Research in Pharmaceutical Sciences, June 2015; 10(3): 206-213
Received: Mar 2014
Accepted: May 2014

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Evaluation of the effect of ethanolic extract of fruit pulp of *Cassia fistula* Linn. on forced swimming induced chronic fatigue syndrome in mice

P. Sarma*, M. Borah, and S. Das

Department of Pharmacology, Assam Medical College, Dibrugarh, Assam, India.

Abstract

The fruit of *Cassia fistula* Linn. is a legume, has antioxidant and lots of other medicinal properties. As oxidants are involved in the pathogenesis of chronic fatigue syndrome, the present study was done to evaluate the effect of ethanolic extract of fruit pulp of *C. fistula* Linn. (EECF) on forced swimming induced chronic fatigue syndrome (CFS). Albino mice of 25-40 grams were grouped into five groups (n=5). Group A served as naive control and group B served as stress control. Group C received EECF 200 mg/kg and group D received EECF 400 mg/kg respectively. Group E received imipramine 20 mg/kg (standard). All animals were treated with their respective agent orally daily for 7 days. Except for group A, animals in other groups were subjected to force swimming 6 min daily for 7 days to induce a state of chronic fatigue. Duration of immobility was assessed on day 1st, 3rd, 5th and 7th. Anxiety level (by elevated plus maze and mirrored chamber) and loco-motor activity (by open field test) were assessed 24 h after last force swimming followed by biochemical estimations of oxidative biomarkers in brain homogenate at the end of study. Treatment with EECF resulted in significant reduction in the duration of immobility, reduced anxiety and increased loco-motor activity. Malondialdehyde level was also reduced and catalase level was increased in the extract treated group and standard group compared to stress control group. The study indicates that EECF has protective effect against experimentally induced CFS.

Keywords: *Cassia Fistula* Linn; Chronic fatigue syndrome; Forced swimming; Imipramine

INTRODUCTION

Chronic fatigue syndrome (CFS) is a disorder characterized by persistent and unexplained fatigue resulting in severe impairment in daily functioning (1). CFS patients complain of headache, gastrointestinal disturbance, paresthesia, cognitive dysfunction, and neuropsychiatric problems including anxiety like behavior (2). The etiology of CFS is unknown. However, raised level of *in-vivo* oxidative stress was seen in patients of CFS (3,4).

CFS is reported to be initiated by different stressors each of which increase level of nitric oxide and its potent oxidant product peroxynitrite. Both nitric oxide and peroxynitrite initiates a local biochemical vicious cycle the NO/ONOO-cycle. This cycle is proposed to be the cause of CFS (5). Elevated peroxynitrite level leads to mitochondrial dysfunction, Hypothalamus pituitary adrenal dysfunction (2), single strand nicks on DNA, depletion of NAD/NADH pools, which produce lower oxygen utilization in the tissues (5). Also it causes decrease in NK cell (natural killer cell) function and other immune dysfunction (5). In mouse model of CFS, chronic fatigue was correlated with markers of oxidative stress (5,6).

A number of anti-oxidants were found to be useful in treatment of CFS (5). Citalopram and imipramine have neuroprotective effect against CFS induced behavioral and biochemical alterations with possible involvement of NO pathway (2).

Cassia fistula Linn. (Golden Shower Tree) is also known as Savarnangah in Sanskrit, Xonaru in Assamese (7). It is native to India, Amazon, Sri Lanka and many other parts of the world. It has pinnate leaves consists of 8-12 pair of leaflets, flowers are yellow in color and long drooping racemes. Pod is cylindrical and pulpy. Seeds are light brown, hard and shiny (8).

*Corresponding author: P. Sarma
Tel: 0091 8822140764
Email: phulen10@gmail.com

This plant is traditionally used in the treatment of biliousness, ulcers, erysipelas, vomiting, vaginal complains, fever, inflammations, leprosy, piles, nose disease, gonorrhea, syphilis, and dysentery (8). Other activities reported are antimicrobial (8) and antiobesity activity (9). Fruit pulp of *C. fistula* Lin. is reported to have hepatoprotective effect (7).

