



## Research Article

### AMELIORATIVE EFFECT OF BROCCOLI POWDER ON RENAL TOXICITY IN MICE CAUSED BY CONTINUOUS EXPOSURE TO ESCITALOPRAM ANTIDEPRESSANT DRUG

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#### ABSTRACT

Antidepressants induced metabolic syndrome is a major public health problem in the treatment of psychiatric disorders which leads to an increased risk of cardiovascular disease, non-alcoholic hepatic steatosis and renal dysfunction. Psychiatric disorder is a mental illness that includes depression, schizophrenia, and anxiety disorder that affect millions of people around the world. The purpose of present study was to investigate the protective role of broccoli powder against continuous ingestion of antidepressant drug induced nephrotoxicity in Swiss albino mice. In present study mice were continuously treated with escitalopram antidepressant drug (20 mg/kg/b.w. orally) for 4, 8 and 12 weeks. The escitalopram drug intoxicated group showed elevation in serum urea and creatinine level. Escitalopram drug intoxicated group also showed degeneration of glomerulus and renal tubule that are comparable with carbon tetrachloride (33 mg/kg) group considered as positive control. Group that received both broccoli powder and escitalopram drug (200 mg/kg + 20 mg/kg) significantly reduced elevated level of serum urea and creatinine with mild degeneration of glomerulus and renal tubule as compared to antidepressant drug intoxicated group.

**Keywords:** Escitalopram drug, CCl<sub>4</sub>, Broccoli powder

#### INTRODUCTION

Selective serotonin re-uptake inhibitors (SSRIs) are a class of antidepressants that have been available since the late 1980s<sup>1</sup> and are considered as the first-line medications for treatment of depression and anxiety. SSRIs drugs are used to treat the symptoms of depressive disorders by correcting chemical imbalances of neurotransmitters within the brain. They are called "selective" because they mainly affect serotonin. This class includes drugs fluoxetine, sertraline, citalopram and escitalopram.

Escitalopram is a pharmacologically active S-enantiomer of citalopram that selectively binds to the human serotonin transporter (SERT). This activity inhibits serotonin (5-HT) reuptake and increases the amount of serotonin in synaptic clefts, which results in antidepressant action<sup>2,3</sup>.

The ability of the SSRIs drug to block neurotransmitters in the brain leads to various side effect of these drug classes such as sexual dysfunction, weight gain, central nervous system, serotonin syndrome, cardiotoxicity, hypertension, liver function tests and renal dysfunction<sup>4-6</sup>.

Medicinal plants, herbs and vegetables have been used for the treatment of various diseases in different parts of the world. Natural products from plants, fungi, bacteria and other organisms continue to be used in pharmaceutical preparations either as pure compounds or as extracts and raw juices<sup>7</sup>.

Broccoli (*Brassica oleracea* L.) is a plant of the cabbage family, *Brassicaceae*, and is widely recognized for its contribution to human nutrition and health benefits. It contains several bioactive compounds, such as isothiocyanates, vitamins C and E, quercetin and kaempferol glycosides and associated with inhibition of chemically induced carcinogenesis in laboratory animals and humans. It also acts as purgative, diuretic<sup>8,9</sup>.

The present study was aimed to investigate the protective effect of broccoli powder against continuous ingestion of antidepressant drug induced nephrotoxicity in Swiss albino male mice.

#### MATERIALS AND METHODS

**Test Animal:** All animal procedures were performed in accordance to the Institutional Animal Ethics Committee (Registration No. 1689/PO/a/13/CPCSEA) and in accordance with the recommendations for the proper care and use of laboratory animals. In this study, Swiss albino male mice (weighing 22-35 g) were used throughout the experiments. Each group had 20 animals. The animals were housed under standard laboratory conditions, were maintained on natural light and dark cycle and had free access to food and water.

**Drug and Chemical:** Escitalopram oxalate drug was used and purchased from Abbott Healthcare, Jaipur, India. The drug was dissolved in isotonic (0.9% NaCl) saline solution immediately before use and the dose of escitalopram drug was selected according to Cimen *et al.*, (2015)<sup>10</sup>. Carbon tetrachloride (CCl<sub>4</sub>)

was obtained from Merck Limited, India and the dose of CCl<sub>4</sub> was selected according to California Public Health Goals for chemicals in drinking water guidance<sup>11</sup>.

