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Original Article

Protective effect of Purslane on liver from reserpine-induced Hepatic toxicity in mice

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ABSTRACT:

Purpose: *(Portulaca oleracea)* is a universal species with a broad range of biological activities. This study aims to estimate the hepatoprotective effects of purslane ethanolic and aqueous extracts on hepatotoxicity induced by reserpine. **Method and Mothods**: Mice were divided into five groups and treated for four weeks. The first group is a negative control with no treatment. The second group is a positive control treated only with reserpine. The other groups were treated with reserpine and also treated with Escitalopram or purslane ethanolic extract, or purslane aqueous extract. Blood serum was used to estimate the concentrations of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Albumin, and Globulin.
Results: The data showed that the serum concentrations of ALT and AST were significantly elevated in all treated groups compared to the negative control group. In contrast, these values were significantly lower than the positive control group. Moreover, a significant decrease was exhibited in serum albumin and globulin in all treated groups as compared to the negative control group, and at the same time, there was a significant increase as compared to the positive control group.
Conclusion: Purslane ethanolic and aqueous extracts have a partial protection from liver toxicity induced by reserpine.

Key words: Purslane, *Portulaca oleracea*, Reserpine, hepatic, liver, toxicity, Mice.

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INTRODUCTION:

Purslane (*Portulaca oleracea*) is a succulent plant in the family Portulacaceae (figure 1A). It has been used as a food since ancient times. The plant is consumed fresh as a salad or cooked in many countries [1]. It is also used in traditional medicine in many countries for treating some skin conditions. and also used as an anti-inflammatory and a diuretic herb, it is also used for healing wounds and burns [2]. Reserpine (figure 1 D) appears naturally in the dried roots of plants such as snake root (*Rauwolfia Serpentina)* and the poison devil 'pepper *(Rauvolfia vomitoria)* (figure 1 B and C). It is an indole alkaloid antipsychotic and antihypertensive drug that has been used for controlling high blood pressure and for the relief of psychotic symptoms [3]. The antihypertensive actions of Reserpine are a result of its ability to deplete catecholamines from peripheral sympathetic nerve endings. These substances are normally involved in controlling heart rate, force of cardiac contraction, and peripheral resistance [4]. On the other hand, reserpine irreversibly blocks the intracellular vesicles' monoamine transporters. This blockage overlaps with the storage of monoamines into the intracellular vesicles, which effect in the depletion of catecholamines in nerve terminals and transient hypo locomotion and muscular rigidity [5]. The accumulation of neurotransmitters in the nerve terminals leads to a rise in the metabolism of these substances by monoamine oxidase. These can lead to the formation of reactive metabolites and hydrogen peroxide, producing free radicals and cellular damage by increased oxidative stress [6].

Figure 1. Purslane, snake root, poison devil 's-pepper, and Reserpine structure.

(A) Purslane (Portulaca oleracea) morphological appearance. (B) Snake root (Rauwolfia Serpentina) morphological appearance. (C) poison devil s-pepper (Rauvolfia vomitoria) morphological appearance. (D) Reserpine molecular structure.

This work aims to assess the hepatoprotective effect of purslane aqueous and ethanolic extracts against reserpine-induced hepatotoxicity in mice.

MATERIALS AND METHODS:

1.Experimental animals:

Adult male albino mice weighing 20-25 g were used. The animals were acclimatized for 7 days to a temperature (22 ±2 °C) and a 12-hour natural light/dark cycle, fed a standard diet, and water was provided.

2.Preparation of purslane extracts:

The aqueous extracts of purslane were prepared according to the method of Gülcin *et al.* (2004), and the ethanolic extracts of purslane were prepared according to the method of Wanyin *et al*. (2012) [7,8].

Experimental design:

Mice were divided into 5 groups. The first group was a negative control (Control). The second group is the positive control treated with reserpine 0.1 mg/kg (Res) as a single dose after 15 days of the beginning of the experiment (which is the dose that induces depression with minimal side effects). The third group was treated with escitalopram 1mg/kg and, after 15 days, treated with reserpine 0.1 mg/kg (Res) as a single dose (ESC+Res). The fourth group was treated with purslane ethanolic extract (PEE) 50 mg/kg, and after 15 days, treated with reserpine 0.1 mg/kg (Res) as a single dose (PEE+Res). The fifth group was treated with purslane aqueous extract (PAE) 50 mg/kg and, after 15 days, treated with reserpine 0.1 mg/kg (Res) as a single dose (PAE+Res).