Studies in our laboratory revealed that ethanolic extract of fruit pulp of *C. fistula* Linn. (EECF) has high antioxidant activity. Therefore, this plant is selected for the study.

MATERIALS AND METHODS

Collection and authentication of Plant Material

Ripe pods of *C. fistula* Linn were collected from Assam Medical College and Hospital campus (AMCH), Dibrugarh and identified by Dr L. R. Saikia, Professor, Department of Life Science, Dibrugarh University (Voucher specimen No DUL.Sc. 461/2013). A voucher specimen was deposited in the Herbarium of the institute.

Preparation of plant extract

Pods of *C. fistula* Linn were peeled off and seeds were separated from fruits. About 150 g of pulp material was obtained which was then packed into Soxhlet apparatus and extraction was done by hot continuous percolation using solvent ethanol (95% v/v). The extract was concentrated using vacuum evaporator (Rotary evaporator). They were further concentrated and dried in desiccators. The yield of ethanolic extract was found to be 11.63% (W/W).

Phytochemical analysis

EECF was subjected to qualitative phytochemical analysis for flavonoids, alkaloids, saponins, tannins, terpinoids, sterols and others as per standard methods (10).

Drugs and chemicals

Imipramine was obtained from Abbott healthcare pvt. Ltd (Solan, India) and diazepam was obtained from Ranbaxy Laboratories Limited (Solan, India). Ethanol was procured from Merck (Mumbai, India). Hydrogen peroxide and tricarobxylic acid were obtained from Sigma Aldrich Private Limited (Bangalore, India). Thiobarbituric acid reagent was obtained from Himedia Laboratories Private limited (Bangalore, India).

Experimental animals

Healthy Swiss albino mice (25-40 g) of either sex were taken from the Central Animal House, Assam Medical College (registration no. 634/02/a/CPCSEA dated 19/05/02). The animals were housed in standard cages under standard conditions of light and dark cycle and maintained under normal room temperature. The animals were fed with normal diet and water *ad libitum*. Before commencing the work permission from the Institutional Animal Ethics Committee was taken and conducted according to guidelines of CPCSEA and declaration of Helsinki.

Acute oral toxicity test

EECF was subjected to acute oral toxicity test following OECD guidelines 425 (up and down method) and was found safe at 2000 mg/kg dose (11). Two arbitrary doses 200 mg/kg and 400 mg/kg were selected for the study.

Experimental design

Animals were randomly assigned to five groups with 5 animals in each (n=5).

Group A- Naive animals (neither subjected to stress nor given any drug or extract)

Group B- Subjected to force swimming (to induce CFS) for 7 days (stress control).

Group C- Subjected to forced swimming + EECF (200 mg/kg) for 7 days.

Group D- Subjected to forced swimming + EECF 400 mg/kg for 7 days.

Group E- Subjected to forced swimming + standard drug (imipramine 20 mg/kg) for 7 days.

Imipramine 20 mg/kg is taken as standard drug (12). Extract and imipramine were administered orally 1 h prior to force swimming.

Induction of CFS

The animals were forced to swim individually in glass jar (25 cm× 12 cm× 25 cm)

containing water at room temperature (22 °C ± 3 °C). Depth of water was adjusted to 15 cm and was kept constant throughout the experiment. The duration of immobility was measured during a total period of 6 min. After an initial period of vigorous activity, each mouse assumed a typical immobile posture. The animals were judged immobile when they ceased struggling movement of their limbs to keep their head above water.

The enhancement in immobility period induced by continued forced swimming was considered as a situation related to CFS (2). The duration of forced swimming to induce CFS is taken as 6 min daily for 7 days. Immobility period was measured on day 1st, 3rd, 5th and 7th (2,6).

