, Pot. Dichromate, FeCl₃ purchased from Hi media (Mumbai, India). Sodium, Sodium Azide, Sodium hydrogen phosphate, Hydrogen peroxide, Glutathione, Di nitrobenzoic acid, Riboflavin, Sodium cyanide, Nitro blue tetrazolium were purchased from SRL, New Delhi, India. Ascorbic acid, 2,4,6-tripyridyl-s-triazine (TPPZ), from Sigma, USA.

Plant collection and identification and extraction

C. fistula pods were collected from trees growing by the roadside in Udaipur, India and were identified by Prof. K.G. Ramawat of the Department of Botany, Mohan Lal Sukhadia University (Udaipur, India). A voucher specimen of the pod was deposited at the herbarium of the department. Pulp was extracted manually from the dried pods by scraping and shade dried at room temperature and ground in an electrical grinder and passed through sieve number 240 to obtain a fine powder of mesh size 60. Soaking 20 gm pulp powder in 100 ml absolute ethanol for 24 h and filtering it through Whatman paper ethanol extract was prepared. Vacuum evaporation yielded 18.35 % w/w of dried extract. Dried extract was stored in the refrigerator till further use.

Phytochemical screening

Total phenolic content and presence of flavonoids in alcohol extract was estimated by methods suggested by Tanner and Brunner^[16] and Zhishen et al.,^[17] respectively.

Ferric reducing antioxidant power (FRAP) assay

Antioxidant activity of *C. fistula* fruit pulp extract was determined by FRAP Assay method of Banzie and Strain^[18]. FRAP assay uses antioxidants as reductant in a redox-linked colorimetric method, employing an easily reduced oxidant system. At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (Which has an intense blue colour) can be monitored, by measuring the change in absorption at 593nm. The change in absorbance is therefore, directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture.

In vivo studies

Departmental Ethical committee as per CPCSEA (Govt. of India) norms (Approval No 973/ac/06/CPCSEA) cleared experiments. Young adult (one month old) Swiss albino inbred mouse strain (n = 40) was used for the study. Animals were randomized and divided into Control (C) and three Experimental groups (E-I, E-II and E-III), each comprising of ten animals and maintained in standard laboratory conditions. Control animals were fed normal diet. Animals of E-I group were subjected to 2 hrs combination of stress (immobilization followed by swimming), daily up to 30 days. This practice was necessary to facilitate the absorption of drug through gastro intestinal tract. E-II animals were fed pulp powder

(100 mg/kg/BW) daily and after one hour were subjected to similar stress. E-III animals were fed pulp powder but were not subjected to stress.

Preparation of tissue and assay of SOD/CAT/GPx/GSH/MDA:

Animals were decapitated and brain, gastronomies muscle, heart, intestine, kidney, lung, liver and stomach were dissected out and placed on chilled glass plate. Each organ was minced thoroughly with the surgical blade. The minced tissue was homogenized thoroughly for 15 min and centrifuged at 10,000 rpm for 15 min. The supernatant was used for assay of SOD [19], CAT [20], GPx [21], GSH [22] and MDA [23].

Statistical Analysis:

One way Analysis of Variance (ANOVA) was used to compare the means of all 4 groups. Tukey's Multiple Comparison Test (Post hoc) test was applied to identify pairs of groups, which differed significantly when the omnibus ANOVA was significant. The Tukey's Multiple Comparison Test (Post hoc) test was applied because all the groups were having equal number of replicates. All the statistical test were performed by using the program GraphPad Prism 3 to assess the effect of experimental condition.

RESULTS

Preliminary phytochemical screening of the extract of *C. fistula* pulp powder revealed the presence of various bioactive components of which phenolic (22mg/kg) and flavonoid (4mg/kg) were most prominent.

In present study, a concentration dependent increase in FRAP values for the pulp powder extract were observed (Table.1). The free radical scavenging activity of the extracts was evaluated based on the ability to reduce the ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. Reduced ferrous form of ferric tripyridyl triazine shows a strong absorption band at 593 nm in visible spectrum (deep blue colour). As the electron became paired of in the presence of free radical scavenging, the absorption increases and the resulting intensity of color stoichiometrically coincides with respect to the number of electrons taken up. The increased absorption is representative of the capacity of the test drugs to scavenge free radicals independently.

Table-1: Antioxidant Activity of the Fruit Pulp Extract by FRAP Assay.

| S. No. | Sample Concentration (Fruit Pulp) | FRAP (μ mol/kg) |
|--------|-----------------------------------|-----------------|
| 1 | 0.1% | 228 |
| 2 | 1.0% | 1028 |
| 3 | 10.00% | 10280 |

Superoxide Dismutase (SOD):

Transfer of a single electron to O₂ generates the potentially damaging superoxide anion free radical (O₂⁻), the destructive effects of which are amplified by its giving rise to free radical chain reactions. The ease with which superoxide can be formed from oxygen in tissues and the occurrence of Superoxide dismutase, the enzyme responsible for its removal in all aerobic organisms (although not in obligate anaerobes) indicate that the potential toxicity of oxygen is due to its conversion to superoxide.

