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**Name of Journal:** *World Journal of Hepatology*

**Manuscript NO:** 70101

**Manuscript Type:** ORIGINAL ARTICLE

*Basic Study*

**Baicalin provides protection against fluoxetine induced hepatotoxicity by modulation of oxidative stress and inflammation**

Ganguly R *et al.* Baicalin provides protection against fluoxetine induced hepatotoxicity

## Abstract

### BACKGROUND

Fluoxetine is one of the most widely prescribed anti-depressant drugs belonging to the category of selective serotonin reuptake inhibitors. Long term fluoxetine treatment results in hepatotoxicity. Baicalin, a natural compound obtained from the Chinese herb *Scutellaria baicalensis* is known for antioxidant, hepatoprotective and anti-inflammatory effects. However, the beneficial effects of baicalin against fluoxetine induced hepatic damage have not been reported so far.

### AIM

To evaluate the protective action of baicalin in fluoxetine induced liver toxicity and inflammation.

### METHODS

The male albino Wistar rats were divided into seven groups. Group 1 was normal control. Fluoxetine was orally administered at 10 mg/kg body weight to groups 2, 3, 4 and 5. In addition, groups 3 and 4 were also co-administered orally with baicalin (50 mg/kg and 100 mg/kg, respectively) while group 5 was fed with silymarin (100 mg/kg), a standard hepatoprotective compound for comparison. Groups 6 and 7 were used as positive control for baicalin (100 mg/kg) and silymarin (100 mg/kg). All the treatments were carried out for 28 d. After sacrifice of rats, biomarkers of oxidative stress [superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione-S-transferase (GST), advanced oxidation protein products (AOPP), malondialdehyde (MDA)], and liver injury [alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, albumin, bilirubin] were studied in serum and tissue using standard protocols and diagnostic kits. Inflammatory markers [tumor necrosis factor (TNF- $\alpha$ ), interleukin (IL)-6, IL-10 and interferon (IFN)- $\gamma$ ] in serum were evaluated using ELISA based kits. Effect of drug on liver was also analyzed by histopathological examination of tissue sections.

## RESULTS

Fluoxetine-treated rats showed elevated levels of the serum liver function markers (total bilirubin, ALT, AST, and ALP) and inflammatory markers (TNF- $\alpha$ , IL-6, IL-10 and IFN- $\gamma$ ), with decline in total protein and albumin levels. The biochemical markers for oxidative stress such as SOD, CAT, GST, GSH, MDA and AOPP in the liver tissue homogenate were also altered indicating surge in reactive oxidative species leading to oxidative damage. Histological examination of liver tissue also showed degeneration of the hepatocytes. Concurrent administration of baicalin (50 and 100 mg/kg) restored the biomarkers of oxidative stress, inflammation and hepatic damage in serum as well as in liver tissues to near normal levels.

## CONCLUSION

The results suggested that long term treatment with fluoxetine leads to oxidative stress *via* formation of free radicals that consequently cause inflammation and liver damage. Concurrent treatment with baicalin alleviated fluoxetine induced hepatotoxicity and liver injury by regulating oxidative stress and inflammation.

**Key Words:** Fluoxetine; Hepatotoxicity; Oxidative stress; Baicalin; Anti-inflammatory; Hepatoprotective and antioxidant

Ganguly R, Kumar R, Pandey AK. Baicalin provides protection against fluoxetine induced hepatotoxicity by modulation of oxidative stress and inflammation. *World J Hepatol* 2022; In press

**Core Tip:** Prolonged treatment with antidepressant drug fluoxetine causes severe hepatic damage. This study evaluated fluoxetine induced liver damage in male albino Wistar rats. Fluoxetine was orally administered (10 mg/kg) for 28 d that caused significant alterations in serum and tissue biomarkers. Baicalin and silymarin were co-

administered to facilitate the amelioration of oxidative stress mediated hepatic damage and inflammation. The biochemical markers (total protein, albumin, total bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase, superoxide dismutase, catalase, glutathione, glutathione-S-transferase, malondialdehyde and advanced oxidation protein products) and inflammatory markers [tumor necrosis factor- $\alpha$ , interleukin (IL)-6, IL-10 and interferon- $\gamma$ ] were considerably restored to near normal levels after treatment with the natural flavonoid compound baicalin. Histopathological examination of liver slices showing cellular degeneration and increased vacuolation in the fluoxetine treated rats also corroborated the results obtained for biomarkers of liver function, oxidative stress and inflammation. The baicalin treated rats demonstrated normal vacuolation and cellular pattern. Thus, baicalin acts as antioxidant, anti-inflammatory and hepatoprotective agent in mitigating fluoxetine induced toxicity. To the best of our knowledge, this is the first study reporting hepatoprotective efficacy of baicalin in fluoxetine induced liver damage.