Elevated plus maze test

The elevated plus maze (EPM) apparatus consisted of two open arms [16 cm × 5 cm] and two covered arms [16 cm × 5cm × 12 cm] (13). The arms are arranged in such a way that the two arms of each type remain opposite to each other (14). The arms extend from a central platform [5 cm × 5 cm] and the maze was elevated to a height of 25 cm from the floor (13). The animals were placed individually at the center of the elevated plus maze with their heads facing towards an open arm. During the 5 min test, Parameters observed are: a) latency to enter open arm, b) time spent in open arm during five minute session (12), c) number of entries to open arm (on entry is defined as all four paw entry to the arm) (15). Reading was taken 24 h after last forced swimming (2).

Mirror chamber test

The mirror chamber apparatus essentially consists of mirrored cube open on one side constructed of 5 pieces of mirrored glass (30 cm × 30 cm × 30 cm) with one side mirrored and opposite side painted dark brown. The container box (40 cm× 40 cm×40 cm) has a white floor and opaque black walls. Placement of the mirrored cube into the container forms a five centimeter corridor which completely surrounds the mirrored chamber. A sixth mirror is placed on the container wall positioned such that it faces the single open side of the mirrored chamber (15).

Animal was placed individually in a fixed corner outside the mirror chamber. During the 5 min test session, following parameters were noted: (a) latency to enter the mirror chamber, (b) number of entry, (c) total time spent in mirror chamber and (d) average time per entry (time/entry) in mirror chamber (15).

Assessment of locomotor activity, open field test

The open field apparatus was constructed with plywood and measured 72 cm × 72 cm with 36 cm walls. Blue lines were drawn on the floor which divided the floor into sixteen 18 cm×18 cm squares. A central square of 18 cm×18 cm was drawn in the middle of the apparatus. Mice were placed randomly into one of the four corners of the open field, facing the centre. Each mouse was allowed to explore the apparatus for 5 min.

The behaviours scored were Line crossing (frequency with which animals crossed one of the grid lines with all four limbs) and Rearing (frequency with which the animals stood on their hind legs in the open field) (16).

Assessment of oxidative stress

Twenty four hour after the last forced swimming experimental animals were sacrificed by decapitation. The whole brain was removed and a 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). For catalase assay, the post nuclear fraction was obtained by centrifugation of the homogenate at 1000 g for 15 min at 4 °C. For malondialdehyde (MDA) assay homogenate was centrifuged at 12000 g for 60 min at 4 °C (17). Biochemical assessment of brain homogenate included the following:

1. Catalase assay

The catalase activity assay was carried out using the method described by Beers and Sizer (18). 2.5 ml of phosphate buffer (65 µM, pH 7.8) was added to 0.1 ml of supernatant and incubated for 20 min. The absorbance was measured at 240 nm spectrophotometrically. Then 650 µl of hydrogen peroxide solution

(7.5 mM) was added to initiate the reaction. The change of absorbance was measured for 3 min. Values are expressed as μmol of H_2O_2/min/mg of proteins.

2. Assessment of lipid peroxidation

MDA level was estimated by Satoh K method (19). 75 mg of thiobarbituric acid was dissolved in 15% trichloroacetic acid (TCA), to this 2.08 ml of 0.2 N HCl was added, the volume was made up to 100 ml using 15% TCA. 3.0 ml of this reagent was added to 0.75 ml of brain homogenate. The test tubes were kept in a boiling water bath for 15 min. They were cooled and centrifuged for 10 min at 10000 rpm. Absorbance of the supernatant was read against the blank at 535 nm. The results were expressed in nmol/mg of proteins.

3. Protein estimation

The amount of protein was measured according to the method of Lowry using bovine serum albumin as standard (20).

Statistical analysis

The statistical significance between groups was analyzed separately using One–way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. The significance was expressed by *P* values, as mentioned in the tables. *P* values of <0.05 were considered as significant.