Statistical Analysis

The data were expressed as means ± SE. One-way ANOVA Statistical analysis was performed, and the least significant difference (LSD) comparisons were performed to assess the significance of differences among various treatment groups. Statistical package for social science "SPSS" for Windows software, Release 18.0 (SPSS, Chicago, IL) was used at a p value ≤ 0.05.

RESULTS:

At the end of the experiment, the blood was collected and the serum was separated. The liver damage and affected protein production functions were measured by investigating the leakage of enzymes from the liver into the blood especially Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), and the protein production was measured by measuring the serum protein contents especially albumin and globulin.

The table below summarizes the results of these tests, and the statistical analysis using one-way ANOVA was performed to ensure that the differences were significant.

Table 1. Serum levels of all the parameters used for all the experimental groups.

Groups	Parameters				
	ALT	AST	Total Protein	Albumin	Globulin
Control	38.0 ± 1.7	49.7 ± 1.3	6.7 ± 0.21	4.2 ± 0.12	2.5 ± 0.07
Res	86.7 ± 2.7 (a)	105 ± 1.2 (a)	4.7 ± 0.14 (a)	2.6 ± 0.08 (a)	2.1 ± 0.06 (a)
ESC + Res	55.3 ± 2.6 (a, b)	76.5 ± 3.1 (a, b)	6.2 ± 0.18 (a, b)	3.5 ± 0.11 (a, b)	2.7 ± 0.08 (b)
PAE + Res	63.5 ± 1.8 (a, b, c)	83.1 ± 2.9 (a, b)	6.1 ± 0.20 (a, b)	3.6 ± 0.11 (a, b)	2.5 ± 0.08 (b)
PEE + Res	58.3 ± 1.7 (a, b)	70.3 ± 4.0 (a, b)	6.3 ± 0.21 (a, b)	3.7 ± 0.11 (a, b)	2.6 ± 0.08 (b)

The table summarizes the results for all the parameters tested for all the experimental groups. Data are expressed as Mean ± S.E.M for all groups.
* (a) significant difference from the negative control group at the same column with one-way ANOVA at $P < 0.05$.

Serum Alanine Aminotransferase (ALT) levels

The ALT levels in serum for all the experimental groups were measured and expressed as g/dL Figure 2. The data showed that the positive control (Res treated group) had the highest ALT level with a mean of 86.7 g/dL, and the negative control (control) had the lowest ALT level with a mean of 38 g/dL, and this was a significant difference with $P<0.05$. The ALT levels of the other treated groups were less than the positive control (Res treated group) but more than the negative control. The ALT level for the ESC+Res-treated group was 55.3 g/dL , and it was significantly lower than Res Res-treated group but also significantly higher than the control group, with $P<0.05$. The ALT level for the PAE+Res-treated group was 63.5 g/dL, and it was also significantly lower than the Res-treated group but also significantly higher than the control group with $P<0.05$. The ALT level for the PEE+Res treated group was 58.3 g/dL, and it was also significantly lower than the Res-treated group, but also significantly higher than the control group, with $P<0.05$. There are no significant differences among the three treated groups. The data suggested that both the ethanolic extract and the aqueous extract, and also ESC, had partial protective effects against the leakage of ALT from hepatic cells.

* (b) significant difference from reserpine-treated group (positive control group) at the same column with one-way ANOVA at $P < 0.05$.
* (c) significant difference from escitalopram and reserpine-treated groups in the same column with one-way ANOVA at $P < 0.05$.

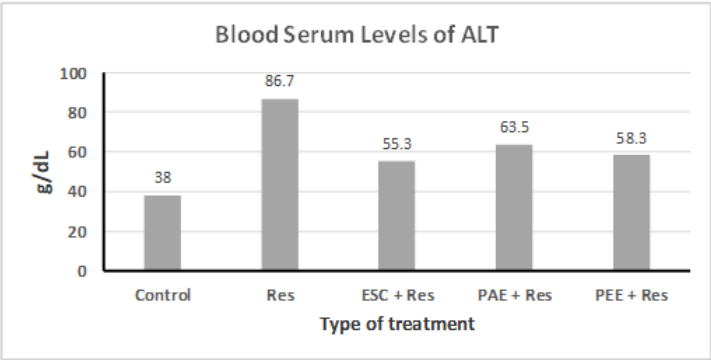

Figure 2. Serum levels of Alanine aminotransferase (ALT) for all the experimental groups.

