

Protective role of broccoli powder against continuous ingestion of escitalopram antidepressant drug induced hepatotoxicity in Swiss albino male mice

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Abstract

To investigate the protective role of broccoli powder "*Brassica Oleracea Italica*" against continuous ingestion of escitalopram antidepressant drug induced hepatotoxicity in Swiss albino male mice. Methods: Mice were divided into different groups. Group 1: Normal control (0.9% NaCl), Group 2: Escitalopram drug treated only (20 mg/kg), Group 3: Broccoli powder with Escitalopram drug treated (200 mg/kg + 20 mg/kg), Group 4: Olive oil vehicle control, Group 5: Carbon tetrachloride (CCl₄) referenced as positive control (33 mg/kg), Group 6: Broccoli powder with CCl₄ treated (200 mg/kg + 33 mg/kg). The effect of these groups on liver tissue was studied after three different time periods for 4, 8 and 12 weeks. The results showed that the treatment with escitalopram drug displayed significantly increased serum SGOT, SGPT, ALP level and alter liver antioxidant enzymes level (LPO, SOD and GSH) that are comparable with CCl₄ intoxicated group considered as positive control. Comparing escitalopram drug treated group with group that received both broccoli powder and escitalopram drug displayed a significant decrease in serum SGOT, SGPT, ALP levels and restored the level of antioxidant enzymes. The protective effect of broccoli powder on escitalopram drug induced hepatotoxicity was also supported by histopathological studies.

Keywords: Escitalopram antidepressant drug, Broccoli powder, CCl₄, Hepatotoxicity

Introduction

Antidepressants are classes of psychotherapeutic drugs that relieve the symptoms of depression. They were first developed in the 1950s and have been used regularly since then [1]. These are classified into 4 groups: Serotonin reuptake inhibitors (SSRIs), Serotonin norepinephrine reuptake inhibitors (SNRIs), Tricyclic antidepressants (TCAs) and Monoamine oxidase inhibitors (MAOIs). The SSRIs are the most commonly prescribed class of antidepressants and are considered as the first-line medications for the treatment of depression and anxiety. They act on a chemical in the brain called serotonin. The SSRIs include drugs such as Citalopram, Escitalopram, Sertraline and Fluoxetine. Escitalopram is the most commonly prescribed antidepressant drug comprising 40% of total prescriptions [2] Escitalopram is the active S-enantiomer of citalopram [3]. All serotonin reuptake inhibitors are metabolized in liver by cytochrome P450 system for reason they competitively inhibit these hepatic enzyme and

increase the levels of other medications metabolized by these enzymes, possibly leading to toxic effects [4], [5], [6] and [7]. Superfoods are excellent source of nutrition that contain large amounts of antioxidants, polyphenols, vitamins, and minerals. Consumption of superfoods may reduce the risk of chronic disease such as cancer, diabetes, heart ailments etc. Superfood such as broccoli is a cruciferous vegetable that belong to the cabbage family Brassicaceae and classified as the Italica cultivar group of the species *Brassica Oleracea* [8] and [9]. Broccoli is an edible green large flowering head and is consumed as a vegetable. The aim of present work was to investigate protective role of broccoli against continuous ingestion of antidepressant drug induced liver damage in Swiss albino male mice.

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Materials and Methods

Drug and Chemical: Escitalopram oxalate drug was obtained from Abbott Healthcare, Jaipur, India. Drug was dissolved in isotonic (0.9% NaCl) saline solution immediately before use. The dose of escitalopram drug was selected according to [10]. Carbon tetrachloride (CCl₄) was obtained from Merck Limited, India. The dose of CCl₄ was selected according to California Public Health Goals for chemicals in drinking water guidance [11]. **Plant Material:** Broccoli florets were used and was purchased from local grocery market in Jaipur, India. The fresh florets were washed under running tap water, air dried, powdered and a suspension was prepared in isotonic saline solution. The dose of broccoli powder was selected according to [12] and [8]. Laboratory grade olive oil was used as a vehicle for the tested compound carbon tetrachloride. The conversion of experimental doses were based on [13] and [14]. **Test Animal:** Swiss albino male mice, weighing 22-35 g were used throughout the experiments. The animals were housed under standard laboratory conditions, were maintained on natural light and dark cycle and had free access to food and water. Each group had 20 animals. All animal procedures were performed in accordance to the Institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals.