RESULTS

Acute toxicity study

EECF was subjected to acute oral toxicity test (as per OECD guidelines 425). It was found safe at 2000 mg/kg dose (11).

Phytochemical analysis

The preliminary phytochemical investigation indicates that the *C. fistula* contains alkaloids, saponins, phenols, tannins and flavonoids.

Effect of extract treatment on duration of immobility on chronically fatigued animals

The results of forced swimming test are depicted in Table 1. Immobility period was significantly increased on day 3rd, 5th and 7th in group B (stress control) compared to group A ($P<0.05$).

EECF (both 200 and 400 mg/kg) showed significant ($P<0.05$) reduction in immobility period when compared to group B. Similar reduction in duration of immobility was observed in imipramine treated animals and there was no significant difference between extract and imipramine treated animals.

Effect of extract treatment on the level of anxiety tested in elevated plus maze

The results are shown in Table 2. There was significant decrease in the number of entry and total time spent in open arm in group B (stress control) as compared to naive group ($P<0.05$). EECF increased the number of entry and total time spent in open arm which was statistically significant ($P<0.05$) when compared to group B (stress control). Similar increase in both parameters was seen in standard group (group E) and no significant difference was seen between EECF and imipramine treated animals.

Latency to enter open arm increased in stressed group as compared to naive ($P<0.05$). EECF treated animals showed a significant ($P<0.05$) decrease in latency period when compared to stress control group. Similar effect was also seen in imipramine treated group and there was no significant difference between extract and imipramine treated animals.

Effect of extract treatment on level of anxiety in animals tested in mirror chamber

Results are shown in Table 3. Latency to enter mirrored chamber increased in stressed group compared to naive ($P<0.05$). EECF treated animals showed significant decrease in latency period when compared to stress control group ($P<0.05$).

Both extract and imipramine treated animals showed similar effect on latency. Again there was significant decrease in the number of entry, total time spent in mirrored chamber and average time per entry in stress control group (group B) compared to naive. All these parameters were found significantly increased in extract treated animals as compared to stress control group ($P<0.05$). There was no significant difference in these parameters between EECF and imipramine treated animals.

Table 1. Effect of extract treatment on duration of immobility of chronically fatigued animals at different intervals of time during 7 days study.

Group	Treatment	Duration of immobility (s)			
		Day 1	Day 3	Day 5	Day 7
A	Naive	149.1 ± 13.38	151.1 ± 6.995	172.9 ± 13.12	171.3 ± 13.02
B	Stress control	149.9 ± 9.493	202.9 ± 16.81[a]	207.1 ± 16.71[a]	205.6 ± 16.4[a]
C	EECF 200 mg/kg	147.3 ± 8.763	142.7 ± 12.75[b]	139.3 ± 12.83[b]	138.9 ± 12.53[b]
D	EECF 400 mg/kg	153.3 ± 5.862	135.1 ± 11.97[b]	132.6 ± 10.96[b]	131.7 ± 10.78[b]
E	imipramine 20 mg/kg	150.6 ± 2.724	143.4 ± 12.14[b]	145.4 ± 13.31[b]	147.4 ± 14.03[b]

All values are expressed as Mean ± SEM. n=5, Analyzed by one way ANOVA followed by Dunnett's multiple comparison tests. [a]; $P<0.05$ when compared to naive group. [b]; $P<0.05$ when compared to stress control group.

Table 2. Effect of extract treatment on the performance of chronically fatigued animals in elevated plus maze.

