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Research Article

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The Protective Effect of Bee Venom and *Echinacea Purpurea* against Liver Injury Induced by Dexamethasone

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Abstract The liver is one of the most important organs in the body, performing a fundamental role in the regulation of diverse processes, among which the metabolism, secretion, storage, and detoxification of endogenous and exogenous substances are prominent. Due to these functions, hepatic diseases continue to be among the main threats to public health. Glucocorticoids are among the most utilized medications around the world, dexamethasone (DEX) is one of the most potent synthetic glucocorticoid that associated with several side effects especially on the liver. Many natural products and plant extracts become a new trend in the traditional and complementary medicines. Bee Venom (BV) and *Echinacea Purpurea* (EP) were used for their chemo-protective and immunomodulatory activities. *Aims and Objectives:* To study the protective effect of BV and EP and their combination on the liver status and liver cell energy against DEX treatment in adult female albino rats. *Method:* For performing the present work fifty-six adult female albino (150 ± 20 gm) were randomly divided into 7 groups (n=8). G1: control group; G2: treated with DEX (5mg i.p /kg BW/day) for three days; G3: orally treated with BV (150 µg/kg BW/day) for 28 days; G4: orally treated with EP (30 mg /kg B.W/day) for 28 days; G5: treated with both DEX+BV; G6: treated with both DEX+EP; G7: treated with DEX+BV+EP, all with the same aforementioned doses and durations. Liver injury was assessed biochemically and histologically. *Results:* Administration of DEX (5mg i.p /kg BW/day) for three days resulted in elevated liver enzymes, protein synthesis and reduction in liver cell energy. On the other hand oral administration of BVfor 28 days (150 µg/kg B.W/day) and EP (30 mg /kg B.W/day) for 28 days with DEX resulted in a great normalization in the levels of liver's protein and significantly decreased ALT and AST serum levels as compared to DEX group. In addition, both BV and EP resulted in ameliorated effect on liver cell energy by increasing their levels in comparing with DEX group. *Conclusion:* The current findings suggested that both BV and EP or their combination, may exert beneficial protective effects against the negative deteriorative influences during DEX treatment through ameliorating liver status and liver cell energy prominence. In conclusion, treatment with DEX resulted in mild liver injuriousness, whose certain manifestations were alleviated on co-treatment with BV and EP biochemically and histologically. Based on these results, patients should be monitored periodically liver functions during DEX therapy.

Keywords Dexamethasone, liver, hepatoprotective, *Echinacea Purpurea*, Bee venom and liver cell energy.

Introduction

Liver is a vital organ in human body and plays important functions in the maintenance, performance as well as regulating homeostasis of the body and detoxification [1]**.** It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [2]. It acts as a center of

metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Therefore, maintenance of a healthy liver is essential for the overall well-being of an individual [3].

Liver enzymes such as AST and ALT are among the marker enzymes for liver function and integrity [4-5], which are usually elevated in the manifestation of acute hepatotoxicity or mild hepatocellular injury [1, 6]. There are various mechanisms through which drugs may damage the liver [7-8].

Liver may be impaired through inflammation or tissue damage or its dysfunction. So, natural products like bee venom and plant extracts, have been traditionally used for protecting against liver diseases. Many natural products have been clinically available as potent hepatoprotective agents against commonly occurring liver diseases [9-10].

Dexamethasone (DEX), a widely prescribed synthetic corticosteroid (CS), has long been the cornerstone for the treatment of inflammation and immunological dysfunctions in Rheumatoid Arthritis (RA) [10-11]. However DEX therapy is associated with a variety of side effects, including liver and kidney dysfunction as well as immunosuppression. The current study reveals the protection consulted by bee venom (BV) and *Echinacea purpura* (EP) against dexamethasone –induced immunosuppression in experimental female rats.

Bee venom possesses a number of beneficial biological activities, particularly for regulating the immune system, BV has long been used as an alternative medicine to alleviate a variety of pathological conditions, such as pain and inflammation (anti-inflammatory and anti-nociceptive effects) [12]. This natural toxin is a complex mixture of proteins (phospholipase A2 and hyaluronidase), peptides (melittin, apamine, mast cell degranulating peptide 401 and adolapine), it was reported that adolapin and mast cell degranulating peptides had anti-inflammatory and anti-nociceptive activity , and low molecular components (histamine, dopamine and norepinephrine) [13].