Experiment Design

Behavioral Test for Antidepressant efficacy

A well-established behavioral test for measuring antidepressant response is the forced swim test (FST), known as the Porsolt test [15]. FST animal model produced physical stress that lead to depression. This model of depression provided a fast and consistent behavior screening test for antidepressant. In FST, mice were forced to swim in a restricted space from which they cannot escape and are induced to immobility [16]. The decrease in the immobility time is accompanied with the increase in swimming time. The FST was conducted as follows: The animals were divided into different groups. The depression in each animal was induced by forced swim test for 6 min for first day [17]. The first group was assigned as depression control receiving 0.9% saline solution, the second group received depression + antidepressant drug (Escitalopram oxalate 20 mg/kg) and the third group received depression + combination of broccoli powder with antidepressant drug (200 mg/kg + 20 mg/kg). All doses were administered orally one hour prior to the swim test. The mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), with water depth of 10 cm at 25±1 °C and it remained there for 6 minute. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above water. The time of immobility was recorded during the last 4 minute of the 6 minute testing period i.e. after 2 min of habituation. Results were expressed as the immobility time during the last 240 seconds test period (mean ± SEM) [18]. A decrease in the duration of immobility indicated an antidepressant like effect.

Result of Behavior Test

The result of behavior test is shown in Table 1.

Table1: The effect of immobility period of FST activity in mice on antidepressant drug (20 mg/kg) and combination with broccoli powder (200 mg/kg+200 mg/kg). The value represent the mean ± SEM (n=20 mice/group).

Treatment	Mean duration of immobility for 240 second (sec)
Depression control (0.9% NaCl)	184.9 ± 9.22
Depression + Escitalopram antidepressant drug treated	109.4 ± 8.10 ****
Depression + Broccoli powder with Escitalopram drug treated	106.9 ± 11.04 ####
Data are shown as mean ± SEM (n=20). **** $P < 0.00001$, #### $P \leq 0.00001$, compared with depression control group.	

The group 2 that was treated with depression + escitalopram drug in the first day was followed by treatment of only escitalopram drug from second day for continuously 12 weeks. Group 3 that was administered with depression + combination of broccoli powder with escitalopram drug in the first day was followed by combination treatment of broccoli powder with escitalopram