**Plant Material:** Broccoli was purchased from local grocery market in Jaipur, India. The fresh broccoli 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 El-Baz *et al.*, (2012)<sup>8</sup> and Subramanian (2011)<sup>12</sup>. Laboratory grade olive oil was used as a vehicle for the tested compound carbon tetrachloride. The conversion of experimental doses was based on CDER (2005)<sup>13</sup> and Shin *et al.*, (2010)<sup>14</sup>.

### Experiment Design

**Group 1:** Saline with vehicle control (0.9% NaCl) for 12 weeks treatment

**Group 2:** Antidepressant drug treated groups

Forced swim test (FST) for Antidepressant efficacy<sup>15</sup>

2a) Depression control receiving 0.9% saline solution

2b) Depression+ antidepressant drug (Escitalopram oxalate 20 mg/kg)

2c) 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.

FST was performed on first day on group 2b and 2c, from second day group 2b and 2c were continuously administered

antidepressant drug and its combination with broccoli powder respectively, for 12 weeks without FST.

**Group 3:** Positive control group

3a) Olive oil (vehicle control) for 12 weeks treatment

3b) Carbon tetrachloride (CCl<sub>4</sub>) (33 mg/kg) for 12 weeks

3c) Broccoli powder with CCl<sub>4</sub> treated (200 mg/kg + 33 mg/kg) for 12 weeks

All experimental doses were continuously administered orally for 12 weeks, once a day according to respective body weight. The effects of experimental groups on animal kidney were studied after three different experimental periods of 4, 8 and 12 weeks. The effects of antidepressant drug were then compared with CCl<sub>4</sub> treated group considered as positive control.

### Forced Swim Test (FST)

FST is a common behavioral test for assessing depression in rodents and testing the efficacy of antidepressants drugs, as originally developed by Porsolt in 1977. In this test rodents are forced to swim in a narrow cylinder from which there is no escape and, thus, are induced to a characteristic behavior of immobility. A decrease in the duration of immobility indicated an antidepressant like effect. The depression in each animal was induced by forced swim test for 6 min<sup>15, 16</sup>.

FST was conducted as mentioned in Chenu *et al.*, (2006)<sup>17</sup>. Result was expressed as the immobility time during the last 240 seconds test period (mean ± SEM) and is shown in Figure 1.

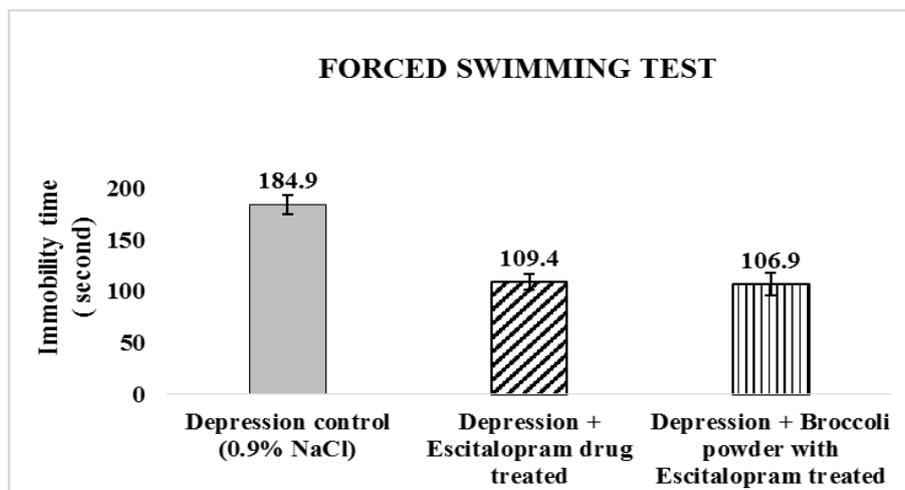


Figure1: The effect of immobility period of escitalopram drug and their combination with broccoli powder for forced swim test. Data are shown as mean ± SEM (n=20)

**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 separated by centrifugation at 3000 rpm for 10 minutes and kept for subsequent evaluation of kidney function tests using commercially available kits (Accurex Biomedical Pvt. Ltd. and Jeev Diagnostic Pvt. Ltd.). The kidney was removed and washed with normal saline and was fixed in 10% formalin for histological examination. The tissues were embedded in paraffin and sectioned at 5 µm and stained with hematoxylin and eosin (H & E)<sup>18</sup>.