Echinacea purpurea one of the most important medical herbs, has been used to treat common cold and infection diseases. It contains a variety of medically important substances that play a role in its therapeutic effects which include alkylamides, caffeic acid derivatives, glycoproteins, polysaccharides, polyacetylenes, phenolic compounds, cinnamic acids, essential oils and flavonoids [14-16]. Several phenolic compounds have been reported to be inhibitors of chemical carcinogenesis and mutagenesis [17]. Moreover, EP has been reported to possess anti-tumor, anti-inflammatory, antibacterial, antiviral, antifungal, wound healing properties, antioxidant activities and immunostimulant properties [17-18].

This study aimed to investigate the possible protective effects of BV and EP and their combination on the liver status and cell energy against dexamethasone–induced immunosuppression in experimental female adult rats.

Materials and Methods

Experimental Animals

The present study was conducted on adult female albino rats of Sprague-Dawley strain of weight 150 ± 20 gm. Animals were obtained from National organization for drug control and research (NODCAR) Giza, Egypt.They were kept under strictly hygienic conditions for acclimatization. They were fed a standard basal diet formulated in accordance with composition authorized by the association of official analytical chemists [19]. The rats were allowed free access to drinking water *adlibitum*, they were placed in cages of adequate size each comprising 8 animals, allowing free spontaneous motility. They were kept through the period of the experiment under properly controlled environmental conditions in the animal house with respect to ambient temperature (22 – 25 °C) and 8 hours daily of light periods. Animal use followed guidelines stated by the Institutional Animal Care and Use Committee (IACUC) guidebook 8th edition (2011).

Tested drugs and doses:

1. **Dexamethasone (DEX)**: Intraperitoneally injected for 3days (5mg/kg/i.p.) and was obtained from Amriya Pharm. Ind. Company, Egypt as sodium phosphate.
2. **Bee Venom (BV):** Orally administrated for 28 days (150 µg/kg B.W. /day) and was procured from the faculty of Agriculture, Cairo University.
3. **Echinacea purpurea (EP):** Orally administrated for 28 days (30 mg/kg B.W/day) and was supplied as capsules each containing 210mg from dry extract by Immunvita from Pharaonia pharmaceuticals company, Egypt**.**

Experimental design

The experiment continued for 31 days. Rats were randomly arranged in seven groups each comprised eight rats as follows (n=8):

The 1st group (C) served as negative control group received stock diet.

The 2nd group (D) intraperitoneally injected with (5mg/kg B.W/i.p.) of DEX for 3days.

The 3rd group (B) orally administrated with 150 µg/kg B.W/day of BV for 28 days s.

The 4thgroup (E) orally administrated with 30 mg/kg B.W/day of EP for 28 day.

The 5th group (D+B) were orally administrated with 150 µg/kg B.W/day of BV for 28 days and 5mg/kg/i.p. of DEX intraperitoneally for 3days.

The 6th group (D+E) orally administrated with 30 mg/kg B.W/day of EP for 28 days and 5mg/kg/i.p. of DEX intraperitoneally for 3days.

The 7th group (D+B+E) were orally administrated with 30 mg/kg B.W/day ofEP, 150 µg/kg B.W/day of BV for 28 days and 5mg/kg/i.p. of DEX intraperitoneally injected for 3days.

Sample Collection

At the end of the experimental period, animals were sacrificed; blood collected for separating serum and the whole liver of each animal was rapidly dissected, thoroughly washed with isotonic saline, dried on filter paper and then weighed. Each liver were weighed and homogenized in 75% aqueous HPLC grade methanol (10% w/v). The homogenate was spun at 4000 r.p.m. for 10 min. The supernatant (10%) was separated and stored at -70 0C with the sera for biochemical analysis. Liver tissues examined for histopathological changes under microscope.

Biochemical Examinations

Determination of Total Protein

Serum total protein was determined spectrophotometrically according to Gornall *et al* [20] by using kits of Stanbio, company, Texas.

Determination of Albumin

Serum albumin was determined spectrophotometrically according to Doumas *et al.* [21] by using the kits of the Biodiagnostic Company.

Determination of globulin

The plasma globulin was calculated by subtracting albumin values from the total protein of each corresponding sample. The Albumin to Globulin ratio (A/G) was calculated by dividing A/G values.

Determination of AST and ALT

According to the method described by Reitman and Frankel [22], serum Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were determined by using the Biodiagnostic's Enzymatic Colorimetric kits.