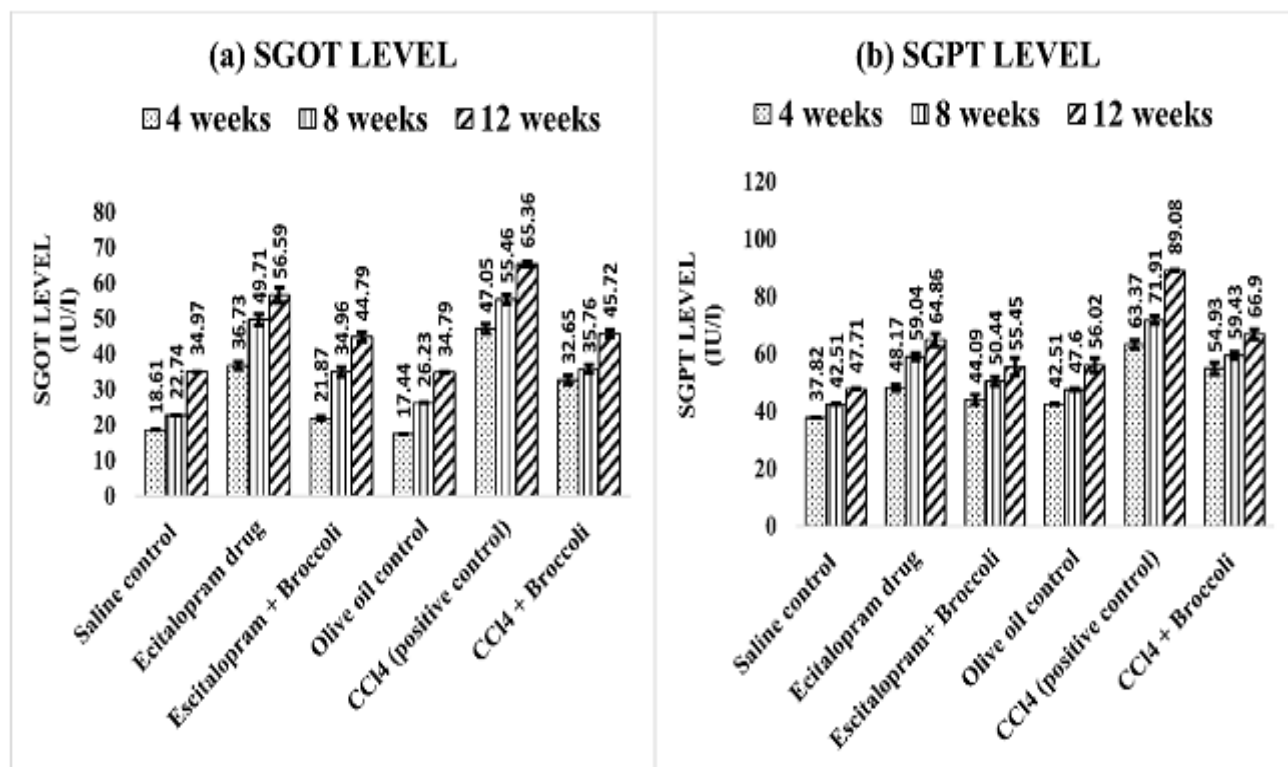
drug from second day for continuously 12 weeks. Mice were divided into different groups. Each group had 20 mice which were treated continuously for 12 weeks as follows: Group 1: Saline with vehicle control (0.9% NaCl). Group 2: Escitalopram antidepressant drug treated only (20 mg/kg) Group 3: Broccoli powder with Escitalopram drug treated (200 mg/kg + 20 mg/kg) Group 4: Olive oil (vehicle control) Group 5: Carbon

tetrachloride (CCl4) referenced as positive control (33 mg/kg) Group 6: Broccoli powder with CCl4 treated (200 mg/kg + 33 mg/kg). All treatments were orally administered continuously using a single dose of body weight, however, only group 2 and group 3 received FST. The effects of these groups on animal liver were studied after three different experimental periods of 4, 8 and 12 weeks. The effects of antidepressant drug were then compared with CCl4 treated group considered as positive control. Biochemical Analysis: Subsequent to completion of 4, 8 and 12 weeks treatment, the mice were killed by cervical dislocation. The blood from heart was immediately collected into sterilized tubes and was allowed to clot for serum separation. Serum was then separated by centrifugation at 3000 rpm for 10 minutes and was kept for subsequent evaluation of biochemical parameters such as serum SGOT, SGPT, ALP. The activities of these biochemical parameters were determined by using commercially

available kits (Accurex Biomedical Pvt. Ltd. and Jeev Diagnostic Pvt. Ltd.). The liver was removed and washed with ice-cold saline solution (0.9%) and stored at -4 oC for analysis of liver antioxidant enzyme activity such as lipid peroxidation (LPO), superoxide dismutase (SOD), and reduced glutathione (GSH) that were determined by methods of Ohkawa et al. [19], Marklund & Marklund [20] and Moron et al. [21], respectively. Statistical Analysis: Data were expressed as Mean ± SEM (Standard Error Mean) using Student's t-test. Statistical significance was considered at P < 0.05. Probability values less than 0.01 were considered as highly significant (P < 0.01).

Results

The result of biochemical analysis and liver antioxidant enzyme levels is shown in Figure 1.