**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 serum urea and creatinine level is shown in Figure 2 and 3. Histopathological analysis is shown in Figure 4 to 6.

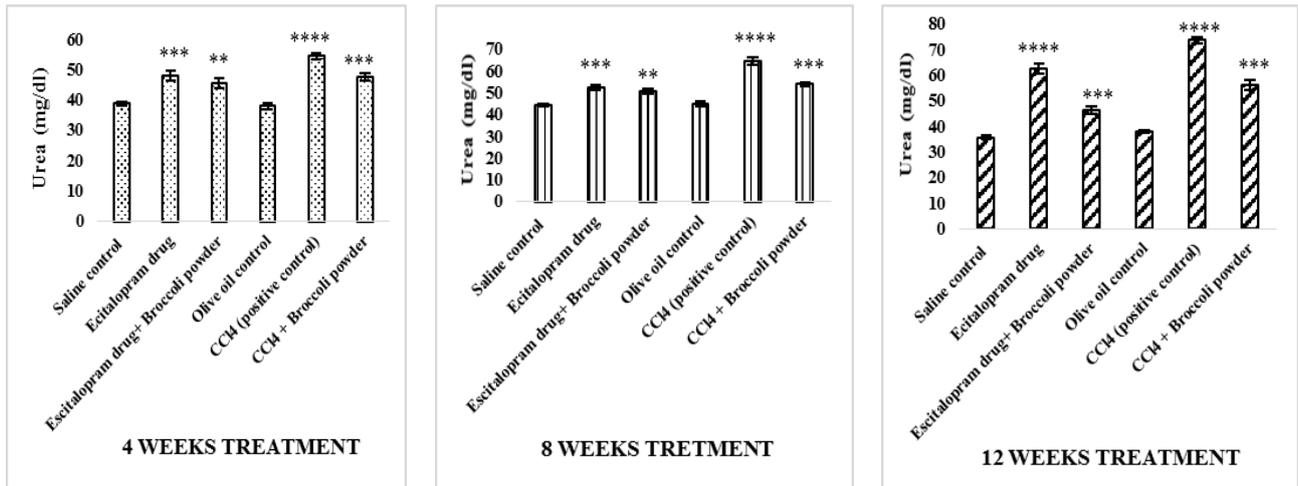


Figure 2: Bar chart depicting serum urea level on different groups of mice after treatment for 4, 8 and 12weeks