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## INTRODUCTION

Fluoxetine {N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propan-1-amine} is the most commonly prescribed drug for depression and other neuropsychotic disorders. It belongs to the category of selective serotonin reuptake inhibitors, and is used widely owing to its higher tolerability and lesser side effects<sup>[1,2]</sup>. Fluoxetine has a long half-life, is metabolized in the liver and excreted *via* urine. The active metabolite of fluoxetine is norfluoxetine. Fluoxetine acts by inhibiting cytochrome P450 (2D6) and its other isozymes, leading to potential drug interactions<sup>[3]</sup>. Although clinically approved, prolonged use of fluoxetine may cause various adverse effects such as anxiety, sleeplessness, nausea, diarrhea, metabolic disorders and sexual dysfunction<sup>[4,5]</sup>. The metabolism of fluoxetine results in excess production of free radicals that consequently causes liver damage. In addition, inflammation in the hepatic tissue is also due to over production of superoxide, hydroxyl, and some non-radical species like hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) along with surplus phagocyte formation which in turn can cause

further tissue damage<sup>[6]</sup>. Liver diseases resulting from the excessive consumption of drugs are a leading cause of mortality worldwide. The mammalian system has evolved several enzymatic and non-enzymatic pathways that can counter the drug induced adverse effects arising from free radicals' action<sup>[7-9]</sup>. In the past decade, several studies have reported the adverse effects caused by fluoxetine such as hyperglycemic effect, hepatic damage, bipolar disorders and even organ failure in extreme cases<sup>[10-13]</sup>. Further studies also showed similar toxicity profile of fluoxetine mediated by oxidative stress and inflammation<sup>[14]</sup>.

Baicalin (5,6,7-trihydroxyflavone 7-O- $\beta$ -D-glucuronide) is a flavonoid primarily obtained from the Chinese herb *Scutellaria baicalensis*. It is known to possess several pharmacological activities including anti-diabetic, anti-inflammatory, hepatoprotective, neuroprotective, antioxidant and anticancer properties<sup>[15]</sup>. Structurally, baicalin possesses a di-ortho hydroxyl functional group on its aromatic rings (Figure 1). The divalent metal ion chelating and free radical scavenging actions of baicalin can be attributed to this structural feature<sup>[16]</sup>. Baicalin extracted from *Scutellaria* roots has exhibited antidepressant properties in mice and rats<sup>[17]</sup>. Several researchers have reported the neuroprotective effects of baicalin in rats with cerebral ischemia<sup>[18,19]</sup>. The antioxidant and hepatoprotective activities of baicalin in mice have also been reported<sup>[20]</sup>. Baicalin is also capable of inducing colon cancer cell apoptosis by inhibiting onco-miRNAs<sup>[21]</sup>. So far, no studies have been done to assess the hepatoprotective activity of baicalin against fluoxetine induced toxicity. Moreover, prolonged treatment with fluoxetine in depression cases might result in liver dysfunction. Thus, the present study was undertaken to demonstrate the protective ability of baicalin against fluoxetine induced hepatotoxicity, inflammation and oxidative stress in male albino Wistar rats.

## **MATERIALS AND METHODS**

### ***Drugs and chemicals***

Fluoxetine hydrochloride and silymarin were procured from Sigma-Aldrich. Baicalin, thioburbituric acid (TBA), trichloroacetic acid (TCA), chloramines-T, 5-5'-Dithio-bis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), pyrogallol, bovine serum albumin (BSA), hematoxylin, and eosin were purchased from TCI chemicals, India. Diagnostics kits were obtained from Erba Diagnostics Mannheim, Germany. Inflammatory marker kits were supplied by Krishgen BioSystems, India.

### ***Animals***

Healthy male albino Wistar rats of similar age groups (weight: 200-250 g) were acclimatized for one week prior to the experiment at  $23 \pm 2$  °C. The animals were given standard pellet diet and water ad libitum. The *in vivo* experiments were carried out as per norms of the Institutional Animal Ethics Committee, University of Allahabad, Allahabad [IAEC/AU/2017(1)/003].

### ***Animal treatment***

The rats were divided into seven groups ( $n = 6$ ). Fluoxetine was used to induce toxicity by oral administration for 28 d. Group 1 rats, treated as normal control, were fed with feed and water. Group 2 rats were administered fluoxetine (10 mg/kg) only. Group 3 (fluoxetine 10 mg/kg + baicalin 50 mg/kg), Group 4 (fluoxetine 10 mg/kg + baicalin 100 mg/kg) and Group 5 (fluoxetine 10 mg/kg + silymarin 100 mg/kg) were drug combination groups. Group 6 and group 7 were treated as positive controls and were only given baicalin (100 mg/kg) and silymarin (100 mg/kg), respectively. Silymarin was used as standard hepato-protectant for comparison. After completion of 28 d treatment, the rats were sacrificed by cervical dislocation. Blood and liver tissue were collected for the evaluation of enzymatic and non-enzymatic biochemical markers.

### ***Measurement of body weight***

The body weight of the rats was measured every day for 28 d until sacrifice.