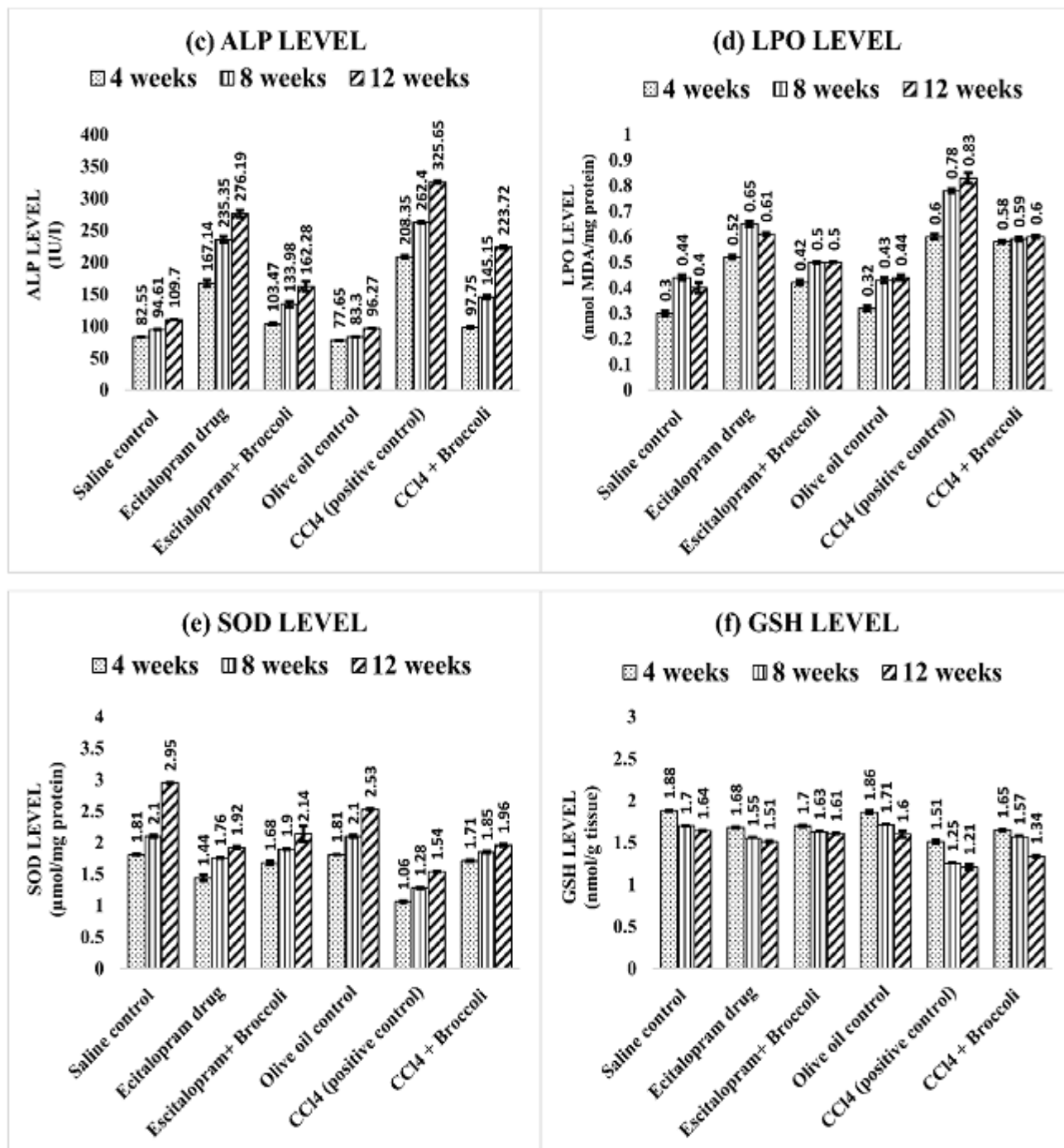


Figure 1: Bar chart depicting biochemical analysis and antioxidant enzyme levels on different groups of mice after treatment for 4, 8 and 12 weeks. (a) serum SGOT level (IU/l); (b) serum SGPT level (IU/l); (c) serum ALP level (IU/l); (d) liver LPO level (nmol MDA/mg protein); (e) liver SOD level (µmol/mg protein) and (f) reduced GSH level (nmol/g tissue). Data are shown as mean ± SEM.

The result of histopathological analysis is shown in Figure 2 and Figure 3.