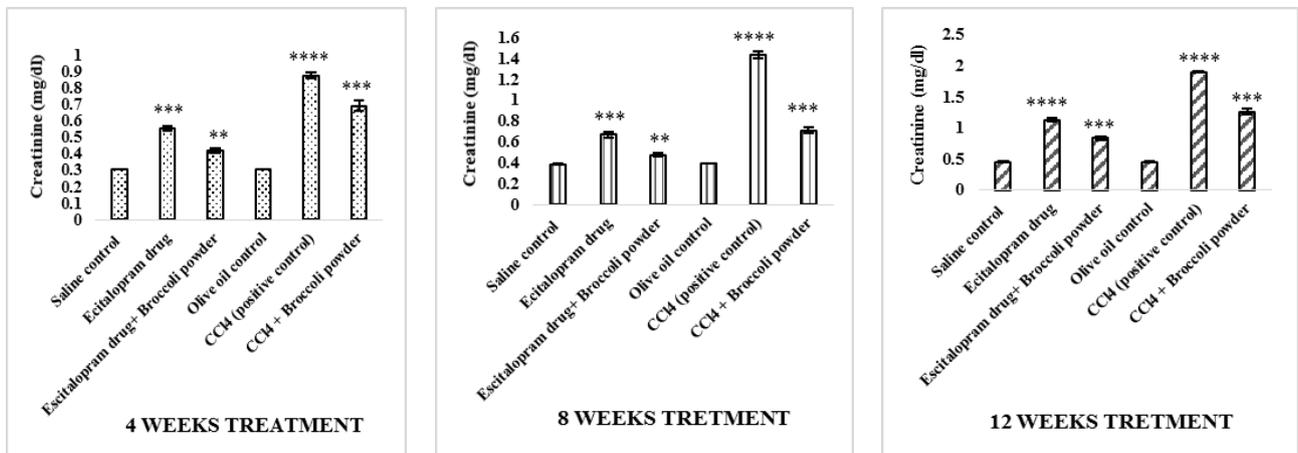


Figure 3: Bar chart depicting serum creatinine level on different groups of mice after treatment for 4, 8 and 12weeks

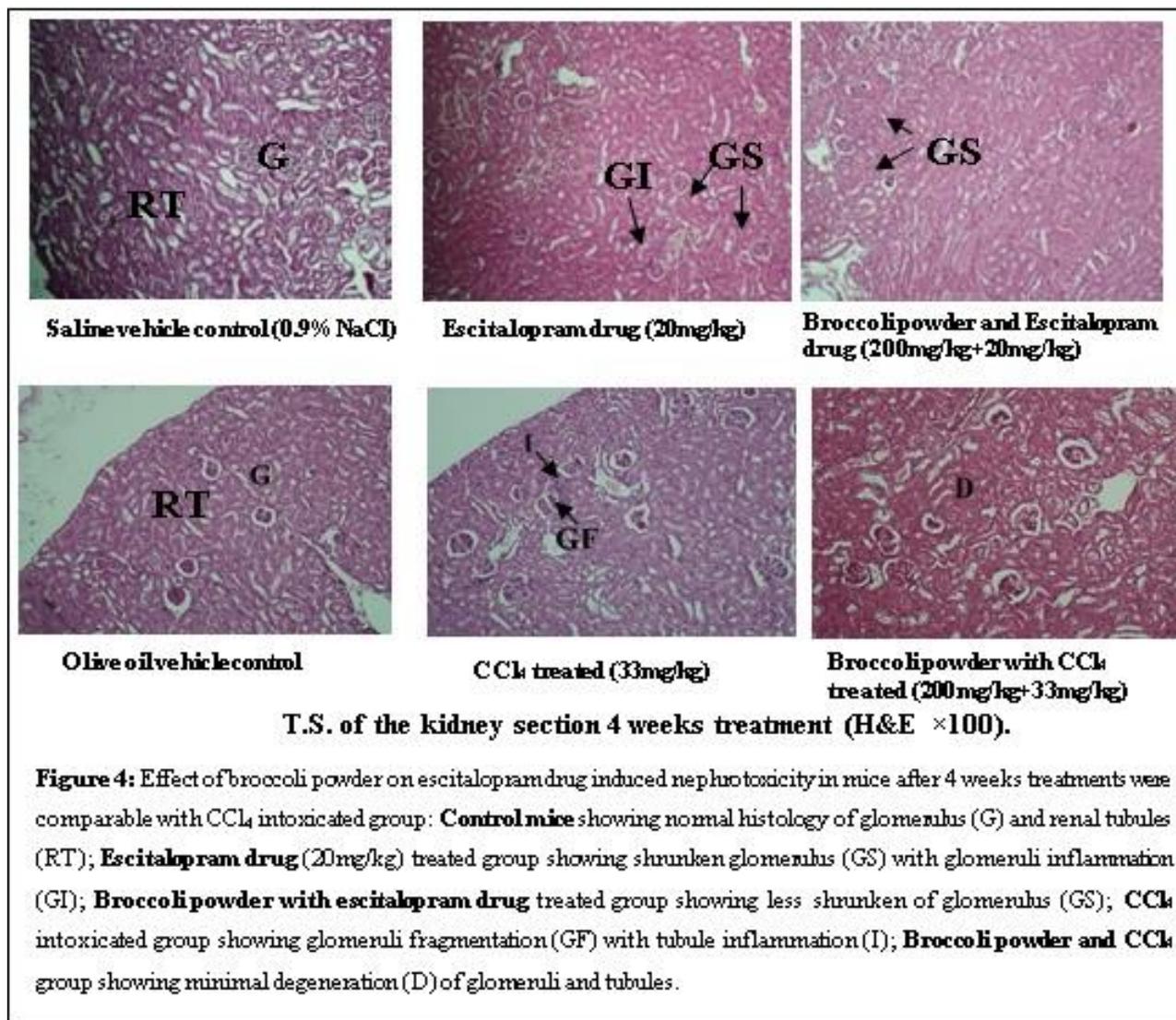
Data are shown as mean  $\pm$  SEM (Figure 2 and 3).