Application of one way ANOVA depicts that levels of Superoxide Dismutase altered significantly after the stress and dose treatment (F=43.5, P<0.0001) in brain. Tukey's Multiple Comparison Test (Post hoc) revealed that there is a significant difference between the groups for Brain (Control and stress MD= 58.9, P<0.001; Control and Stress+Dose MD= 20.0, P<0.05; Stress and Stress+Dose MD= -38.9, P<0.001; Stress and Dose MD= -62.8, P<0.001; Stress+Dose and Dose MD= -23.9, P<0.01) except when control was compared to dose (MD= -3.9, P>0.05). Similarly when SOD content was analyzed in stomach One way ANOVA depicted significant difference (F=59.2, P<0.0001). Post hoc analysis between the experimental and control groups exhibited that superoxide dismutase altered significantly (Control and stress MD= 56.7, P<0.001; Control and Stress+Dose MD= 32.2, P<0.05; Stress and Stress+Dose MD= -24.4, P<0.001; Stress and Dose MD= -44.4, P<0.001; Stress+Dose and Dose MD= -20.0, P<0.01). Expected non significant difference was found between control and dose (MD= 12.2, P>0.05). SOD content in Intestine was significantly different (F=13.9, P<0.0001). Comparison between groups illustrates that non significant mean difference between Control and Dose (MD= 15.0, P>0.05) Stress+Dose and Dose (MD=-11.1, P>0.05). Rest of all other groups exhibited significant mean difference (Control and Stress MD=55.0, P<0.001; Control and Stress+Dose MD=26.2, P<0.05; Stress and Stress+Dose MD= -28.9, P<0.05; Stress and Dose MD= -40.0, P<0.001). Superoxide Dismutase content difference in lungs was significant (F=46.8, P<0.0001). Comparison of groups when analyzed with post hoc test was found to

be significantly different in all the groups ($P < 0.001$). SOD concentration in Liver was significantly different ($F = 514$, $P < 0.001$). Tukey's Multiple Comparison Test revealed that mean difference between the experimental and control groups was significant except Control Vs Dose ($MD = 3.86$, $P > 0.05$). Comparison between all other groups was significant ($P < 0.001$). Analysis of Kidney revealed significant difference ($F = 236$, $P < 0.001$). Post hoc analysis exhibited significant mean difference between all groups. Control and Stress, Control and Stress+Dose, Control and Dose, Stress and Stress+Dose were highly significant ($P < 0.001$). Stress and Dose, Stress+Dose and Dose were significant ($P < 0.01$). SOD concentration in Muscle was significantly different ($F = 333$, $P < 0.001$). Tukey's Multiple Comparison Test revealed that mean difference between the experimental and control groups were significant ($P < 0.001$). Superoxide Dismutase content difference in Heart was significant ($F = 100.0$, $P < 0.0001$). Comparison of groups when analyzed with post hoc test was found to be significantly different in control and experimental groups. Control Vs Stress ($MD = 51.7$, $P < 0.001$); Control Vs Stress+Dose ($MD = 10.6$, $P < 0.05$), Stress Vs Stress+Dose ($MD = -41.1$, $P < 0.001$); Stress Vs Dose ($MD = -47.8$, $P < 0.001$). Comparison of Control and Dose ($MD = 3.89$, $P > 0.05$) Stress+Dose Vs Dose ($MD = -6.66$, $P > 0.05$) was non significant.

Catalase (CAT):

Hydroperoxidases use hydrogen Peroxide or an organic peroxide As substrate. Two type of enzymes found both in animals and plants fall into this category: peroxidases and catalase. Hydroperoxidases protect the body against harmful peroxides. Accumulation of peroxides can lead to generation of free radicals, which in turn can disrupt membranes and plays role in causation of disease.

Catalase is a hemoprotein containing four heme groups. In addition to possessing peroxidase activity, it is able to use one molecule of H_2O_2 as a substrate electron donor and another molecule of H_2O_2 as an oxidant or electron acceptor. Under most conditions in vivo, the peroxidase activity of catalase seems to be favored. Catalase is found in blood, bone marrow, mucous membranes, kidney, and liver. Its function is assumed to be the destruction of hydrogen peroxide formed by the action of oxidases.

Effect of stress and dose treatment altered catalase levels significantly. After stress treatment catalase levels exhibited decrease in concentration and when stressed animals were treated with dose levels of enzyme were commensurate to control animals. This trend was pervasive in all tissues and was significant although post hoc analysis depicted variations in significance level.