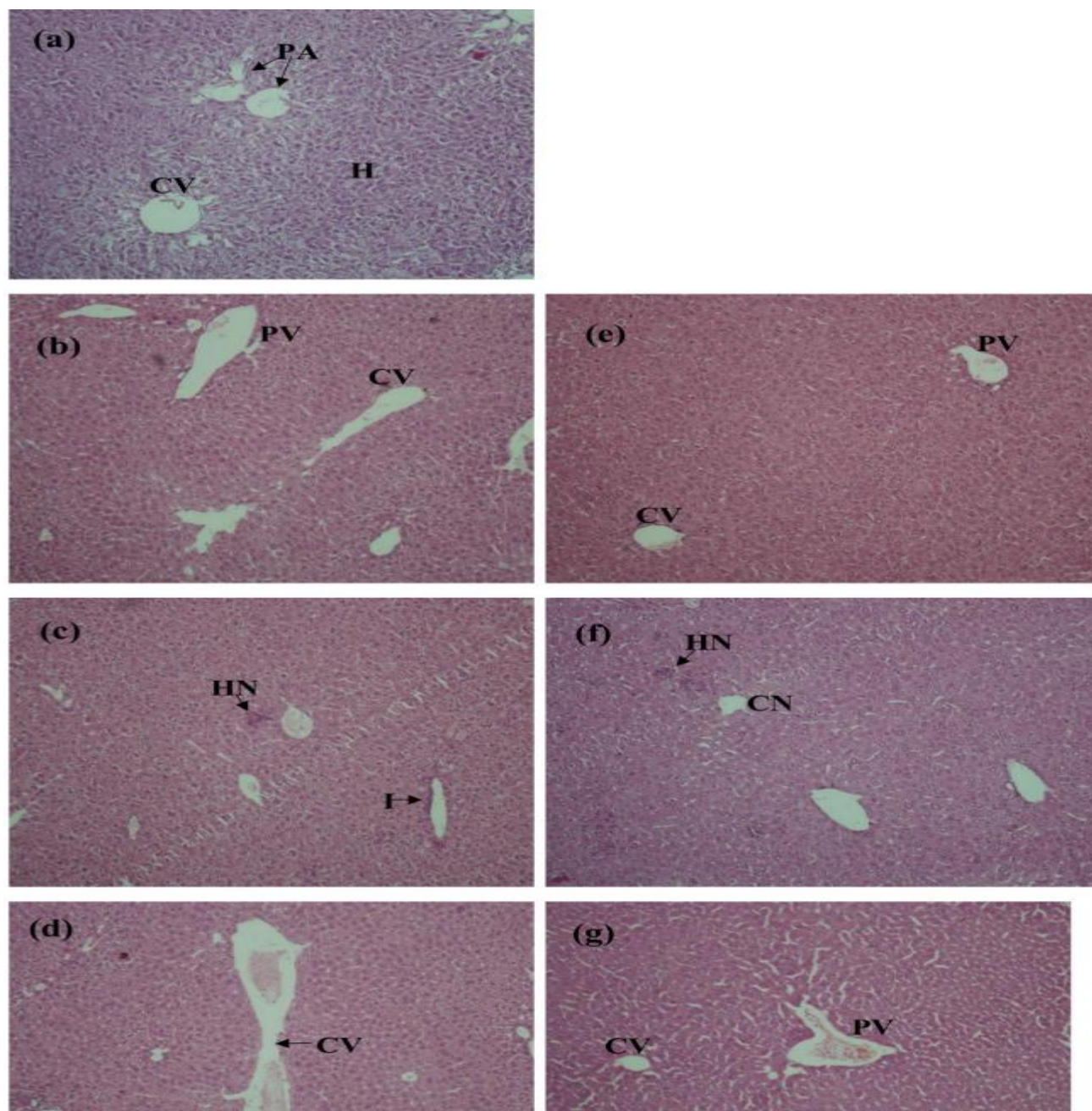


Figure 2: Effect of broccoli powder on escitalopram drug induced liver toxicity in mice after treatment for 4, 8 and 12 weeks. (a) T.S. of the liver from a control (0.9% NaCl) mice showing normal central veins (CV) and portal area (PA) with normal hepatic cells (H) after 4, 8 and 12 weeks treatments; (b, c, d) Escitalopram drug treated T.S. of liver showing dilation, congestion of central vein (CV) and portal vein (PV) (after 4 weeks, b), hepatocyte necrosis (HN) and mononuclear cells infiltration (I) in hepatic area (after 8 weeks, c), severe dilation and congestion of central vein (CV), congested portal vein (PV) (after 12 weeks, d) ; (e, f, g) T.S. of liver treated with broccoli powder and drug showing minimal congestion of central vein (CV) and portal vein (PV) (after 4 weeks, e), minimal hepatocyte necrosis (HN) near the central vein (CV) (after 8 weeks, f) and dilation of portal area (PA) with less congested central vein (CV) (after 12 weeks, g) compared with escitalopram drug treated group (H&E 100).