#### *Serum and plasma collection from rat blood*

At the end of experiment, approximately 5 mL blood was drawn by heart puncture. About half the blood was left for clotting for 20 min at room temperature about 25 °C and serum was obtained at 5000 rpm for 10 min. The rest of the blood was collected in heparin containing vials. This was centrifuged in a cooling centrifuge at 4000 rpm for 20 min, and clear non-hemolyzed plasma was obtained. The plasma and serum samples thus separated were transferred to fresh microfuge tubes and preserved at -80 °C, until further assessment.

#### *Evaluation of serum markers*

The serum was used for the assessment of enzymes viz., alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and non-enzymatic parameters such as total bilirubin, albumin and total protein using Erba Diagnostics kits. The levels of serum inflammatory markers tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6, IL-10 and interferon (IFN)- $\gamma$  were evaluated using ELISA kits (Krishgen BioSystems).

#### *Evaluation of hepatic and oxidative stress markers in liver homogenate*

**Tissue homogenization:** 10% (w/v) liver tissue homogenate was prepared in 0.1M phosphate buffer with 0.15M KCl, pH 7.4. The clear supernatant was separated after centrifugation at  $4000 \times g$  for 15 min at 4 °C and was used for further analysis.

**Evaluation of malondialdehyde in liver homogenate:** The amount of lipid peroxidation in the liver tissues was determined by the method of Niehaus and Samuelsson<sup>[22]</sup>. 100  $\mu$ L tissue homogenate was mixed with 2 mL TBA reagent comprising TBA 0.37%, 15% TCA and 0.25N HCl, and the tubes were placed in hot water bath for 10 min and cooled at room temperature followed by centrifugation. The supernatant was used for spectrophotometric assessment at 532 nm against reference



blank. Extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$  was used and the results were denoted as nmol malondialdehyde (MDA)/mg.

**Evaluation of advanced oxidation protein products in liver homogenate:** The advanced oxidation protein products (AOPP) levels were determined by method of Witko-Sarsat [23]. To 2 mL of liver homogenates [1:5 diluted in phosphate buffer solution (PBS)], 100  $\mu\text{L}$  KI (1.16 M) was added followed by addition of 200  $\mu\text{L}$  glacial acetic acid after two minutes. The absorbance of the reaction mixture was read spectrophotometrically at 340 nm against a reference blank. The blank contained the same reaction mixture, except that homogenate sample was replaced by 2 mL of PBS. The concentrations of AOPP were denoted as  $\mu\text{mol/L}$  of chloramine-T equivalents.

**Evaluation of reduced GSH in liver homogenate:** GSH content in liver homogenates was determined by the method of Ellman *et al* [24]. 250  $\mu\text{L}$  of liver homogenate was added to the reaction mixture comprising 100  $\mu\text{L}$  of 6 mmol/L DTNB, 300  $\mu\text{L}$  of 0.2 M phosphate buffer (pH 8.0) and 50  $\mu\text{L}$  0.3 M NaOH. Absorbance of the reaction mixture was determined at 412 nm. GSH was used as standard and the results were represented as  $\mu\text{g mg}^{-1}$  protein.

**Determination of activity of antioxidant enzymes in liver homogenate:** The activity of glutathione-S-transferase (GST) was measured by the method of Habig *et al* [25]. The activity assay was performed in a reaction mixture of 1 mL comprising 0.1 M phosphate buffer (pH 6.5), 1 mmol/L CDNB, 1 mmol/L GSH and 100  $\mu\text{L}$  supernatant of liver homogenate. The change in absorbance on account of conjugate formation of GSH and CDNB was measured at 340 nm. GST activity was represented as  $\mu\text{mol min}^{-1} \text{mg}^{-1}$  protein.

The superoxide dismutase activity (SOD) was evaluated by the method of Marklund and Marklund [26]. The colored complex formation takes place due to auto-oxidation of pyrogallol. This is measured for 3 min at the interval of 60 s at 412 nm in the presence or

absence of the enzyme. 1 unit of the enzyme activity was represented as 50% inhibition of auto-oxidation of pyrogallol per minute.

The catalase (CAT) activity was assessed by the method of Beers and Sizer<sup>[27]</sup>. The decreasing absorbance of H<sub>2</sub>O<sub>2</sub> consumption is measured at 240 nm at the interval of 60 s for 3 min. 1 unit of CAT activity was expressed in  $\mu\text{mols}$  of H<sub>2</sub>O<sub>2</sub> decomposed per minute with extinction coefficient of H<sub>2</sub>O<sub>2</sub> as  $43.6\text{M}^{-1}\text{cm}^{-1}$ .

#### ***Total protein assay***

The total protein content in serum and tissue samples was estimated by the method of Lowry *et al*<sup>[28]</sup> with BSA as standard.