Application of one way ANOVA depicts that levels of Catalase altered significantly after the stress and dose treatment ($F = 204$, $P < 0.0001$). Tukey's Multiple Comparison Test (Post hoc) revealed that there is a significant difference between the groups for Brain (Control and stress $MD = 29$, $P < 0.001$; Control and Stress+Dose $MD = 5.0$, $P < 0.05$; Control and Dose $MD = -9.25$, $P < 0.001$; Stress and Stress+Dose $MD = -24.0$, $P < 0.001$; Stress and Dose $MD = -38.3$, $P < 0.001$; Stress+Dose and Dose $MD = -14.3$, $P < 0.001$). When CAT content was analysed in stomach One way ANOVA resulted into significant difference ($F = 269$, $P < 0.0001$). Post hoc analysis between the experimental and control groups exhibited that change in catalase concentration was significant (Control and stress $MD = 40.5$, $P < 0.001$; Control and Stress+Dose $MD = 17.3$, $P < 0.001$; Stress and Stress+Dose $MD = -23.3$, $P < 0.001$; Stress and Dose $MD = -39.5$, $P < 0.001$; Stress+Dose and Dose $MD = -16.3$, $P < 0.001$). Non significant difference was found between control and dose ($MD = 1.0$, $P > 0.05$). Analysis of Catalalase content inferred that there is significant difference ($F = 248$, $P < 0.0001$). Post hoc test between control and experimental groups upshot into significant difference between groups, excluding Control Vs Stress+Dose ($MD = 2.25$, $P > 0.05$). Catalase activity was significantly decreased in lungs after stress treatment and up concentration trend was found after drug treatment and all this was found to be significant after one way ANOVA ($F = 145$, $P < 0.0001$). Post hoc analysis represented significant mean difference among the groups (Control Vs Stress $MD = 30.5$, $P < 0.001$; Control Vs Stress+Dose $MD = 13.8$, $P < 0.001$; Stress Vs Stress+Dose $MS = -16.8$, $P < 0.001$; Stress Vs Dose $MD = -33.8$, $P < 0.001$; Stress+Dose Vs Dose $MD = -17.0$, $P < 0.001$) but when control was compared with dose no significant difference was observed ($MD = -3.25$, $P > 0.05$). Liver concentration of catalasewas altered significantly after stress and drug treatment ($F = 146.0$, $P < 0.0001$). Comparison between groups revealed that Mean concentration of catalase altered significantly except Control Vs Dose ($MD = -2.25$, $P > 0.05$). Catalase change in kidney was significantly altered after stress and drug treatment ($F = 162$, $P < 0.001$). Comparison among groups has revealed that mean difference was significant in Control Vs Stress ($MD = 29$, $P < 0.001$) Stress Vs Stress+Dose ($MD = -26.5$, $P < 0.001$) and Stress Vs Dose ($MD = -29.8$, $P < 0.001$). No significant difference was found in Control Vs Stress+Dose, Control Vs Dose and Stress+Dose Vs Dose). Muscles analyzed for catalase content exhibited significant difference ($F = 160$, $P < 0.001$). Comparison among groups was also significant at $P < 0.001$ except Control Vs Dose. Concentration of catalase in heart muscle altered significantly after stress and *Cassia fistula* fruit pulp extract treatment ($F = 289$, $P < 0.0001$). Post

hoc analysis revealed that mean difference was significant among following groups; Control Vs Stress (MD= 36.3, $P < 0.001$) Control Vs Dose (MD= -5.25, $P < 0.05$) Stress Vs Stress+Dose (MD= -38.4, $P < 0.001$) and Stress Vs Dose (MD= -41.5, $P < 0.001$). No significant difference was observed in Control Vs Stress+Dose (MD= -2.16, $P > 0.05$) and Stress+Dose Vs Dose (MD= -3.09, $P > 0.05$).

Glutathione Peroxidase (GPx):

The enzyme glutathione peroxidase, containing selenium as a prosthetic group, catalyzes the destruction of H_2O_2 and lipid hydroperoxides by reduced glutathione, protecting membrane lipids against oxidation by peroxides. Also act on reduced glutathione (GSH) and H_2O_2 to produce oxidized glutathione (GSSG) and H_2O ; this enzyme can also use other peroxides as substrates. $OH\cdot$ and OH^- can be formed from H_2O_2 in a nonenzymatic reaction catalyzed by Fe_2^+ (the Fenton reaction). O_2 and H_2O_2 are the substrates in the iron-catalyzed Haber-Weiss reaction (reaction that also produces $OH\cdot$ and OH^-).

In the present study Glutathione peroxidase content was significantly decrease after stress treatment and this was further increased after the *Cassia fistula* fruit pulp extract treatment in all the tissues.