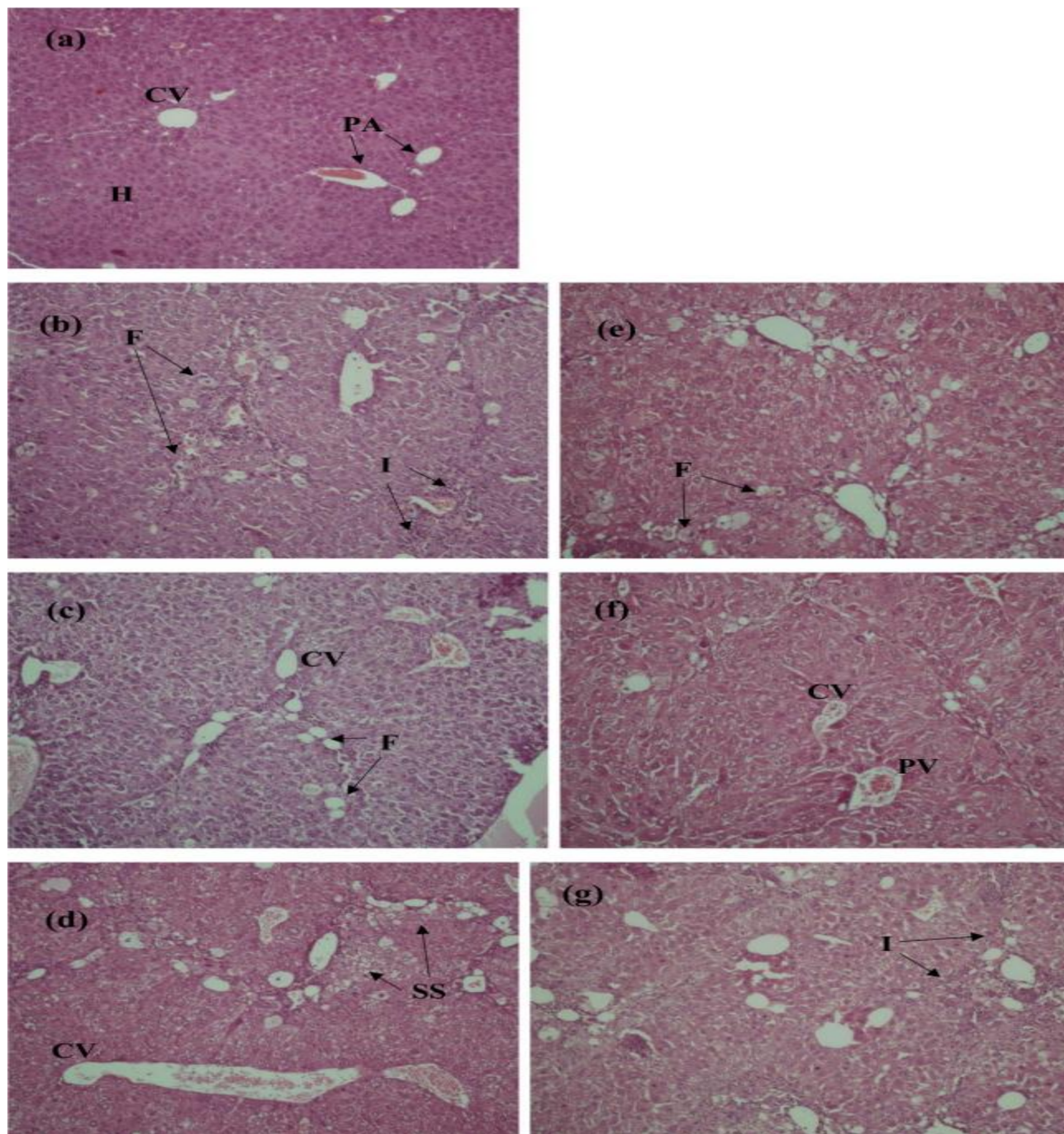


Figure 3: Effect of broccoli powder on CCl₄ induced liver toxicity in mice after treatment for 4, 8 and 12 weeks. (a) T.S. of the liver section from a control group (olive oil) showing normal hepatic architecture with normal central vein (CV), portal vein (PV) and hepatocytes (H) after 4, 8 and 12 weeks treatments; (b, c, d) T.S. of liver tissue from a mice received CCl₄ showing fatty degeneration (F) of hepatocytes with aggregation of inflammatory cells infiltration (I) surrounding the lobule (after 4 weeks, b), centrilobular fatty changes (F) with congestion of central vein (CV) (after 8 weeks, c), severe steatosis degeneration (SS) with dilated and congested central vein (CV) (after 12 weeks, d); (e, f, g) Liver T.S. treated with broccoli powder and CCl₄ showing less fatty degeneration of hepatocytes (F) (after 4 weeks, e), slightly dilation of central vein (CV) and portal vein (PV) (after 8 weeks, f) and inflammatory cells infiltration (I) surrounding the lobule when compared with CCl₄ treated group (after 12 weeks, g) (H&E 100).

Discussion

The FST immobility result revealed that antidepressant drug (Escitalopram oxalate) treated group and co-administrated with broccoli powder showed significant decrease in immobility time as compared to control group (Table 1). The effect of escitalopram drug on biochemical parameter and antioxidant enzymes in liver was studied for three time periods of 4, 8 and 12 weeks. The drug treated group was then compared with CCl₄ treated group, since CCl₄ is a standard hepatotoxic agent and therefore, was considered as positive control. Our results are in agreement with Abdelmajeed [22], Zlatkovi *et al.*, [23] and Yilmaz [24]. The present study of escitalopram drug treated group showed significantly increased SGOT level ($P < 0.01$, $P < 0.01$, $P < 0.01$), SGPT level ($P > 0.05$, $P > 0.05$, $P > 0.05$), ALP level ($P < 0.01$, $P < 0.001$, $P < 0.001$), LPO level ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$), significantly decreased SOD ($P < 0.001$, $P < 0.00001$, $P < 0.00001$) and reduced GSH level ($P < 0.00001$, $0.00001 < P < 0.001$) as compared to saline control group after 4, 8 and 12 weeks treatment (Figure 1) respectively. Similar conclusion was reported by CCl₄ hepatotoxic group that was considered as positive control. The present study showed that escitalopram drug induced elevated level of SGOT, SGPT, ALP, LPO, reduced level of SOD and GSH were comparable with CCl₄ treated group ($P < 0.001$, $P < 0.0001$, $P < 0.00001$), ($P < 0.05$, $P < 0.001$, $P < 0.001$), ($P < 0.001$, $P < 0.001$, $P < 0.001$), ($P < 0.00001$, $P < 0.001$, $P < 0.00001$) ($P < 0.00001$, $P < 0.00001$, $P < 0.00001$) and ($P < 0.00001$, $P < 0.001$, $P < 0.00001$) after treatment of 4, 8 and 12 weeks respectively. Thus, the present results revealed that escitalopram antidepressant drug showed hepatotoxicity and were comparable to CCl₄ intoxicated group (Figure 1). Hashem *et al.* (2013) observed that broccoli showed good hepatoprotective activity against liver damage induced by paracetamol [24]. The present study provided an evidence for a beneficial effect of the broccoli powder with escitalopram antidepressant drug decreasing toxicity in liver caused by escitalopram drug. The result showed that broccoli powder co administered with escitalopram drug significantly decreased elevated level of SGOT ($P < 0.05$, $P < 0.01$, $P < 0.01$), SGPT ($P > 0.05$, $P > 0.05$, $P > 0.05$), ALP ($P < 0.01$, $P < 0.01$, $P < 0.01$), LPO ($P < 0.001$, $P < 0.01$, $P < 0.01$), significantly increased in the SOD ($P < 0.01$, $P < 0.0001$, $P < 0.0001$) and reduced GSH level ($P < 0.0001$, $P < 0.001$, $P < 0.05$) as compared to only escitalopram drug-intoxicated group after 4, 8 and 12 weeks treatments (Figure 1) respectively. The present investigation also indicated the effectiveness of broccoli powder that was continuously administered with CCl₄ for 12 weeks to mice showed significant reduction in the level of SGOT ($P < 0.001$, $P < 0.05$, $P < 0.001$), SGPT ($P > 0.05$, $P < 0.01$, $P < 0.05$), ALP ($P < 0.001$, $P < 0.05$, $P < 0.01$), LPO level ($P < 0.00001$, $P < 0.0001$, $P < 0.0001$), significantly increased low level of SOD ($P < 0.01$, $P < 0.001$, $P < 0.00001$) and reduced GSH level ($P < 0.001$, $P < 0.001$, $P < 0.01$) as compared to CCl₄ treated group (Figure 1). The present results of beneficial effects of broccoli on