#### ***Histopathological study of liver tissue***

After the sacrifice of rats, the tissue samples were washed with cold saline and fixed in 10% formalin. The samples were then processed further for the preparation of paraffin wax blocks. Sectioning was done with a rotatory microtome and stained with hematoxylin and eosin<sup>[29]</sup>. The slides were then examined under the light microscope at 40 × magnification to observe the protective effect of baicalin against fluoxetine mediated oxidative damage in liver tissue.

#### ***Statistical analysis***

The statistical analysis was performed using GraphPad Prism 5 software. Unpaired t test was used for statistical comparisons and the results were expressed as mean  $\pm$  SD. The *P* value < 0.05 was considered significant.

### **RESULTS**

#### ***Change in body weight***

There was considerable decline in body weight of group 2 rats (220 g to 173 g) within the span of 28 d, while a constant increase was observed in group 1 rats (213 g to 245 g) (*P* < 0.05). In group 3 rats, there was a decline (240 g to 213 g) till the second week, and

the body weight increased thereafter (213 g to 229 g) ( $P < 0.05$ ). Similar pattern was observed in group 4 (245 g to 224 g up to second week and 224 g to 238 g thereafter) ( $P < 0.005$ ) and group 5 (250 g to 231 g up to second week and 231 g to 245 g thereafter) ( $P < 0.0005$ ). Co-administration of baicalin and silymarin in groups 3, 4 and 5 helped gaining body weight from day 14 onwards, after initial weight loss due to fluoxetine treatment. Groups 6 and 7 exhibited a normal pattern of body weight similar to the control group (Figure 2).

#### *Effect of baicalin on serum liver function enzymes*

To evaluate the protective effect of baicalin against fluoxetine-induced liver hepatic injury, liver function marker enzymes were assessed in serum. Fluoxetine treatment for consecutive 28 d caused a significant increase in serum AST levels in group 2 rats (129.29 IU/L) in comparison to the control group (64.28 IU/L). Concurrent supplementation of baicalin significantly alleviated serum AST in group 3 (113.49 IU/L) and group 4 (94.51 IU/L) rats (Figure 3A). Group 5 rats treated with silymarin, a standard hepato-protectant, also showed decline in AST levels to near normal (88.82 IU/L). ALT showed a similar pattern with a significant rise in group 2 (70.40 IU/L) as compared to the control group 1 (25.63 IU/L). Oral supplementation of baicalin decreased ALT levels in group 3 (55.84 IU/L) and group 4 (42.67 IU/L) rats (Figure 3B). Silymarin treated group 5 rats also showed reduction in ALT (38.22 IU/L). Like ALT and AST, group 2 rats also exhibited a marked increase (218.95 IU/L) in the level of serum ALP in comparison to group 1 (102.66 IU/L). Concurrent administration of baicalin or silymarin produced a little decline in serum ALP levels in group 3 (191.26 IU/L), while significant decline in group 4 (147.33 IU/L) and group 5 (134.46 IU/L) was observed (Figure 3C). The highest ameliorative potential of the serum enzymes was observed in group 5 rats treated with standard hepato-protectant silymarin, while the group 4 rats treated with baicalin (100 mg/kg) clearly exhibited greater restoration ability compared to group 3 rats with baicalin (50 mg/kg). The groups 6 and 7 served as

positive controls and showed no significant changes compared to control (group 1) for all the biomarkers.

#### ***Effect of baicalin on serum bilirubin, total protein and albumin***

The serum total bilirubin levels in group 2 rats (1.44 mg/dL) were significantly increased compared to group 1 (0.35 mg/dL). Baicalin treatment along with fluoxetine led to decline in bilirubin level in group 3 (0.81 mg/dL), and group 4 (0.64 mg/dL) (Figure 4A). Similarly, co-administration of silymarin with fluoxetine in group 5 caused reduction in bilirubin (0.57 mg/dL). However, the level of total protein in group 2 (3.53 g/dL) was significantly decreased compared to group 1 rats (7.52 g/dL). Concurrent administration of baicalin exhibited significant improvement in the total protein levels of group 3 (4.55 g/dL), group 4 (4.89 g/dL), and group 5 (5.52 g/dL) rats (Figure 4B). Similarly, the level of albumin was also decreased in group 2 (2.39 g/dL) compared to group 1 (3.97 g/dL), which increased with baicalin administration in group 3 (3.02 g/dL), group 4 (3.61 g/dL), and group 5 (3.75 g/dL) (Figure 4C).

#### ***Effect of baicalin on hepatic antioxidant enzymes in liver homogenate***

The enzymatic activities of SOD, CAT and GST were evaluated in the liver tissue homogenates of the rats treated with fluoxetine, baicalin and silymarin. The change in activities in the different groups has been shown in Table 1. About 55% reduction in the activity of SOD was observed in group 2 (6.357 U/mg) compared to group 1 (14.04 U/mg). The groups 3, 4, and 5 (7.73 U/mg, 11.11 U/mg and 10.37 U/mg, respectively) showed significant restoration in the presence of baicalin and silymarin. Similar pattern was observed in CAT activity with group 2 showing decline (3.17 U/mg) as compared to control group 1 (7.26 U/mg). Co-administration of baicalin (50 and 100 mg/kg) and silymarin accounted for improved CAT activity in groups 3, 4 and 5 (3.74 U/mg, 4.94 U/mg and 5.37 U/mg, respectively). GST activity in group 2 rats (1.29 U/mg) showed decrease in activity compared to group 1 (2.14 U/mg). In groups 3, 4 and 5, the activity

of GST was increased towards normal values (1.32 U/mg, 1.41 U/mg, 1.54 U/mg, respectively).