After stress treatment 40 percent decrease was noted in stressed animals brain as compared to control. This decrease was recovered after dose treatment in animals, which were subjected to stress and dose simultaneously. One way ANOVA revealed significant difference in Glutathione content in Brain ($F=8.50$, $P < 0.0002$). Comparison among groups demonstrated that there is significant difference between Control Vs Stress (MD= 1.65, $P < 0.05$) and Stress Vs Dose ($F= -2.95$, $P < 0.001$). Remaining all the groups Control Vs Stress+Dose, Control Vs Dose, Stress Vs Stress+Dose and Stress+Dose Vs Dose did not exhibit significant difference. Stomach depicted comparatively lesser significant data in comparison to brain F value was 7.00 and $P < 0.0008$. Post hoc analysis resulted in significant difference among following groups Control Vs Stress (MD= 3.2, $P < 0.001$), Stress Vs Stress+Dose (MD= -2.0, $P < 0.05$), Stress Vs Dose (MD= -2.0, $P < 0.05$). Control Vs Stress+Dose (MD=1.2, $P > 0.05$) Control Vs Dose (MD=1.2, $P > 0.05$) and Stress+Dose Vs Dose resulted in non significant mean difference. Similar trend was observed for intestine. One way ANOVA analysis was significant ($F=9.58$, $P < 0.0001$). But post hoc analysis for Control Vs Stress+Dose Control Vs Dose and Stress+Dose Vs Dose resulted in non significant mean difference although significant mean difference was observed for Control Vs Stress (MD= 1.85, $P < 0.01$), Stress Vs Stress+Dose (MD= -1.5, $P < 0.05$), Stress Vs Dose (MD= -2.35, $P < 0.001$). Lungs

depicted approximately 51.6 percent decrease in GPx content this was further returned to values comparable to control animals after treatment with drug. This change was significant ($F=46.34$, $P < 0.0001$). Post hoc analysis resulted into significant mean difference in all the experimental groups except Control Vs Dose.

Reduced glutathione (GSH):

Reduced glutathione removes H_2O_2 in a reaction catalyzed by glutathione peroxidase, an enzyme that contains the selenium analogue of cysteine (selenocysteine) at the active site. This reaction is important, since accumulation of H_2O_2 may decrease the life span of the cell by causing oxidative damage to the cell membrane, leading to cell death.

Treatment with stress and dose significantly altered reduced glutathione levels in all the tissues. This was decreased by 33.34 percent in brain, 41.72 in stomach, 43.23 in intestine, 15.78 in lungs, 47.32 in liver, 39.83 in kidney, 33.07 in muscle and in heart it was decreased by 41.93 percent. After dose treatment in brain (42.35%), stomach (58.02%), intestine (55.26%), Lungs (41.66%), liver (40.56%), kidney (47.29%), muscle (28.23) and in heart (52.65%) increase in reduced glutathione content was observed. One way ANOVA revealed significant difference in brain ($F=19.28$, $P < 0.0001$), stomach ($F=29.87$, $P < 0.001$), intestine ($F=188.7$, $P < 0.0001$), lungs ($F=9.51$, $P < 0.001$) liver ($F=19.91$, $P < 0.0001$), kidney ($F=18.60$, $P < 39.83$), muscle ($F=19.98$, $P < 0.008$), heart ($F=67.69$, $P < 0.001$).

Post hoc analysis of brain revealed significant mean difference in control Vs Stress (MD=2.15, $P < 0.001$), Stress Vs Stress+Dose (MD= -1.8, $P < 0.001$) and in Stress Vs Dose (MD= -1.65, $P < 0.001$) and non significant mean difference among Control Vs Stress+Dose (MD= 0.35, $P > 0.05$), Control Vs Dose (MD=0.05, $P > 0.05$), and Stress+Dose Vs Dose (MD= -1.5, $P > 0.05$).

In stomach significant mean difference in control Vs Stress (MD=2.9, $P < 0.001$), Control Vs Dose (MD= 0.95, $P < 0.05$), Stress Vs Stress+Dose (MD= -2.35, $P < 0.001$) and in Stress Vs Dose (MD= -1.95, $P < 0.001$) and non significant mean difference among Control Vs Stress+Dose (MD= 0.55, $P > 0.05$), and Stress+Dose Vs Dose (MD= 0.40, $P > 0.05$) was observed after Tukey's Multiple Comparison Test (Post hoc) test.

Comparison of groups with post hoc test for intestinal tissue revealed significant mean difference in all the groups ($P < 0.001$) except Stress+Dose Vs Dose (MD= -0.15, $P > 0.05$). Post hoc analysis for lungs depicted significance at two levels Stress Vs Stress+Dose (MD= -2.0) and Stress+Dose (MD= -1.3) were significant at $P < 0.001$ and Control Vs Stress (MD=0.90) and Control Vs Stress+Dose (MD= -1.1)

were significant at $P < 0.05$. Control Vs Dose (MD= -4.0) and Stress+Dose Vs Dose (MD= 0.70) were non significant. Post hoc analysis for liver exhibited significant difference among groups viz. Control Vs Stress (MD=4.8, $P < 0.001$), Control Vs Stress+Dose (MD=2.65) and Stress+Dose Vs Dose (MD= -2.8) were significant at $P < 0.01$; Stress Vs Stress+Dose (MD= -2.15) Stress Vs Dose (MD= -4.95) were having P value less than 0.05. Control Vs Dose (MD= -0.15) was found to be non significant. Tukey's test for kidney depicted significance level $P < 0.01$ for Control Vs Stress (MD=2.45), Stress Vs Dose (MD= -3.10). Stress Vs Stress+Dose (MD= -1.75, $P < 0.01$) and Stress+Dose Vs Dose (MD= -1.35, $P < 0.05$) were also found to be significant. Control Vs Stress+Dose and Control Vs Dose was non significant. Post hoc analysis for muscle was significant for all the groups except Control Vs Dose. Mean difference for heart exhibited significance between $P < 0.001$ and $P < 0.05$ for all the groups.