CCl₄ induced hepatotoxicity were in agreement with AlHowiriny [26], El-Baz *et al.* [12] and Subramanian [8]. The histopathological examination revealed that the escitalopram drug treated group showed severe dilation and congestion of central vein, congested portal vein, mononuclear cell infiltration with hepatocyte necrosis (HN) in close proximity to hepatic vein (Figure 2b, 2c and 2d). A similar histopathological alteration was reported by Zlatkovi *et al.* [22], Abdelmajeed [23] and Yilmaz [24]. Zlatkovi *et al.* (2014) observed that chronic administration of fluoxetine (15 mg/kg/day) or clozapine (20 mg/kg/day) in rat liver showed necrosis and fatty changes of hepatocytes with inflammatory infiltrate of lymphocytes and macrophages near central venule [22]. Abdelmajeed (2009) worked on the oxidative tissue damage induced in liver in response to citalopram for three different experimental periods of 10, 20 and 30 days showing severe degenerative change in hepatocytes and congestion of central vein [23]. Histopathological observations of escitalopram drug treated group showed degenerative change in hepatocytes, congestion of central vein and mononuclear cell infiltration of hepatic vein that were comparable with CCl₄ treated group. CCl₄ intoxicated group showed severe fatty degeneration of hepatocytes with aggregation of inflammatory cells infiltration surrounding the lobule and severe steatosis degeneration (Figure 3b, 3c and 3d). Histopathological results of CCl₄ were similar to Al-Ghamdi [27], Eidi *et al.*, [28], Soujanya *et al.*, [29], Cordeiro and Kaliwal [30], Althnain *et al.*, [31] and Abdel *et al.*, [32]. From the histopathological examination of the present work it could be concluded that broccoli powder with escitalopram drug treated group showed minimal congestion of central vein and portal vein with minimal hepatocyte necrosis near the central vein [24] (Figure 2e, 2f and 2g) when compared with escitalopram drug intoxicated group. The broccoli powder and CCl₄ group showed less fatty degeneration of hepatocytes with slight dilation of central vein and portal vein with inflammatory cells infiltration surrounding the lobule [12] (Figure 3e, 3f and 3g) when compared with CCl₄ treated group.

Conclusion

The results showed that escitalopram antidepressant drug exhibited hepatotoxicity comparable to CCl₄ intoxicated group considered as positive control. However, combination therapy of broccoli powder with escitalopram antidepressant drug and CCl₄ showed significant hepatoprotective effect against continuous ingestion of drug and CCl₄ treated groups alone. The results demonstrated that powder of *Brassica Oleracea Italica* (BROCCOLI, 200 mg/kg) had potent hepatoprotective effect against escitalopram antidepressant drug and CCl₄ induced liver damage in Swiss albino male mice.



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Nil.

Conflict of Interest

There is no conflict of interest.

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