#### ***Assessment of the non-enzymatic antioxidant markers in liver tissue***

The treatment of fluoxetine caused increase in the levels of MDA and AOPP, along with decline in GSH as compared with the control group (Table 1). Fluoxetine administration led to decline in GSH in group 2 animals (52.12 nM/mg) by more than 50% compared to group 1 (115.76 nM/mg). The levels of GSH in group 3 (78.87 U/mg), group 4 (97.02 U/mg) and group 5 (104.91 U/mg) animals were subsequently elevated to near normal levels upon supplementation of baicalin and silymarin. The level of MDA in group 2 exhibited about three-fold increase (2.07 nM/mg) from control group 1 (0.68 nM/mg). This was again restored partially in groups 3, 4 and 5 (1.73 nM/mg, 1.32 nM/mg and 0.96 nM/mg, respectively). The AOPP levels also exhibited an identical pattern of around three-fold increase in group 2 animals (1.12 nM/mg) from control group 1 (0.48 nM/mg). The groups 3, 4 and 5 treated with baicalin and silymarin showed restoration of the AOPP levels to near normal (0.83 nM/mg, 0.64 nM/mg and 0.56 nM/mg, respectively) suggesting appreciable antioxidant potential of baicalin. The results were found comparable to silymarin.

#### ***Assessment of inflammatory markers in serum***

The serum TNF- $\alpha$  level in fluoxetine fed group 2 (163.75 pg/mL) significantly increased as compared to group 1 (49.9 pg/mL). Baicalin and silymarin treatments accorded improvement in the groups 3 (120.36 pg/mL), 4 (98.67 pg/mL) and 5 (88.8 pg/mL) (Figure 5A). Similarly, serum IL-6 was elevated in group 2 (331.81 pg/mL) relative to group 1 (200.07 pg/mL) but there was subsequent decline in groups 3 (298.18 pg/mL), 4 (258.24 pg/mL) and 5 (260.39 pg/mL) (Figure 5B). IL-10 levels mildly increased in group 2 (84.06 pg/mL) compared to group 1 (55.24 pg/mL), while the baicalin and silymarin treated groups 3 (93.7 pg/mL), 4 (85.36 pg/mL) and 5 (77.47 pg/mL) showed comparable results to group 2 (Figure 5C). The serum IFN- $\gamma$  levels followed a similar

pattern. There was a rise in fluoxetine treated group 2 (365.14 pg/mL) compared to group 1 (256.73 pg/mL), whereas groups 3 (298.17 pg/mL), 4 (258.24 pg/mL) and 5 (260.39 pg/mL) showed considerable restoration to near normal levels (Figure 5D). In all treatment groups, baicalin at a dose of 100 mg/kg showed better efficacy as compared to baicalin at 50 mg/kg.

### ***Histopathological studies***

The histopathological examination of the control liver section revealed normal cellular architecture with mild vacuolation, presence of central vein and sinusoidal spaces with intact hepatic cells (Figure 6A). The fluoxetine treated liver slices showed irregular pattern of hepatic cells, increased vacuolation, dilation of hepatic sinusoids, inflammatory cell infiltration, cellular disintegration and initial stage of bridging necrosis that links the terminal veins to the portal tracts (Figure 6B). Co-administration of baicalin and silymarin prevented the disarrangement of hepatic cells, and normal sinusoidal spaces were observed without any abnormal alterations (Figures 6C, 6D and 6E). The positive control groups 6 and 7 did not exhibit any alterations and showed normal cellular pattern (Figures 6F and 6G).

### **DISCUSSION**

Liver is associated with the biotransformation of the entire xenobiotic load in the body. Drugs and various chemicals ingested in the living system are transformed with the help of cytochrome P450 in the liver. The active metabolites produced during biotransformation elevate the levels of free radicals and reactive oxygen species (ROS) thus disrupting the redox homeostasis<sup>[30,31]</sup>. Hepatic injury and toxicity can be produced by the use of numerous chemical agents or drugs, heavy metals and pesticides<sup>[32]</sup>. In the present study, the dose dependent protective efficacy of baicalin was evaluated in fluoxetine induced hepatotoxicity in male Wistar rats. Baicalin is well known to be used in the treatment of several liver related anomalies. It ameliorates the effects of estrogen induced liver injury by up-regulation of the expression of hepatic efflux transporters

and down-regulation of hepatic uptake transporters<sup>[33]</sup>. The neuroprotective and antidepressant properties of baicalin and its derivatives have also been widely studied. Baicalin facilitates stimulation of neurogenesis, production of neurotrophic factors and modulation of hypothalamic-pituitary-adrenal axis, which further counter oxidative stress, and inflammation. Similar to fluoxetine and its metabolite norfluoxetine, baicalin also elicits anti-depressant effect by regulation of the gamma-aminobutyric acid (GABA) neurotransmitter system, and upregulating the GABA receptors<sup>[17,34,35]</sup>.