Group	Treatment	No. of entries to open arm	Time spent in open arm (s)	Latency to enter open arm (s)
A	Naive	3 ± 0.44	26.2 ± 3.323	105.2 ± 1.36
B	Stress control	0.8 ± 0.2[a]	6.6 ± 1.364[a]	249.6 ± 3.970[a]
C	EECF 200 mg/kg	2.2 ± 0.37[b]	20.4 ± 4.226[b]	80.4 ± 3.641[b]
D	EECF 400 mg/kg	2.8 ± 0.66[b]	25.6 ± 3.415[b]	69 ± 13.92[b]
E	imipramine 20 mg/kg	3 ± 0.31[b]	27.8 ± 4.620[b]	66.8 ± 11.64[b]

All values are expressed as Mean ± SEM. Analyzed by one way ANOVA followed by Dunnett's multiple comparison tests. [a]; $P<0.05$ when compared to naive group. [b]; $P<0.05$ when compared to stress control group.

Table 3. Effect of extract on performance of chronically fatigue animals in mirror chamber.

Group	Treatment	Latency (s)	No of entry	Total time Spentin mirror chamber (s)	Average time Spent in mirror chamber (s)
A	Naive	139.8 ± 19.47	2.6 ± 0.24	34.6 ± 7.39	12.92 ± 2.01
B	Stress control	207.2 ± 18.72[a]	0.8 ± 0.2[a]	3.8 ± 1.24[a]	4 ± 1.24[a]
C	EECF 200 mg/kg	138.8 ± 7.11[b]	2.4 ± 0.24[b]	28.8 ± 4.02[b]	12.77 ± 2.55[b]
D	EECF 400 mg/kg	112.8 ± 6.2[b]	2.8 ± 0.66[b]	41.20 ± 4.62[b]	14.6 ± 0.2[b]
E	imipramine 20 mg/kg	128.4 ± 9.11[b]	3 ± 0.31[b]	44.6 ± 2.874[b]	14.9 ± 2.63[b]

All values are expressed as Mean ± SEM. Analyzed by one way ANOVA followed by Dunnett's multiple comparison tests. [a]; $P<0.05$ when compared to naive group. [b]; $P<0.05$ when compared to stress control group.

Table 4. Effect of extract treatment on the locomotor activity of chronically fatigue animals.

Group	Treatment	Total lines crossed	Rearing
A	Naive	62.40 ± 4.082	10.06 ± 3.17
B	Stress control	25.20 ± 2.375[a]	1.20 ± 0.37[a]
C	EECF 200 mg/kg	71.80 ± 4.994[b]	7.60 ± 1.03[b]
D	EECF 400 mg/kg	73.06 ± 5.995[b]	8.80 ± 1.24[b]
E	imipramine 20 mg/kg	78.40 ± 3.140[b]	8.80 ± 0.80[b]

All values are expressed in Mean ± SEM. Analyzed by one way ANOVA followed by Dunnett's multiple comparison tests. [a]; $P<0.05$ when compared to naive group. [b]; $P<0.05$ when compared to stress control group.

Table 5. Effect of extract treatment on levels of catalase and MDA in brain tissue.

Group	Treatment	Catalase (µmol/min/mg of proteins)	MDA (nmol/mg of proteins)
A	Naive	2.262 ± 0.9254	0.193 ± 0.01632
B	Stress control	1.023 ± 0.3931[a]	0.954 ± 0.04906[a]
C	EECF 200 mg/kg	2.862 ± 0.1626[b]	0.336 ± 0.02882[b]
D	EECF 400 mg/kg	2.984 ± 0.8469[b]	0.242 ± 0.02618[b]
E	imipramine 20 mg/kg	3.162 ± 0.3612[b]	0.134 ± 0.02193[b]

All values are expressed as Mean ± SEM. Analyzed by one way ANOVA followed by Dunnett's multiple comparison tests. [a]; $P<0.05$ when compared to naive group. [b]; $P<0.05$ when compared to stress control group.

Assessment of locomotor activity by open field test

Results are shown in Table 4. There was a decrease in total line cross and rearing in stress control group when compared to naive (P<0.05). EECF treatment significantly increased both the parameters of ambulatory activity when compared to group B (P<0.05). Effect of EECF was comparable to imipramine and no statistically significant difference was seen between these two.