The data showed that Res Res-treated group had the highest ALT levels. The other treated groups had much lower ALT levels compared to the Res group, but higher than the control group. The ESC+Res, PAE+Res , and PEE+Res treated groups had ALT levels that were significantly higher than the control group but also significantly lower than the Res Res-treated group.

Serum Aspartate Aminotransferase (AST) levels

The AST levels in serum for all the experimental groups were measured and expressed as g/dL Figure 3. The data showed that the positive control (Res treated group) had the highest AST level with a mean of 105 g/dL, and the negative control (C group) had the lowest AST level with a mean of 49.7 g/dL, which was significantly different from the positive control group with a P<0.05. The AST levels of the other treated groups were less than the negative control but more than the positive control (Res treated group). The AST level for the ESC+Res-treated group was 76.5 g/dL, and it was significantly lower than the Res-treated group but also significantly higher than the control group, with P<0.05. The AST level for the PAE+Res-treated group was 83.1 g/dL, and it was also significantly lower than the Res-treated group, but also significantly higher than the control group, with P<0.05. The AST level for the PEE+Res-treated group was 70.3 g/dL, and it was also significantly lower than Res Res-treated group but also significantly higher than the control group with P<0.05. There are no significant differences among the three treated groups. The data suggested that both of the ethanolic extract and the aqueous extract, and also ESC had partial protective effects against the leakage of AST from hepatic cells.

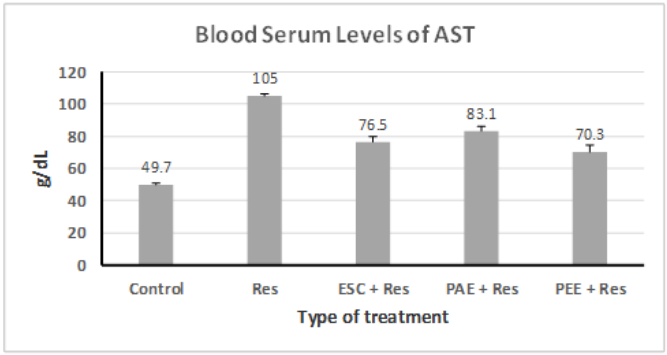

Figure 3. Serum levels of Aspartate aminotransferase (AST) for all the experimental groups.

The data showed that the Res Res-treated group had the highest ALT levels. The other treated groups had much lower ALT levels compared to the Res group, but higher than the control group. The ESC+Res, PAE+Res, and PEE+Res treated groups had AST levels that were significantly higher than the control group but also significantly lower than the Res Res-treated group.

Serum albumin levels

The albumin levels in serum for all the experimental groups were measured and expressed as g/dL Figure 4. The data showed that the positive control (Res treated group) had the lowest albumin level with a mean of 2.6 g/dL, and the negative control (C group) had the highest albumin level with a mean of 4.2 g/dL which was significantly different from the positive control group with a P<0.05. The albumin levels of the other treated groups were less than the positive control but more than the negative control. The albumin level for the ESC+Res-treated group was 3.5 g/dL, and it was significantly higher than the Res-treated group but also significantly lower than the control group with P<0.05. The albumin level for the PAE+Res-treated group was 3.6 g/dL , and it was also significantly higher than the Res-treated group, but also significantly lower than the control group, with P<0.05. The albumin level for the PEE+Res-treated group was 3.7 g/dL, and it was also significantly higher than the Res-treated group, but also significantly lower than the control group, with P<0.05. There are no significant differences among the three treated groups. The data suggested that both the ethanolic extract and the aqueous extract, and also ESC had partial protective effects on albumin secretion from hepatic cells.

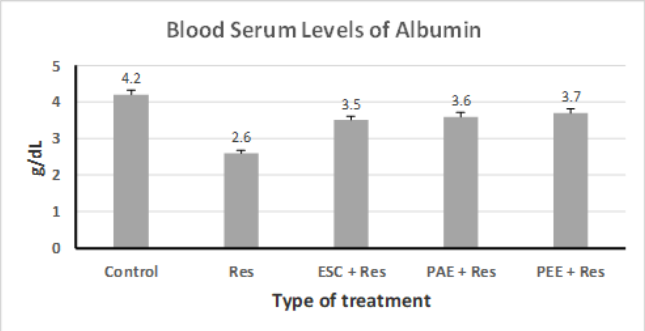

Figure 4. Serum levels of albumin for all the experimental groups.

The data showed that Res Res-treated group had the lowest albumin level. The other treated groups had much higher albumin levels compared to the Res group, but lower than the control group. The ESC+Res, PAE+Res, and PEE+Res treated groups had albumin levels that were significantly lower than the control group but also significantly higher than the Res Res-treated group.