The long-term fluoxetine treatment in male albino Wistar rats exhibited reduced food intake over the span of 28 d thereby resulting in weight loss (50 g in 28 d). Fluoxetine increases serotonin signaling in the brain, and higher serotonin levels help in activating the satiety neurons thus decreasing appetite. In addition, the active metabolite of fluoxetine, norfluoxetine is slow metabolizing and causes anorexia<sup>[36]</sup>. However, co-administration of baicalin prevented excess weight loss from the second week onwards. Baicalin has been reported to alleviate anorexia by inhibiting the over expression of pro inflammatory cytokines TNF- $\alpha$  and IL-6<sup>11</sup>. The hypothalamic region in brain that regulate food intake are stimulated by the cytokine levels thus helping to increase appetite and body weight<sup>[37]</sup>.

Fluoxetine showed significant increase in the serum levels of ALT, AST and ALP in rats. This could be attributed to the fluoxetine-induced membrane alterations resulting from ROS action leading to cellular disintegration and necrosis of hepatic cells. Since these enzymes reside in the cytoplasm, their elevated levels in the serum indicate breakdown of the hepatocellular membrane<sup>[31,38]</sup>. ALT is a liver injury biomarker while ALP is a marker of hepatic biliary injury and cholestasis. These results are further corroborated by earlier reports that showed enhancement in the level of ALT, AST and ALP upon fluoxetine treatment<sup>[9,11]</sup>. Oral administration of baicalin led to substantial decrease in serum ALT, AST and ALP levels in fluoxetine treated rats, indicating its hepatoprotective action. Baicalin at 100 mg/kg was more effective in restoring the enzyme levels near normal than at 50 mg/kg. The efficacy of higher dose baicalin treatment was comparable to the effect of silymarin (100 mg/kg), a standard hepato-



protectant used in experimental studies. Silymarin can bind to the receptors on the hepatocyte membrane to prevent the entry of toxic substances in the liver. The antioxidant properties of silymarin can reduce ROS, thus inhibiting cellular damage<sup>[39]</sup>. Fluoxetine treatment led to elevation in serum bilirubin along with reduction in total protein and albumin. Higher serum bilirubin is an indicator of lower hepatic clearance suggesting abnormality in liver function that may cause jaundice and other hepatic symptoms<sup>[40]</sup>. Upon treatment with baicalin, the serum bilirubin levels were considerably restored to near normal.

Decline in the total protein and albumin levels also point towards anomaly in protein synthetic machinery leading to alteration in cellular physiology and hepatocellular function in fluoxetine treated rats. This subsequently causes a decline in cytochrome P450 activity. Baicalin treatment increased the total protein and albumin levels, and thereby improved the cellular functions. Previous studies also demonstrated the potential of baicalin as a hepato-protectant that helps in restoring the total protein and albumin levels in mice<sup>[17]</sup>. Fluoxetine treatment caused elevated AOPP levels along with increased lipid peroxidation as indicated by higher MDA content in the liver tissue suggesting the overproduction of ROS and free radicals. Moreover, decrease in the levels/activity of GSH, SOD, CAT and GST in liver tissue further supports enhanced oxidative stress in fluoxetine treated rats.

GSH plays a key role in intracellular defense against free radical induced oxidative stress. It is an intermediate in the pathways involving antioxidant enzymes like GSH peroxidase and GSH reductase. The other important biological roles of GSH include regulation of signal transduction, transport of sulfate, modulation of cell growth and division, metabolite conjugation, protein and nucleic acid synthesis, xenobiotic detoxification, promoting metal ion chelation and enzymatic reactions<sup>[41]</sup>. Low GSH acts as an indicator of oxidative stress and tissue damage and adversely affects redox equilibrium with increasing oxidized state of the system<sup>[42,43]</sup>. GSH acts as a free radical scavenging and membrane stabilizing agent. It is capable of minimizing the radical linked membrane damage and prevents lipid peroxidation. Thus reduced GSH content