Biochemical estimation of effect of extract treatment on level of catalase and malondialdehyde in mice brain

The results are shown in Table 5. Group B animals recorded significant decrease in catalase levels when compared to group A (P<0.05). Significant elevation of catalase level was seen in EECF and imipramine treated animals when compared to stress control group. Effect of EECF was comparable to Group E (imipramine treated group) and there was no significant difference between these two.

Again group B animals recorded statistically significant elevated levels of MDA compared to group A (P<0.05). Dose dependent reduction in EECF (200 and 400 mg/kg) as well as imipramine treated animals showed significant reduction in the level of MDA when compared to group B (stress control). There was no significant difference between EECF and imipramine treated animals.

DISCUSSION

CFS is a disorder of unknown etiology with symptoms like persistent and relapsing fatigue, somatic complains like headache, joint pain, cognitive dysfunction and paresthesia and neuropsychiatric problems like anxiety (2). The chronic illness is largely produced by the $NO/ONOO^-$ cycle may be initiated by potent oxidant peroxynitrite (5). A hyper secretion of proinflammatory cytokines is also seen in patients with CFS (21). This may be due to stimulation of nuclear factor kappa beta (NFkB) which leads to elevated levels of IL-1β, IL-6, IL-8, TNF-α and IFN-γ (5).

In the present study continued swimming of 6 min daily for 7 days in the stress control group (group B) resulted in significant changes in behavioral parameters such as increased immobility period, elevation of anxiety level and decreased locomotor activity. In brain homogenate decrease in catalase and increased lipid peroxidation markers (MDA) were seen which indicates increased oxidative stress. As a result, we can definitely say that continuous forced swimming for 7 days produced CFS like condition in mice.

The method of 7 days exposure of mouse to forced swimming is a well validated animal model of CFS (17). In this model, mice are chronically exposed to aversive situation (swimming) from which there is no possibility to escape. The animals eventually stop struggling and assume a typical immobile posture that is suggestive of behavioral depression and fatigue (12). In our present study chronic forced swimming for seven days significantly increased immobility period (in forced swimming test) and decreased locomotor activity (in open field test) in the stress control group. However extract and imipramine treated animals showed significant increase in locomotor activity and decrease in immobility period when compared to stress control group. The increased fatigue seen in CFS may be attributed to mitochondrial energy metabolism dysfunction due to oxidation of cardiolipin molecules in the inner mitochondrial membrane by superoxide, leading to lowered complex I, III and IV activity. This results in lowered oxygen utilization in the tissues (5). Peroxynitrite, superoxide and nitric oxide also depletes ATP (5). The protective action of EECF seems to be due to presence of flavonoids which scavenges peroxynitrite and superoxide radical (5).

Chronic swimming also led to increased anxiety behavior of animals in elevated plus maze and mirror chamber. Elevated plus maze is a sensitive behavioral test that reveals animal's neophobia or anxiety (15). In elevated plus maze, the numbers of entry to open arm, total time spent in open arm was significantly increased in the extract treated and standard treated groups when compared to stress control group. Mirror chamber method

is another rapid, sensitive and quantitative method which is used to evaluate animal's anxiety. Many animals exhibit approach-avoidance conflict upon placement of a mirror within their environment (15). In this test, latency to enter mirror chamber was significantly increased while number of entry to mirror chamber, total time in mirror chamber and average time spent in mirror chamber were significantly decreased in the stress control group. However extract and imipramine treatment significantly reversed these parameters when compared to stress control group. This indicates decreased anxiety of the extract and imipramine treated animals compared to stress control group. This anti-anxiety activity of *C. fistula* Linn. may be attributed to the flavonoids present in the extract. Flavonoids modulate $GABA_A$ receptors. Flavonoids can activate $GABA_A$ receptors even in absence of GABA (22). In intact animals activation of this receptor is associated with anti-anxiety actions (15).