Serum globin levels

The globin levels in serum for all the experimental groups were measured and expressed as g/dL Figure 5. The data showed that the positive control (Res treated group) had the lowest albumin level with a mean of 2.1 g/dL, and the ESC+Res treated group (ESC+Res) had the highest globulin level with a mean of 2.7 g/dL, which was significantly different from the positive control group with a $P<0.05$. The globulin levels of the other groups were less than the positive control but more than the negative control. The globulin level for the ESC+Res-treated group was 2.7 g/dL, and it was significantly higher than the Res-treated group with $P<0.05$, but there was no significant difference between this group and the control group. The globulin level for the PAE+Res-treated group was 2.5 g/dL, and it was also significantly higher than Res Res-treated group with $P<0.05$, but the difference between this group and the control group was not significant. The globulin level for the PEE+Res-treated group was 2.6 g/dL, and it was also significantly higher than the Res-treated group with $P<0.05$, but the difference between this group and the control group was not significant. There are no significant differences among the three treated groups. The data suggested that both of the ethanolic extract and the aqueous extract, and also ESC had partial protective effects on globulin secretion from hepatic cells.

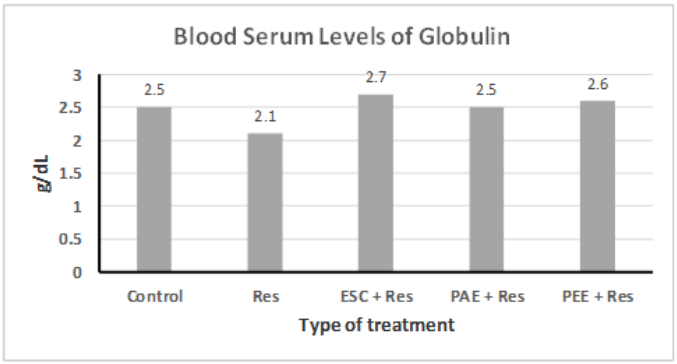

Figure 5. Serum levels of globulin for all the experimental groups.

The data showed that the Res Res-treated group had the lowest globulin level. The other treated groups had higher globulin levels compared to the Res group. The ESC+Res, PAE+Res, and PEE+Res treated groups had globulin levels that were significantly higher than the Res-treated group.

DISCUSSION:

In this study, there were significant increases in serum Alanine aminotransferase level (ALT) and Aspartate aminotransferase level (AST) of mice groups administered with reserpine (0.1 mg/kg body weight) as compared to the control group and significant decreases were noticed in serum levels of albumin and globulin of mice administered reserpine as compared to the control group.Serum aminotransferase enzymes (ALT and AST) are the most sensitive biomarkers employed in the diagnosis of hepatic damage because these enzymes are cytoplasmic in location and are released into circulation after the cellular damage [9]. ALT is more specific to the liver and a better parameter for detecting liver damage, whereas AST is abundant in many other tissues, including kidneys, heart, and testes [10]. ALT and AST are hepatocyte cytosolic enzymes; the increased levels of ALT and AST usually indicate liver cell damage and leakage of these enzymes into the main circulation [11]. This resulted from cell membrane damage and mitochondrial damage respectively, followed by release of more than 80% of total hepatic enzymes from the mitochondria [12]. In agreement with the present results, Zhu *et al*. (2014) noticed the capacity of reserpine in depleting biogenic amines (serotonin, glutamate, and dopamine) in the central nervous system in mice. With an increase in serum levels of alanine transaminase (ALT) and aspartate transaminase (AST) that indicate hepatic lesion [13]. Li and colleagues found that reserpine has degenerative effects on organs other than the brain, including liver, kidneys, and heart [14]. The significant increase of serum levels of ALT and AST as compared to the normal control group after administration of reserpine (in rats) was also reported by Ahmed and her colleagues in 2014 [15]. The activity of ALT, AST is significantly increased in such cases and escapes to the plasma from the injured hepatic cells. In addition, ALT level is of value, also indicating the existence of liver diseases, as this enzyme is present in large quantities in the liver. It increases in the serum when cellular degeneration or destruction occurs in this organ. Oxidative stress induces liver injury that causes a rise in serum transaminase activity and changes in hepatic activity, including a decrease in total protein and albumin levels, and an increase in serum ALT and AST levels [16,17].The results of the present study agree with Afzal and colleagues, who found that in reserpine-treated rats, serum transaminases ALT, AST were increased while the total protein and albumin levels showed significant decreases. These results indicate the liver damage induced by reserpine, which causes an increase in the serum enzyme levels resulting from their leakage from damaged and necrotic hepatocytes as a result of toxicity [18]. Most proteins are synthesized and secreted into blood by the liver; the decrease in blood protein contents can be considered a useful index of the severity of cellular dysfunction in liver diseases [19]. The results showed significant decreases in serum Alanine aminotransferase activity (ALT) and Aspartate aminotransferase (AST) and significant increases in serum proteins in the treated groups (EEP + Res group), (AEP + Res group), and (ESC + Res group) when compared to the reserpine only group. Purslane contains antioxidant enzymes through which it might lead to reduced oxidative stress caused by reserpine [20]. It becomes evident that liver injury, caused by stress, is the result of enhanced free radical generation and altered antioxidant enzyme activities [21]. The results showed that purslane aqueous extract caused a decrease in serum activities of ALT and AST which is supported by the findings of Oliveira *et al.* (2009) and Hozayen *et al.* (2011) where they concluded that the protective effect is due to the presence of omega-3, omega-6 and phenolic compounds as antioxidants in the purslane aqueous extract [22,23].The protective effects due to treatment with purslane extract strongly indicate the possibility of the extract being able to prevent and/or mitigate any leakages of marker enzymes into circulation condition the hepatocytes to accelerate regeneration of parenchymal cells, and preserve the integrity of the plasma membranes, and hence restores these enzyme levels [24]. Several laboratories investigated the potential protective or repairing effects of purslane against various acute and chronic models of liver damage in rodents. Sharmila-Banu *et al.* in 2009 reported that oral administration of the ethanolic extract of purslane lowered serum enzymatic activities of aspartate aminotransferase (AST), alanine aminotransferase (AL,T), and increased serum albumin and total protein levels [25]. These effects may be due to the antioxidant properties of purslane that give protection against free radicals and damage to the liver *in vivo* rodent models [26]. In 2011, El-Sayed and colleagues reported that the usage of ethanolic extract of *P. oleracea* significantly restored the hepatic marker enzymes and total bilirubin to near-normal values, which demonstrates hepatoprotective activity in hepatic injured rats [27]. The results of this study agree with these studies and indicate that purslane improves the functions of the damaged liver of mice by decreasing ALT and AST close to normal levels and increasing globulin and albumin synthesis by the

liver. Both albumin and globulin are serum proteins. Since albumin is synthesized in the liver, it is used to monitor liver function. Okafor and colleagues in 2014 reported that feeding rats with a mixture of maize seeds and purslane extract led to an increase in serum albumin and total protein contents [28]. These results agree with our findings regarding the effect of both the ethanolic and aqueous extracts of purslane on serum albumin levels. Oyedeji and Bolarinwa in 2013 and Modaresi in 2014 found that purslane extract could strengthen the immune system since it increases the serum levels of globulins in mice, suggesting that the purslane extract can have an impact on the activity of the immune system[29,30]. Our findings agree with their results since both the ethanolic and aqueous extracts of purslane showed a significant increase in serum globulin levels.Selective serotonin reuptake inhibitor (SSRI) antidepressants like escitalopram increase the amount of circulating serotonin available in the brain. This may help to increase the desire to eat and therefore get bigger amounts of nutrients important in supplements containing amino acids. Also, it has been found to reduce symptoms, as they are converted to neurotransmitters, which in turn alleviate depression and other mental health problems and decrease neurodegeneration that causes damage in the brain and liver. Thus, it can reduce serum levels of ALT and AST[31]. These findings agree with our results, where escitalopram caused a decrease in ALT and AST levels compared to the reserpine-treated group.Dale *et al.* (2015) recorded that reserpine reduced the levels of protein and albumin in the serum, but escitalopram caused an improvement in the general mood and nutrition, and resulted in raising the serum levels of albumin and plasma proteins [32]. These results agree with our findings that escitalopram improved the serum levels of albumin and globulins compared to the reserpine-treated group.

CONCLUSION:

Purslane is a promising natural product that could be useful for the prevention of hepatic toxicity and other chronic diseases caused by oxidative stress. Purslane gives better hepatic protective results than escitalopram. Both of the ethanolic extract and the aqueous extract of purslane had potent hepatoprotective activity. In general, purslane provided a new herbal remedy of hepatic protection and hepatic healing against hepatic toxicity and some other chronic diseases.

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