in fluoxetine treated rats could be responsible for increase in lipid peroxidation. Elevated levels of MDA, a product of lipid peroxidation, in the tissue also signify the oxidized state of the system that is beyond the control of the antioxidant defense system<sup>[44-48]</sup>. The higher levels of AOPP also point towards oxidative stress causing protein damage on prolonged fluoxetine treatment in rats. Proteins are often targeted by the ROS that cause modification of the amino acids which are measured quantitatively to determine the extent of oxidative damage. Since fluoxetine is prescribed as antidepressant in psychotic disorders, it is possible that its long-term intake causes oxidative damage to proteins in hepatocytes. Chloramines are oxidants that are produced in the neutrophils by the enzyme myeloperoxidase. These oxidants result in the formation of advanced oxidation di-tyrosine cross-linked protein products. They are estimated quantitatively and act as biomarkers for protein oxidation<sup>[49]</sup>. The excessive production of AOPP is also suggestive of the onset of numerous diseases like Alzheimer's disease, rheumatoid arthritis, muscular dystrophy and respiratory diseases<sup>[50]</sup>. Hence, the decline in MDA and AOPP in rats treated with baicalin could be attributed to its antioxidant and oxidative stress lowering potential. SOD causes dismutation of the superoxide radicals ( $O_2^-$ ) generated in tissue into  $H_2O_2$  and  $O_2$ . CAT, in the peroxisomes further converts the excess  $H_2O_2$  thus produced from SOD action into water and  $O_2$ <sup>[51,52]</sup>. During the study, rats treated with baicalin and silymarin exhibited restoration of GSH, along with enhancement in SOD, CAT and GST activities. Nuclear respiratory factor 2 (Nrf2) is a major factor involved in maintaining cellular redox homeostasis. Activated Nrf2 helps in maintaining the mitochondrial redox balance, increases the expression of antioxidant enzymes, and also promotes mitochondrial biogenesis by increased transcription of Nrf1<sup>[53,54]</sup>. The rise in SOD and CAT activities could be due to increased activation of Nrf2 factor by baicalin and silymarin<sup>[55,56]</sup>. This further supports the role of baicalin treatment in improving and maintaining the redox balance in fluoxetine treated rats.

GST is a cytosolic enzyme that helps to detoxify the toxic metabolites generated from cellular processes. It acts as a defense mechanism against oxidative stress, and regulates

GSH homeostasis *via* mitogen activated protein kinase (MAPK) pathways that is involved in cellular response to stress<sup>[57]</sup>. The decline in activity of GST is an indicator of oxidative stress. In current study, fluoxetine treatment in rats led to reduction in GST activity suggesting increased ROS production in the system. Thus, decreased GST activity further hinders the detoxification process<sup>[58]</sup>. The co-administration of baicalin (50 and 100 mg/kg) resulted in increased GST activity signifying reduction in oxidative stress.

Baicalin also showed considerable efficacy against fluoxetine induced inflammatory response. The levels of serum inflammatory markers TNF- $\alpha$ , IL-6, IL-10 and IFN- $\gamma$  were considerably elevated upon fluoxetine treatment. Baicalin administration significantly restored the levels of TNF- $\alpha$ , IL-6, IL-10 and IFN- $\gamma$  in fluoxetine treated rats. The anti-inflammatory effects of baicalin and its derivative baicalein have been reported in several studies<sup>[59]</sup>. Baicalin helps in reducing the elevated levels of cytokines TNF- $\alpha$  and IL-6 by regulating the p38 MAPK signaling cascade<sup>[60]</sup>. Notably, IL-10 is an anti-inflammatory cytokine that binds to the IL receptor proteins and induces the STAT3 signaling cascade. In previous reports, fluoxetine administration has elevated the level of IL-10 in depressive patients as it inhibits the synthesis of other pro-inflammatory cytokines such as IL-6 and IFN- $\gamma$ <sup>[61]</sup>. However, in this study the IL-10 level in fluoxetine treated rats was mildly increased compared to the control group. In liver cells, IFN- $\gamma$  is produced by the nature kill cells and T lymphocytes. During hepatic injury and inflammation, the IFN- $\gamma$  receptor expression is up-regulated which stimulates the secretion of IFN- $\gamma$ . This in turn activates the macrophages, producing other cytokines like TNF- $\alpha$  in abundance<sup>[62]</sup>. Thus, the elevated levels of IFN- $\gamma$  in fluoxetine treated rats indicate hepatic injury and oxidative stress. Baicalin treatment led to restoration of the IFN- $\gamma$  levels to near normal. Better efficacy for baicalin was observed at a dose of 100 mg/kg than 50 mg/kg. The histopathological examination of liver sections of rats treated with fluoxetine and baicalin showed signs of improvement as indicated by the reduced vacuolation in cells, decreased cellular degeneration, fewer infiltrated inflammatory cells and regular cellular architecture.

Thus, long term intake of fluoxetine caused hepatotoxicity because of increased production of ROS during its biotransformation in liver. Baicalin, acting as an oxidative stress mitigator, led to improvement at the structural and functional aspects of liver as shown by biochemical and histopathological indices. In general, baicalin supplementation at 100 mg accounted for appreciable reduction in the fluoxetine-induced hepatic damage and inflammation in rats by restoration of the liver function markers and inflammatory cytokines to near normal levels. This was comparable to the effect of silymarin, a standard hepato-protectant at the same dose. In addition, it has been reported that baicalin possesses higher oral bioavailability than silymarin. Baicalin inhibits efflux transporters to increase the bioavailability of silymarin<sup>[63]</sup>. Therefore, baicalin can be used over silymarin as an alternative hepatoprotective compound to prevent fluoxetine induced liver toxicity. Further, it also improved the antioxidant status of liver that consequently diminished ROS production and associated injury. Thus, co-treatment of baicalin with prolonged fluoxetine treatment proved beneficial for liver and overall health status of the rats.