Determination of tissue ATP, ADP, and AMP by HPLC

Determination of liver tissue ATP, ADP and AMP were performed by HPLC according to Teerlink *et al.* [23] with some modifications. The separation of tissue ATP, ADP and AMP was performed with an Agilent HP 1200 series HPLC apparatus (USA). The analytical column was Ultrasphere ODS EC 250 x 4.6 mm column. Mobile phase A consisted of 0.06 mol/l K2HPO4; 0.04 mol/l KH2PO4 dissolved in deionized water and adjusted to pH 7.0 with 0.1 mol/l KOH, while mobile phase B consisted of 100 % acetonitrile. The flow rate of the mobile phase was 1.2 ml/min. ATP in the samples was identified by comparison with standards purchased from Sigma Aldrich. The report and chromatograms were taken from chemstation

Histopathological Examination

Liver samples of all animals were dissected immediately after death. The specimens were then fixed in 10 % neutral-buffered formalin saline for 72 hours at least. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 6µm thick were cut and stained with haematoxylin and eosin [24] for histopathological examination. Images

were examined and photographed under a digital camera (Microscope Digital Camera DP70, Tokyo, and processed using Adobe Photoshop version 8.0).

Statistical Analysis

For statistical analysis of the data, the SPSS for Windows software package version 20.0 (SPSS, Chicago, USA) was used. Data was given in the form of arithmetical mean values and standard deviation. One way analysis of variance (ANOVA) was performed and variant groups were determined by means of the Post hoc test. P value was assumed to be significant at 0.05.

Results

Total protein, albumin, globulin and A/G ratio were estimated in the different studied groups and data represented in table (1) and figures (1 and 2). Results showed that injection with DEX (D group) caused a significant elevation (P > 0.05) in the serum total protein, albumin, globulin and A/G ratio as compared to the control group. Administration of EP (E group) or BV (B group) showed a great normalization in the levels of liver's protein. Moreover, (B+D) or (E+D) showed a great amelioration in the levels of total protein, albumin, globulin and A/G ratio as compared to the DEX group (D group). Interestingly, the treatment with (D+E+B) caused the serum total protein and albumin levels of stay close to DEX group.

Data shown in table (2) and figure (3) revealed the changes in the liver enzyme levels in the sera of different studied groups. Treatment with DEX caused a significant elevation (P > 0.05) in the serum levels of both ALT and AST as compared to the control group. While administration of EP or BV showed significantly decreased in ALT and AST serum levels as compared to DEX group. It was interestingly that EP group recorded values near to the control group (table 2). A significant reduction in the serum levels of ALT and AST in the (D+E), (D+B) and (D+E+B) groups were observed as compared to the D group. It was observable that combination of EP and BV with DEX induced a slight improvement of the liver enzymes changes compared to EP or BV in the individual form. In tables (3 & 4) and figures (4) data showed that DEX resulted in a significant reduction (P > 0.05) in the cell energy represented by decreased adenylate energy charge (AEC) and increased AMP/ATP ratio as compared to the control group. On the other hand, BV and EP showed a great amelioration and normalization effect on the cell energy by increasing their levels in comparing with the DEX group. It was interestingly that (D+E+B) group recorded values near to the control group.

Table 1: Prophylactic Effects of BV and EP and their combination on total protein, albumin, globulin and A/G ratio in the serum of rats treated with DEX

Groups	Parameters			
	Total protein (U/L)	**Albumin (U/L)**	**Globulin (U/L)**	**A/G ratio**
C	7.0 ± 0.4	4.4 ± 0.2	2.6 ± 0.2	1.7 ± 0.1
D	11.0 ± 0.5 [a]	6.6 ± 0.3 [a]	4.3 ± 0.7 [a]	1.5 ± 0.3
E	7.2 ± 0.5 [b]	4.6 ± 0.2 [ab]	2.5 ± 0.6 [b]	1.9 ± 0.5
B	7.1 ± 0.5 [b]	4.3 ± 0.2 [b]	2.7 ± 0.5 [b]	1.6 ± 0.4
(D+E)	7.9 ± 0.5 [ab]	4.9 ± 0.1 [ab]	2.9 ± 0.3 [b]	1.7 ± 0.2
(D+B)	8.8 ± 0.4 [ab]	5.7 ± 0.2 [ab]	3.1 ± 0.5 [ab]	1.8 ± 0.3
(D+E+B)	10.1 ± 0.6 [ab]	6.1 ± 0.1 [ab]	3.8 ± 0.5 [a]	1.6 ± 0.2

Data was expressed as mean ± SD, n= 8 rat/group, (a) Significantly different from the normal control, (b) Significantly different from DEX group at P > 0.05.Where C: control group, D: DEX group, E: EP group, B: BV group, (D+E): DEX & EPgroup, (D+B): DEX & BV group, (D+E+B): DEX, EP &BV group.