Malondialdehyde (MDA):

Lipid peroxidation has often been regarded simply as an undesirable side reaction, but it is also a normal part of metabolism. Lipid radicals also readily react with oxygen, with the subsequent process, termed lipid peroxidation, producing damage to the membranes and enzymes. The resulting lipid peroxy radicals decompose to aldehydes, the most abundant being malondialdehyde and 4-hydroxy-2,3-nonenal.

After treatment with stress and *Cassia fistula* fruit pulp powder malondialdehyde content was increased in all the tissues analyzed. One way ANOVA depicted that increase was significant at $P < 0.0001$ in all the tissues. Post hoc analysis for brain exhibited significant mean difference when analysis was performed among groups. Only Control Vs Dose was found to be non significant. When same test was applied for stomach all the comparisons among the groups resulted into significant mean difference ($P < 0.001$). Intestinal tissue was affected in the same manner as stomach but significance level varied between $P < 0.001$ and 0.05. Stress+Dose Vs Dose was not significant for intestine. Post hoc for other tissues namely lungs, kidney, liver and muscle was not much promising in all the tissues comparison between Control Vs Stress+Dose, Control Vs Dose and Stress+Dose Vs Dose was non significant. Rest all the comparisons between Control Vs Stress, Stress Vs Stress+Dose and Stress Vs Dose were significant at $P < 0.001$. Heart followed the same pattern as it was for intestine. Significant mean difference was found between Control Vs Stress ($P < 0.001$), Control Vs Dose ($P < 0.01$), Control Vs Stress+Dose ($P < 0.01$), Stress Vs Stress+Dose ($P < 0.001$), Stress Vs Dose ($P < 0.001$) but

found to be non significant for Stress+Dose Vs Dose ($P > 0.05$).

DISCUSSION

In present study, a significant decrease in SOD, CAT, GPX and reduced GSH activity was observed in all tissues of mice subjected to combination of stress as compared to control. A significant increase in SOD, CAT, GPx, and reduced glutathione levels were observed in all the tissues of stressed animals fed with pulp powder extract. In tissues of stressed mice, a significant increase in MDA level was observed which was significantly inhibited after feeding pulp powder extract. Highly significant increase in SOD activity was noticed in lung, liver, kidney, lungs and muscle; CAT activity was observed in all the tissues except kidney. Increase in GPx and GSH was not much promising after post hoc analysis. Measurement of Lipid peroxidation was found to be more significant in brain, liver and intestine as compared to other tissues analyzed. This suggests that as leaves, bark or flowers^[6,7,24,25], pulp of *C. fistula* pod also has potent antioxidant power. Acute or chronic stress negatively alters antioxidant status of body tissues. Excess release of glucocorticoids during severe stress is related with release of free radicals this ultimately alters endogenous antioxidant status of body tissues including brain, liver, muscle, kidney etc.,^[13, 14]. This in vitro and vivo antioxidant activity has been of the *C. fistula* has been further supported by other workers who reported dose dependent protective effects of aqueous and methanolic extract of the *C. fistula* bark against lipid peroxidation and free radical generation in liver and kidney homogenates^[6]. Amongst the vegetative organs of *C. fistula*, bark has the highest antioxidant potential followed by the old leaves, young leaves and the twigs^[9, 24]. Both phenolic and flavonoid components are reported to be associated with antioxidative action in biological system as they scavenge singlet oxygen and free radicals^[26, 27]. Polyphenolic contents have been reported to function as good electron and hydrogen atom donors and therefore should be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products^[28]. Free radicals formed in biological systems have been implicated as a highly damaging species, capable of damaging almost every molecule found in living cells. These radicals have capacity to join nucleotides in DNA and cause strand breakage; in addition they are initiators of lipid peroxidation process, abstracting hydrogen atoms from fatty acids in biological membranes. Peroxidation inhibiting activity has been reported in various solvent extracts of different parts of *C. fistula*^[24].