## **CONCLUSION**

Fluoxetine is a commonly prescribed antidepressant drug used for long term treatment. The study revealed that long term fluoxetine treatment induced oxidative stress, hepatotoxicity and inflammation in rats. Baicalin supplementation prevented the fluoxetine-induced liver damage and inflammation in rats through alleviation of the liver function biomarkers and inflammatory cytokines. Further, it also thwarted ROS mediated damage by strengthening the antioxidant defense system at enzymatic and non-enzymatic levels. Baicalin exhibited considerable hepatoprotective activity at a dose of 100 mg/kg and it was found comparable to the standard compound silymarin at the same dose. Hence, to defend against the oxidative stress and hepatotoxicity resulting from prolonged fluoxetine treatment, baicalin supplementation could be a drug of choice. However, further research is needed for better understanding of the key pathways and mechanisms that could explain the protective effects of baicalin against

fluoxetine induced liver injury, oxidative damage and particularly the anti-inflammatory response.

## **ARTICLE HIGHLIGHTS**

### ***Research background***

Fluoxetine is one of the most commonly prescribed drugs for depression and anxiety disorders. Prolonged use of fluoxetine results in hepatic toxicity. Baicalin is a natural compound obtained from the ancient Chinese herb *Scutellaria baicalensis*. Baicalin is known to possess several antioxidant, anti-inflammatory, anticancer, neuroprotective, cardioprotective and hepatoprotective effects.

### ***Research motivation***

In previous reports, the hepatotoxic effects of fluoxetine upon prolonged treatment have been reported. Since baicalin has anti-inflammatory and hepatoprotective properties, therefore this study was aimed to evaluate the hepatoprotective and anti-inflammatory properties of baicalin when co-administered with fluoxetine.

### ***Research objectives***

The objective of the study was to assess the protective action of baicalin in fluoxetine induced liver toxicity and inflammation.

### ***Research methods***

Male albino Wistar rats were divided into seven groups. Group 1 was normal control. Fluoxetine was orally administered at 10 mg/kg body weight to groups 2, 3, 4 and 5. In addition, groups 3 and 4 were also co-administered orally with baicalin (50 mg/kg and 100 mg/kg, respectively) while group 5 was fed with silymarin (100 mg/kg). Groups 6 and 7 were used as positive control for baicalin (100 mg/kg) and silymarin (100 mg/kg). All the treatments were carried out for 28 d. Biomarkers of oxidative stress [superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione-

S-transferase (GST), advanced oxidation protein products (AOPP), malondialdehyde (MDA)], and liver injury [alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, albumin, bilirubin] were studied in serum and tissue using standard protocols and diagnostic kits. Inflammatory markers [tumor necrosis factor (TNF- $\alpha$ ), interleukin (IL)-6, IL-10 and interferon (IFN)- $\gamma$ ] in serum were evaluated using ELISA based kits. Effect of drug on liver was also analysed by histopathological examination of tissue sections.

### ***Research results***

Fluoxetine-treated rats showed elevated levels of the serum liver function markers (total bilirubin, ALT, AST, and ALP) and inflammatory markers (TNF- $\alpha$ , IL-6, IL-10 and IFN- $\gamma$ ), with decline in total protein and albumin levels. The biochemical markers for oxidative stress such as SOD, CAT, GST, GSH, MDA and AOPP in the liver tissue homogenate were also altered indicating surge in reactive oxidative species leading to oxidative damage. Histological examination of liver tissue also showed degeneration of the hepatocytes. Concurrent administration of baicalin (50 and 100 mg/kg) restored the biomarkers of oxidative stress, inflammation and hepatic damage in serum as well as in liver tissues to near normal levels.

### ***Research conclusions***

The results suggested that prolonged fluoxetine treatment leads to oxidative stress *via* formation of free radicals that consequently cause inflammation and liver damage. Co-administration of baicalin alleviated fluoxetine induced hepatotoxicity and liver injury by regulating oxidative stress and inflammation.

### ***Research perspectives***

Baicalin exhibited considerable hepatoprotective activity at a dose of 100 mg/kg and it was found comparable to the standard compound silymarin at the same dose. Therefore, baicalin can be used as an alternate supplementation along with fluoxetine to

prevent hepatic toxicity and inflammation. However, further research is needed for better understanding of the key pathways and mechanisms that could explain the protective effects of baicalin against fluoxetine induced liver injury, oxidative damage and particularly the anti-inflammatory response.

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