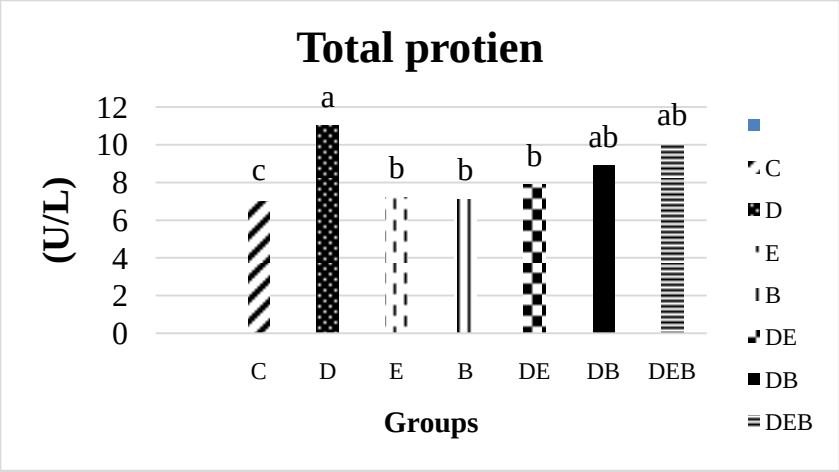

Figure 1: Effects of BV and EP and their combination on total protein in the serum of the adult female rats treated with DEX. Where C: control group, D: DEX group, E: EP group, B: BV group, (D+E): DEX & EPgroup, (D+B): DEX & BV group, (D+E+B): DEX, EP & BV group.

Table 2: Prophylactic Effects of BV and EP and their combination on liver function (ALT and AST) in the serum of adult female rats treated with DEX

Groups	Parameters	
	ALT (U/L)	**AST (U/L)**
C	19.7 ± 0.8	12.3 ± 0.7
D	63.2 ± 0.7 [a]	44.8 ± 0.9 [a]
E	20.1 ± 0.6 [b]	12.2 ± 1.1 [b]
B	15.7 ± 0.5 [ab]	17.2 ± 0.5 [ab]
(D+E)	36.1 ± 0.2 [ab]	23.7 ± 0.9 [ab]
(D+B)	33.9 ± 0.2 [ab]	20.9 ± 1.2 [ab]
(D+E+B)	49.9 ± 0.9 [ab]	41.3 ± 0.9 [ab]

Data was expressed as mean ± SD, n= 8 rat/group, (a) Significantly different from the normal control, (b) Significantly different from DEX group at P > 0.05.Where C: control group, D: DEX group, E: EP group, B: BV group, (D+E): DEX & EPgroup, (D+B): DEX & BV group, (D+E+B): DEX, EP & BV group.