Table-2: Superoxide dismutase (SOD) activity in various tissues of control and experimental animals.

| Group | Treatment | Super oxide dismutase (SOD) (Percentage inhibition of NBT reduction ± SD) | | | | | | | |
|-------|-------------|---|---------------------------|---------------------------|---------------------------|---------------------------|----------------------------|---------------------------|----------------------------|
| | | Brain | Stomach | Intestine | Lungs | Liver | Kidney | Muscle | Heart |
| I. | Control | 76.66±6.41 | 72.77±16.04 | 72.21±26.94 | 86.33±0.39 | 95.27±0.32 | 87.22±3.21 | 81.11±1.28 | 76.66±3.85 |
| II. | Stress | 17.77±3.85 ⁺⁺⁺ | 16.11±5.77 ⁺⁺⁺ | 17.22±4.49 ⁺⁺ | 20.55±1.92 ⁺⁺ | 27.77±3.85 ⁺⁺⁺ | 30.00±16.67 ⁺⁺⁺ | 30.44±1.79 ⁺⁺⁺ | 24.99±4.49 ⁺⁺⁺ |
| III. | Stress+Dose | 56.66±19.24 ⁺⁺ | 40.55±7.05 ⁺⁺ | 46.11±12.18 ⁺⁺ | 56.66±6.41 ⁺⁺⁺ | 81.11±6.42 ⁺⁺⁺ | 72.78±8.34 ⁺⁺⁺ | 56.66±6.41 ⁺⁺⁺ | 66.11±10.90 ⁺⁺⁺ |
| IV. | Only Dose | 80.56±16.03 ⁺⁺⁺ | 60.55±5.77 ⁺⁺⁺ | 57.22±22.45 ⁺ | 68.89±3.84 ⁺⁺⁺ | 91.11±3.84 ⁺⁺⁺ | 64.44±2.57 ⁺⁺ | 67.22±1.93 ⁺⁺⁺ | 72.77±7.05 ⁺⁺⁺ |

All values are represented as Mean ± SD (n = 10)

P value:

+ < 0.001;

++ < 0.01;

+++ < 0.05 When compared with control untreated animals.

Table-3: Catalase (CAT) activity in various tissues of control and experimental animals.

| Group | Treatment | Catalase (µ Moles of H ₂ O ₂ utilized /min./mg of protein ± SD) | | | | | | | |
|-------|-------------|---|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | | Brain | Stomach | Intestine | Lungs | Liver | Kidney | Muscle | Heart |
| I. | Control | 48.50±4.04 | 57.75±4.33 | 45.00±3.46 | 58.75±4.33 | 66.25±6.06 | 48.50±4.04 | 62.00±4.62 | 54.50±4.04 |
| II. | Stress | 19.50±1.73 ⁺⁺⁺ | 17.25±1.44 ⁺⁺⁺ | 15.00±1.15 ⁺⁺⁺ | 28.25±2.60 ⁺⁺⁺ | 28.25±2.60 ⁺⁺⁺ | 19.50±1.73 ⁺⁺⁺ | 25.75±2.02 ⁺⁺⁺ | 18.25±1.44 ⁺⁺⁺ |
| III. | Stress+Dose | 43.50±2.89 ⁺⁺⁺ | 40.50±2.89 ⁺⁺⁺ | 42.75±3.18 ⁺⁺⁺ | 45.00±3.46 ⁺⁺⁺ | 51.25±3.75 ⁺⁺⁺ | 46.00±3.46 ⁺⁺⁺ | 42.75±3.17 ⁺⁺⁺ | 56.66±3.17 ⁺⁺⁺ |
| IV. | Only Dose | 57.75±4.33 ⁺⁺⁺ | 56.75±4.33 ⁺⁺⁺ | 51.25±3.75 ⁺⁺⁺ | 62.00±4.62 ⁺⁺⁺ | 68.50±5.20 ⁺⁺⁺ | 49.25±3.75 ⁺⁺⁺ | 66.25±6.02 ⁺⁺⁺ | 59.75±4.33 ⁺⁺⁺ |

All values are represented as Mean ± SD (n = 10)

P value:

+ < 0.001;

++ < 0.01;

+++ < 0.05 When compared with control untreated animals.

Table 4: Glutathione peroxidase (GPx) activity in various tissues of control and experimental animals.

| Group | Treatment | Glutathione peroxidase (GPX) (Moles of GPx utilized /mg protein/min ± SD) | | | | | | | |
|-------|-------------|---|-------------------------|------------------------|--------------------------|--------------------------|--------------------------|-------------------------|--------------------------|
| | | Brain | Stomach | Intestine | Lungs | Liver | Kidney | Muscle | Heart |
| I. | Control | 4.05±0.87 | 4.65±2.48 | 4.25±0.87 | 4.45±0.52 | 8.10±1.62 | 4.65±0.40 | 4.65±0.40 | 5.35±1.67 |
| II. | Stress | 2.40±0.35 ⁺ | 1.45±0.40 ⁺ | 2.40±0.69 ⁺ | 2.15±0.63 ⁺⁺ | 3.40±0.11 ⁺⁺ | 1.80±0.46 ⁺⁺⁺ | 2.70±0.69 ⁺⁺ | 2.00±0.23 ⁺⁺ |
| III. | Stress+Dose | 3.95±1.10 ⁺ | 3.45±1.33 ⁺ | 3.90±0.92 ⁺ | 3.65±0.63 ⁺ | 4.35±0.40 ⁺⁺ | 5.05±1.79 ⁺ | 3.60±0.23 ⁺ | 2.80±0.11 ⁺⁺⁺ |
| IV. | Only Dose | 5.35±2.02 ⁺ | 3.45±0.98 ⁺⁺ | 4.75±1.33 ⁺ | 5.05±0.40 ⁺⁺⁺ | 6.80±1.50 ⁺⁺⁺ | 4.35±0.17 ⁺⁺⁺ | 4.60±0.11 ⁺⁺ | 4.10±0.35 ⁺⁺⁺ |

All values are represented as Mean ± SD (n = 10)

P value:

+ < 0.001;

++ < 0.01;

+++ < 0.05 When compared with control untreated animals.