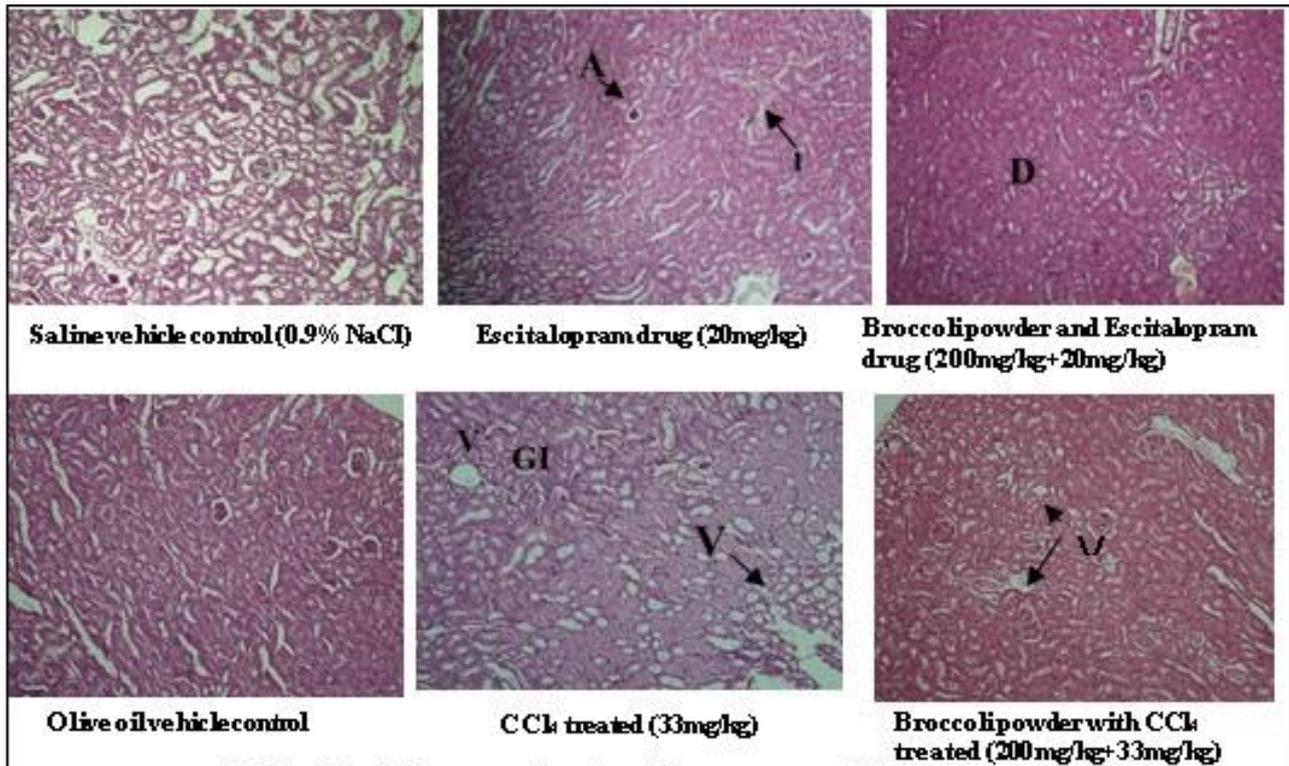
\*\* – the difference is statistically significant, compared with respective control at  $0.001 \leq P < 0.01$ .