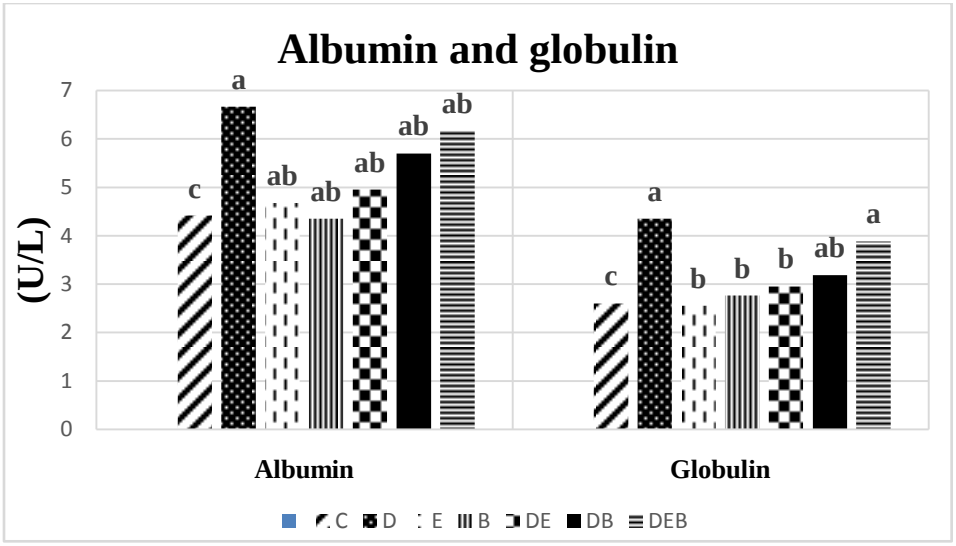

Figure 2: Effects of BV and EP and their combination on albumin and globulin in the serum of the adult female rats treated with DEX. Where C: control group, D: DEX group, E: EP group, B: BV group, (D+E): DEX & EP group, (D+B): DEX & BV group, (D+E+B): DEX, EP & BV group

Table 3: Prophylactic effects of BV and EP and their combination on liver cell energy (ATP, ADP and AMP) of the adult female rats treated with DEX

Groups	parameters		
	ATP	ADP	AMP
	µg/g tissue	µg/g tissue	µg/g tissue
C	30.8 ± 2.2	23.1 ± 0.9	8 ± 0.7
D	16.7 ± 0.7 [a]	20.9 ± 0.6 [a]	6.8 ± 0.5 [a]
E	32.9 ± 1.2 [ab]	22.8 ± 0.4 [b]	8.9 ± 0.5 [ab]
B	33.4 ± 0.7 [ab]	29.9 ± 0.8 [ab]	10.9 ± 0.4 [ab]
(D+E)	21.7 ± 0.3 [ab]	21.2 ± 1 [a]	7.7 ± 0.9 [b]
(D+B)	26.3 ±0.9 [b]	22.4 ± 0.4 [ab]	8.3 ± 0.6 [b]
(D+E+B)	30.8 ± 2.2 [b]	21.7 ± 0.4 [ab]	7.6 ± 1.1 [b]

Data was expressed as mean ± SD, n= 8 rat/group, (a) Significantly different from the normal control, (b) Significantly different from DEX group at P > 0.05.Where C: control group, D: DEX group, E: EP group, B: BV group, (D+E): DEX & EP group, (D+B): DEX & BV group, (D+E+B): DEX, EP &BV group.

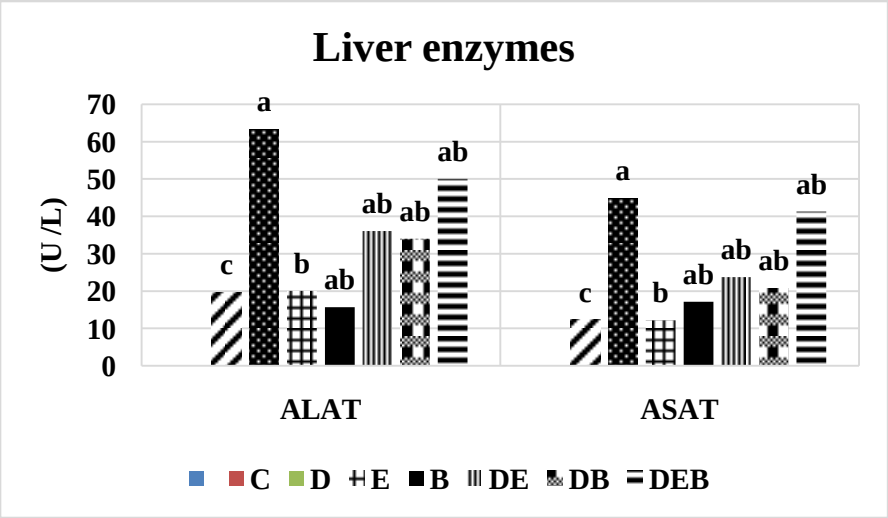

Figure 3: Effects of BV and EP and their combination on liver enzymes ALT and AST in the serum of the adult female rats treated with DEX. Where C: control group, D: DEX group, E: EP group, B: BV group, (D+E): DEX & EP group, (D+B): DEX & BV group, (D+E+B): DEX, EP & BV group.