Table 5: Reduced glutathione (GSH) in various tissues of control and experimental animals.

| Group | Treatment | Reduced Glutathione (GSH) (GSH mg/g of tissue ± SD) | | | | | | | |
|-------|-------------|---|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|-------------------------|--------------------------|
| | | Brain | Stomach | Intestine | Lungs | Liver | Kidney | Muscle | Heart |
| I. | Control | 6.40±0.46 | 6.95±0.17 | 6.70±0.35 | 5.70±0.46 | 10.10±2.31 | 6.15±1.21 | 6.35±0.75 | 6.90±0.81 |
| II. | Stress | 4.25±0.29 ⁺⁺⁺ | 4.05±0.87 ⁺⁺⁺ | 3.80±0.35 ⁺⁺⁺ | 4.80±0.35 ⁺ | 5.30±0.35 ⁺⁺ | 3.70±0.35 ⁺⁺ | 4.25±0.87 ⁺ | 3.80±0.23 ⁺⁺⁺ |
| III. | Stress+Dose | 6.05±0.87 ⁺⁺ | 6.40±0.81 ⁺⁺ | 5.90±0.23 ⁺⁺⁺ | 6.80±1.50 ⁺ | 7.45±0.75 ⁺⁺ | 5.45±1.10 ⁺ | 5.45±0.29 ⁺ | 5.85±0.40 ⁺⁺⁺ |
| IV. | Only Dose | 5.90±0.81 ⁺⁺ | 6.00±0.69 ⁺ | 6.05±0.06 ⁺⁺⁺ | 6.10±0.23 ⁺⁺⁺ | 10.25±2.02 ⁺⁺ | 6.80±0.81 ⁺⁺⁺ | 6.30±0.58 ⁺⁺ | 5.15±0.17 ⁺⁺⁺ |

All values are represented as Mean ± SD (n = 10)

P value:

+ < 0.001;

++ < 0.01;

+++ < 0.05 When compared with control untreated animals.

Table 6: Malondialdehyde (MDA) in various tissues of control and experimental animals.

| Group | Treatment | Malondialdehyde (MDA) (n moles / ml ± SD) | | | | | | | |
|-------|-------------|---|--------------------------|-------------------------|------------------------|-------------------------|------------------------|-------------------------|-------------------------|
| | | Brain | Stomach | Intestine | Lungs | Liver | Kidney | Muscle | Heart |
| I. | Control | 1.35±0.01 | 0.79±0.29 | 0.91±0.37 | 1.95±0.64 | 2.50±0.30 | 1.74±0.74 | 1.47±0.90 | 1.54±0.17 |
| II. | Stress | 3.13±0.46 ⁺⁺⁺ | 4.07±0.21 ⁺⁺⁺ | 3.89±1.03 ⁺⁺ | 4.14±1.18 ⁺ | 4.91±0.88 ⁺⁺ | 4.25±1.33 ⁺ | 4.04±0.85 ⁺⁺ | 3.31±0.66 ⁺⁺ |
| III. | Stress+Dose | 2.45±0.29 ⁺ | 2.44±0.37 ⁺⁺⁺ | 2.17±0.26 ⁺ | 2.65±0.05 ⁺ | 2.59±0.61 ⁺⁺ | 1.98±0.63 ⁺ | 2.31±0.46 ⁺ | 2.33±0.32 ⁺ |
| IV. | Only Dose | 1.21±0.02 ⁺⁺⁺ | 1.58±0.18 ⁺⁺⁺ | 1.70±0.25 ⁺⁺ | 2.45±0.47 ⁺ | 1.99±0.62 ⁺⁺ | 2.04±0.29 ⁺ | 2.23±0.15 ⁺⁺ | 2.29±0.31 ⁺ |

All values are represented as Mean ± SD (n = 10)