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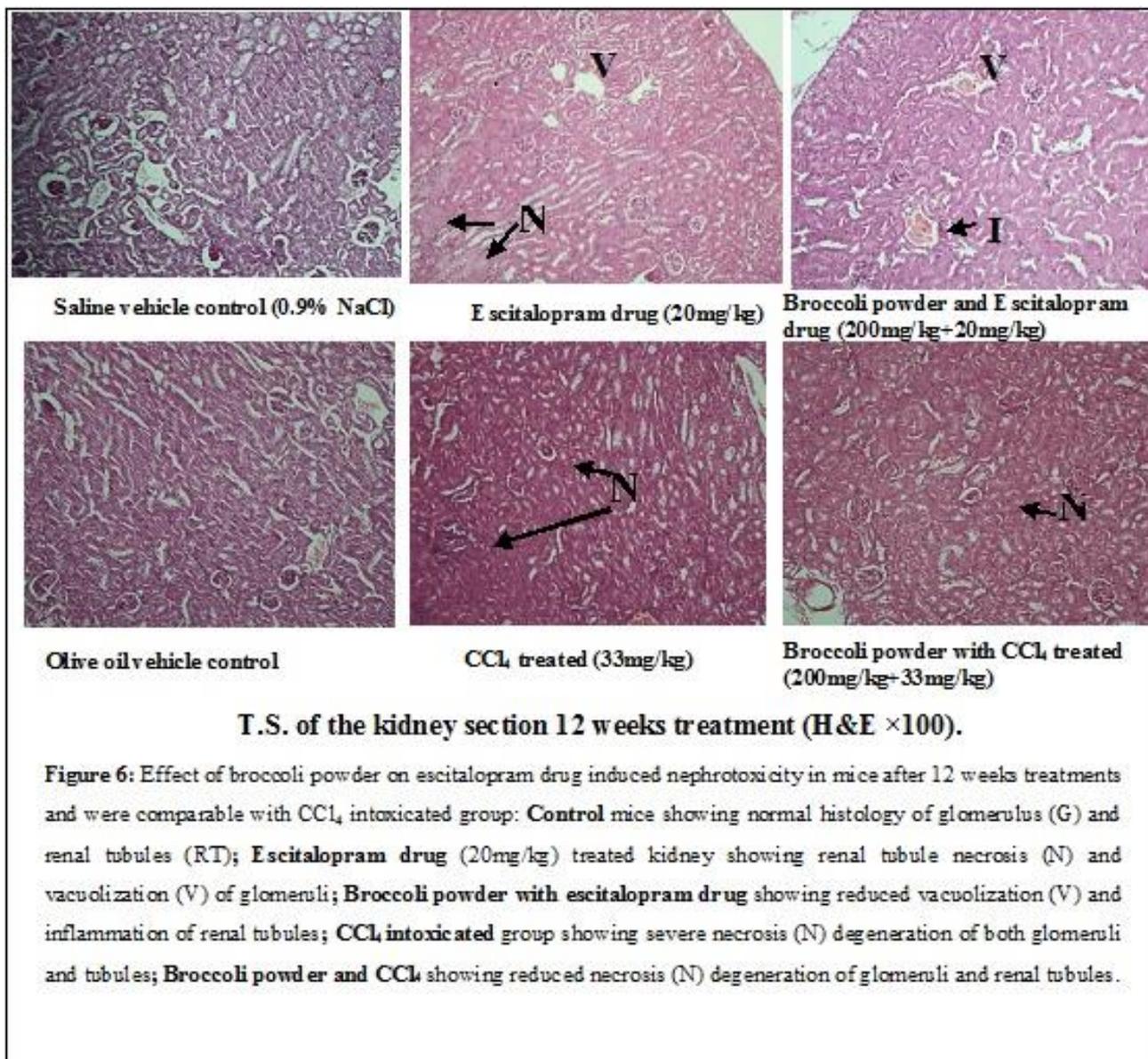
Above data were also referenced with positive control group (CCl<sub>4</sub>-intoxicated group).