Table 4: Prophylactic effects of BV and EP and their combination on adenylate energy charge and AMP/ATP ratio in liver tissues of the adult female rats treated with DEX

Groups	Parameters	
	AMP/ATP	AEC
C	0.26 ± 0.03	0.68 ± 0.01
D	0.41 ± 0.03 [a]	0.61 ± 0.01 [a]
E	0.27 ± 0.01 [b]	0.68 ± 0.003 [b]
B	0.32 ± 0.01 [ab]	0.65 ± 0.004 [ab]
(D+E)	0.35 ± 0.04 [ab]	0.63 ± 0.01 [ab]
(D+B)	0.31 ± 0.02 [ab]	0.65 ± 0.008 [ab]
(D+E+B)	0.25 ± 0.03 [b]	0.68 ± 0.01 [b]

Data was expressed as mean ± SD, n= 8 rat/group, (a) Significantly different from the normal control, (b) Significantly different from DEX group at P > 0.05.Where C: control group, D: DEX group, E: EP group, B: BV group, (D+E): DEX & EP group, (D+B): DEX & BV group, (D+E+B): DEX, EP &BV group.

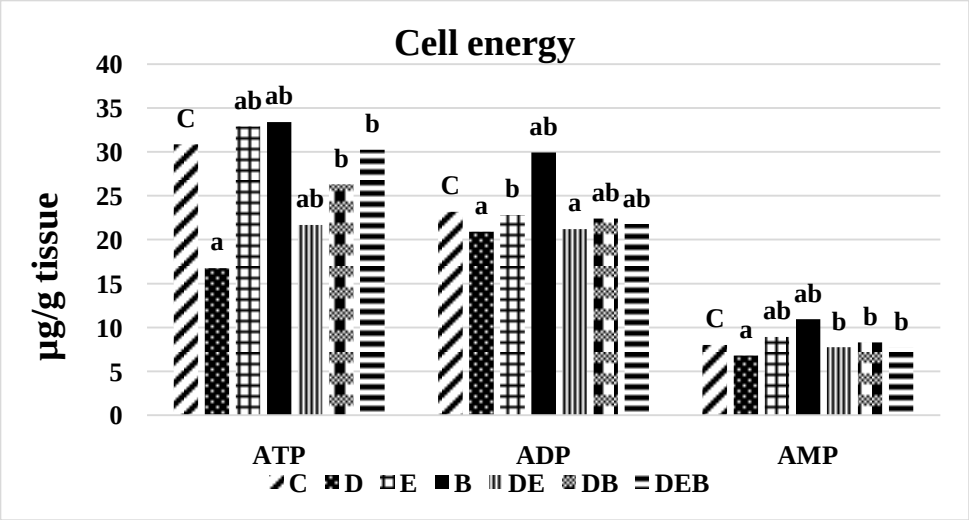

Figure 4: Effects of BV and EP and their combination on liver cell energy (ATP, ADP and AMP) in the serum of the adult female rats treated with DEX. Where C: control group, D: DEX group, E: EP group, B: BV group, (D+E): DEX & EP group, (D+B): DEX & BV group, (D+E+B): DEX, EP &BV group