Free radical generation is a part of normal respiration and other routine cell activities including microbial defense and also a consequence of chemical and radiation injury (23). Lipid peroxidation provides further supply of free radicals, which initiates further peroxidation leading to breakdown of erythrocyte membranes and oxidation of protein and DNA (12). MDA is one of the most frequently used indicators of lipid peroxidation (24). It reacts with DNA to form adducts to deoxyguanosine and deoxyadenosine (25,26). Evidence of oxidative damage of DNA and lipids in the vastus lateralis muscle points towards oxidative stress in CFS (14). Catalase is a tetrameric ubiquitous heme protein (12). It directs degradation of hydrogen peroxide to water and molecular oxygen (23) thereby acting as an antioxidant. In our experiment, stress control group recorded significant decrease in catalase level and an increase in MDA level compared to naive group which is suggestive of oxidative stress. Treatment with imipramine and EECF resulted in significant increase in the level of catalase and a decrease in the level of MDA when compared to stress control group. This may be attributed to antioxidant property of the extract which protected the animals from forced swimming induced oxidative stress.

Studies in our lab and many other in vitro and in vivo studies showed that EECF has potent antioxidant property (27,28,29). The antioxidant property of *C. fistula* Linn. may be due to presence of phenols and flavonoids (29). Phenolic and flavone content are high in pulp (30). Flavonoids are chain breaking antioxidants, scavenges peroxynitrite and superoxide. They also lower NF-kB activity, help to restore tetrahydrobiopterin level (5). Tetrahydrobiopterin is important for synthesis of serotonin and melatonin (5). Melatonin stimulates endogenous anti-oxidant enzymes and scavenges peroxynitrite. It also inhibits the enzyme nitric oxide synthase. (17). Thus flavonoid content of EECF may be responsible for the beneficial effects of EECF in CFS.

CONCLUSION

The present study concludes that the ethanolic extract of fruit pulp of *C. Fistula* Linn. possesses significant protective effect against CFS. This may be due to antioxidant property of the extract. Further study is required for isolation and identification of active constituents to confirm exact mechanism.

ACKNOWLEDGMENTS

The authors are thankful to Mr. Bipul Thakuria, Mr. Mechikanta Mech, Dr. Anusuya Bhattacharyya and Dr. Disha Gupta for their constant encouragement and technical help.

REFERENCES

1. Bleijenberg G, Meer JWMV. Chronic fatigue syndrome. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J. editors. Harrison's principles of Internal Medicine. 18th ed. Newyork: Mc Graw Hill Medical publishing division; 2012. p. 3519-3521.
2. Kumar A, Garg R, Gaur V, Kumar P. Nitric oxide modulation in protective role of antidepressants against chronic fatigue syndrome in mice. Indian J Pharmacol. 2011;43:324–329.
3. Kennedy G, Space VA, McLaren M, Hill A, Underwood C, Belch JJ. Oxidative stress levels are