**T.S. of the kidney section 8 weeks treatment (H&E ×100).**

**Figure 5:** Effect of broccoli powder on escitalopram drug induced nephrotoxicity in mice after 8 weeks treatments were comparable with CCl<sub>4</sub> intoxicated group: **Control** mice showing normal histology of glomerulus (G) and renal tubules (RT); **Escitalopram drug** (20mg/kg) treated T.S. of kidney showing atrophied glomeruli (A) with renal tubule inflammation (I); **Broccoli powder with escitalopram drug** treated group showing reduced dilation of renal tubule (D); **CCl<sub>4</sub> intoxicated group** showing glomeruli inflammation (GI) vacuolation (V) of glomeruli and renal tubule; **Broccoli powder and CCl<sub>4</sub>** treated group showing reduced vacuolation (V) of glomeruli and renal tubule.



## DISCUSSION

FST animal model result showed that escitalopram drug (escitalopram oxalate) treated group and broccoli powder (200 mg/kg) with escitalopram drug (20 mg/kg) treated group showed significant decrease immobility time as compared to depression control group (Figure 1).

The present study was undertaken to demonstrate the ameliorative effect of broccoli powder against renal damage induced by antidepressant drug in Swiss albino mice.

In this study, continuously administration of antidepressant drug significantly increased creatinine and blood urea level with histopathological alterations as compared with normal saline control group (Figure 2 to 6).

A high blood level of urea and creatinine indicates that the kidneys may not be functioning properly and the drug might have interfered with creatinine metabolism that caused renal toxicity in mice<sup>19, 20</sup>. A comparable renal toxicity was reported by Abdelmajeed (2009) and Gulec *et al.*, (2011). Abdelmajeed

(2009) reported that exposure of citalopram antidepressant caused oxidative tissue damage in rats showed significantly increased level of serum creatinine and uric acid with severe necrotic degeneration of both glomeruli and renal tubules for 10, 20 and 30 days treatments<sup>21</sup>. Gulec *et al.*, (2011) was also recorded similar histopathological result. Gulec *et al.*, (2011) noted that chronically administered olanzapine and risperidone caused nephrotoxic effects in male rats. Olanzapine and risperidone are members of the antipsychotic family and showed significant nephrotoxic effects with losing regularities in both tubular and glomerular structure of kidneys<sup>22</sup>.

In current work, similar conclusion was reported by CCl<sub>4</sub> intoxicated group referenced as positive control. CCl<sub>4</sub> intoxicated group also showed significantly increased serum urea and creatinine level with severe necrotic degeneration of both glomeruli and tubules (Figure 2 to Figure 6). The results of CCl<sub>4</sub> were similar to Sakrand and Lamfon<sup>23</sup>, Andrițoiu *et al.*,<sup>24</sup>, Mohamed *et al.*,<sup>25</sup> and Hermenean *et al.*,<sup>26</sup>

Thus, the present results revealed that escitalopram antidepressant drug showed nephrotoxicity and were comparable to CCl<sub>4</sub> intoxicated group.

The current study revealed that broccoli powder alleviated the renal toxicity of antidepressant drug. This was shown by normal appearance of kidney tissues and decreased levels of creatinine and urea (Figure 2 to 6). Similarly, Negrette-Guzmán<sup>1</sup> *et al.* (2013) reported that sulforaphane (SFN) treatment protects kidneys and renal tubular cells against gentamicin-induced nephrotoxicity. SFN is an extraordinary potent isothiocyanate naturally occurring in Cruciferae. It is because of precursor of SFN that is contained in broccoli, several experiments had been conducted with dietary ingestion of this cruciferous vegetable<sup>27</sup>.

The present investigation also indicated the effectiveness of broccoli powder that was continuously administered with CCl<sub>4</sub> for 12 weeks to mice and it showed significant reduction in the level of urea and creatinine with reduction of necrotic degeneration in glomeruli and tubules (Figure 2 to 6).

Thus, the present study provided an evidence for a beneficial effect of the broccoli powder that decreased toxicity in kidney caused by escitalopram drug.

## CONCLUSION

Our study suggests that broccoli powder may be considered as protective agent against antidepressant drug induced renal damage in mice. This effect may be attributed to high sulforaphane contents in broccoli.

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