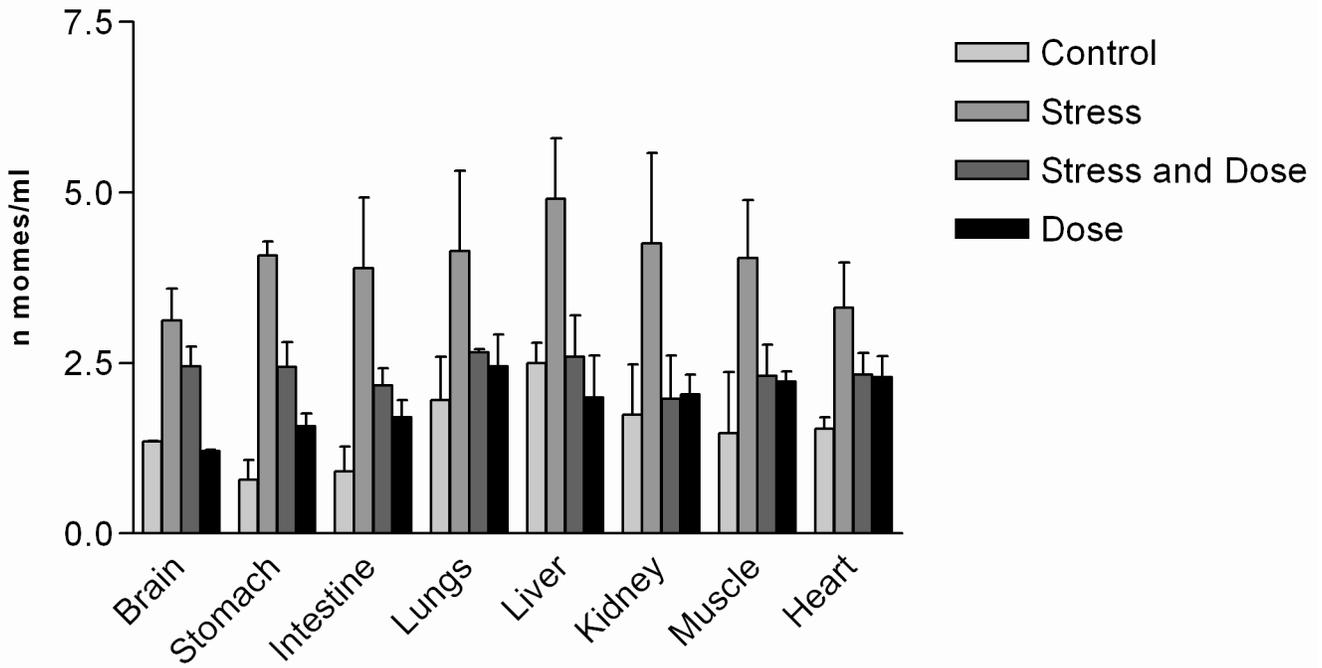
P value:

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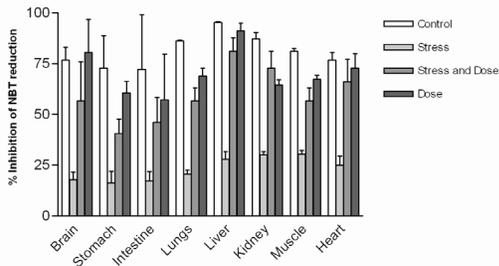
++ < 0.01;

+++ < 0.05 When compared with control untreated animals.

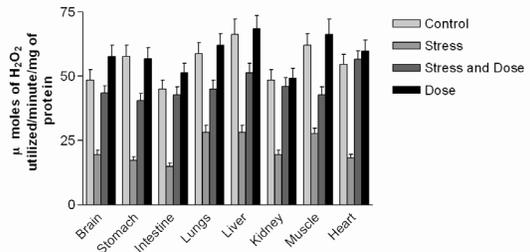
Malanoldialdehyde



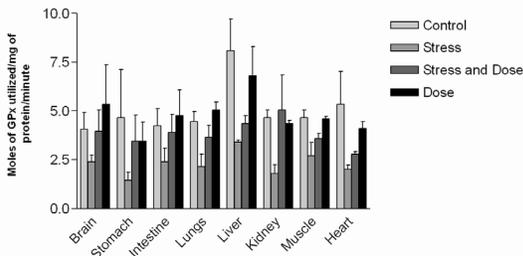
Superoxide Dismutase



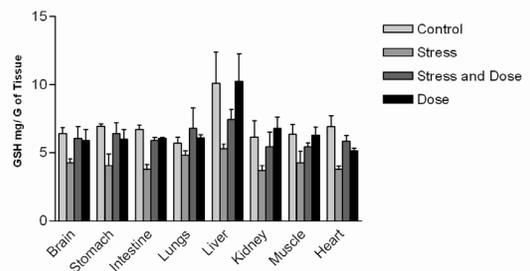
Catalase



Gluthione Peroxidase



Reduced Glutathione



Over the past few years there has been exponential growth in the number of reports indicating that excessive free radical production and lipid per oxidation are actively involved in the pathogenesis of a wide number of diseases. Consequently there has been a growing interest in potential health promoting properties of phytochemicals of plant origin. Special attention has been given to phenolic derivatives^[29-31].

The results of present study with *Cassia fistula* bark extracts have good correlations with the pharmacological property of *Cassia fistula* pulp powder as antioxidant. Plants which belong to Caesalpinaceae family are rich in flavonoids and bio flavonoids are known for their antioxidant activities. Further studies for elucidation of antioxidant effects of active compound (s) present in the extract is under progress.

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