raised in chronic fatigue syndrome and are associated with clinical symptoms. Free redic biol med. 2005;39:584-589.
4. Logan AC, Wong C. Chronic fatigue syndrome: oxidative stress and ditary modifications. Altern Med Rev. 2001;6:450-459.
5. Pall ML. The NO/ONOO-vicious cycle mechanism as the cause of chronic fatigue syndrome/myalgic encephalomyelitis. In: Svoboda E, Zelenjcik K, editors. Chronic fatigue syndrome: symptoms, causes and prevention. Washington: Nova science publishers; 2010. p. 27-56.
6. Singal A, Kaur S, Tirkey N, Chopra K. Green tea extract and catechin ameliorate chronic fatigue-induced oxidative stress in mice. J Med Food. 2005;8:47-52.
7. Das S, Sarma G, Barman S. Hepatoprotective activity of aqueous extract of fruit pulp of *Cassia fistula* (AFCF) against carbon tetrachloride (CCL_4) induced liver damage in albino rats. J Clin Diagn Res. 2008;2:1133-1138.
8. Nealam Ch, Ranjan B, Komal Sh, Nootan Ch. Antimicrobial activity of *Cassia fistula* Linn. Legumes. International research journal of Pharmacy. 2011;2:100-102.
9. Hernandez CLC, Leonido FMG. Weight-lowering effects of *Caesalpinia pulcherrima*, *Cassia fistula* and *Senna alata* leaf extracts. J. Med. Plants Res. 2011;5:452-455
10. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sciencia. 2011;1:98-106.
11. OECD guidelines for testing of chemicals (internet). Section 4, health effects; test no.425; acute oral toxicity; up and down procedure. France: OECD publishing; 2006. p. 1-27. Available from; http;//www.oecdbookshop.org/oecd/index. (Accessed on 2nd August 2011).
12. Lalremurta V, Prasanna GS. Evaluation of protective effect of aegle marmelos in an animal model of chronic fatigue syndrome. Indian J Pharmacol. 2012;44:351-356.
13. Gupta S, Joanofarc J, Chowdhary P. Comparative study of polyherbal combinations on oxidative sterss associated dementia. International journal of pharmaceutical sciences and research. 2013;4:2799-2806.
14. Gholamzadeh S, Zare S, Ilkhanipoor M. Evaluation of the anxiolytic effect of *Echium amoenum* petals extract, during chronic treatment in rat. Res Pharm Sci. 2007;2:91-95.
15. Kulkarni SK, Practical pharmacology and clinical pharmacy. 1st ed (Reprint).Delhi: Vallabh Publications; 2009.
16. Ajibade AJ, Adenowo TK, Akintunde OW, Fakunle PB, Oyewo OO, Ashamu EA, *et al*. Suppression of exploration and locomotion in adult Wister rats following quinine administration. J Neurosci Behav Health. 2011;3:32-37
17. Singh A, Garg V, Gupta S, Kulkarni SK. Role of antioxidants in chronic fatigue syndrome in mice. Indian J Exp Biol. 2002;40:1240-1244.
18. Beers RF, Sizer IW. A spectrophotographic method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952;195:133-40.
19. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin.Chim Acta. 1978;90:37-43.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-275.
21. Kumar A, Garg R. Protective effect of antidepressants against chronic fatigue syndrome induced behavioral changes and biochemical alterations. Fundam Clin Pharmacol. 2009;23:89-95.
22. Hanrahan JR, Chebib M, Johnston GA. Flavonoid modulation of GABA (A) receptors. Br J Pharmacol. 2011;163:234-245.
23. Mitchell RN, Cotran RS. Cell injury, adaptation and death. In: Kumar V, Cotran RS, Robbins SL, editors. Robbins Basic pathology. 7th ed. New Delhi: Elsevier; 2004. p .3-31.
24. Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR. Grandjean P. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. Clin Chem. 1997;43:1209-1214.
25. Marnett LJ. Lipid peroxidation-DNA damage by malondialdehyde. Mutat Res. 1999;424:83-95.
26. Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, Marnett LJ. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. J Biol Chem. 2003;278:31426-31433.
27. Md. I, Md. Z, Singh M, Rizvi MMA. Comparative analysis of the antioxidant activity of *Cassia fistula* extracts. International journal of medicinal chemistry. 2012, 2012:ID 157125.
28. Siddhurajua P, Mohanb PS, Becker K. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. Food Chem. 2002;79:61–67.
29. Bhalodia NR, Nariya PB, Acharya RN, Shukla VJ. *In vitro* antioxidant activity of hydro alcoholic extract from the fruit pulp of *Cassia fistula* Linn. Ayu. 2013;34:209-214.
30. Rajagopal PL, Premaletha K, Kiron SS, Sreejith KR. Phytochemical and pharmacological review on *Cassia fistula* linn. "the golden shower". International journal of pharmaceutical, chemical and biological sciences. 2013;3:672-679.