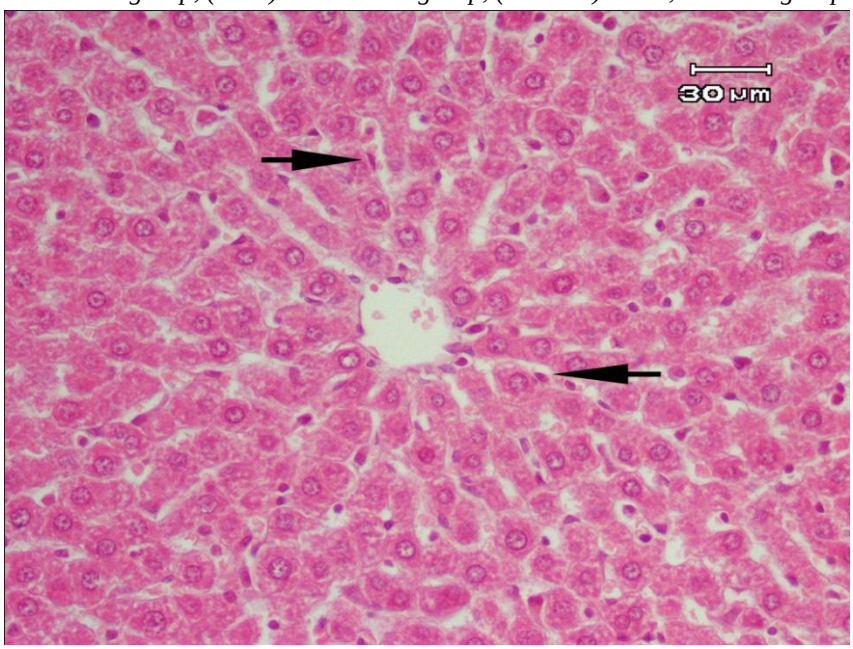

Figure 5: A photomicrograph of a liver section of control normal rat shows the hepatocytes arranged in cords radiating from the central vein. These cords are separating from each other by blood sinusoids in which nuclei of Kupffer cells are noticed (arrow). The cells have granular cytoplasm and large vesicular nucleus.

Histopathological Results on liver tissues

A photomicrograph of liver sections of control, EP and BV treated rats showed normal architectural hepatocytes arranged in cords radiating from the central vein (fig. 5). These cords are separating from each other by blood sinusoids in which normal nuclei of Kupffer cells are noticed. The cells have granular cytoplasm and large vesicular nucleus (fig. 5, 7 & 8).

Liver tissue section from DEX treated rats (fig. 6) showed proliferation of bile duct lining epithelium, dilatation and congestion of blood sinusoids were observed. Liver hepatocytes treated with both DEX plus EP showed dilatation of central vein with absence of blood sinusoids dilatation or congestion, a quite normal portal area is

observed (fig.9).Whereas, liver hepatocytes treated rats with both DEX plus BV(fig.10) showed noticeable dilatation of central vein but with no dilatation nor congestion of blood sinusoids.

On the other hand, liver tissue section from a DEX treated rat with a combination of the BV & EP showed slight thickening of blood vessels wall in portal area with normal bile duct and a normal central vein is noticed (fig.11).

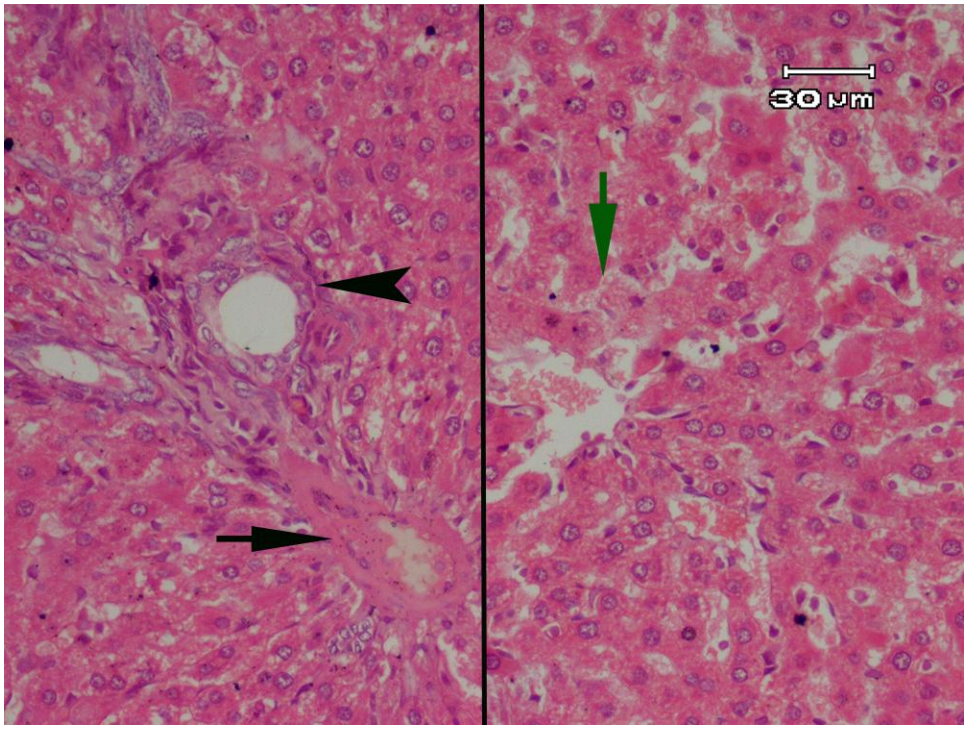

Fig.6: A photomicrograph of a liver tissue section from a DEX rat shows on the left side thickening of blood vessels wall in portal area (arrow) and proliferation of bile duct lining epithelium(arrowhead). On the right side dilatation and congestion of blood sinusoids are observed (green arrow).

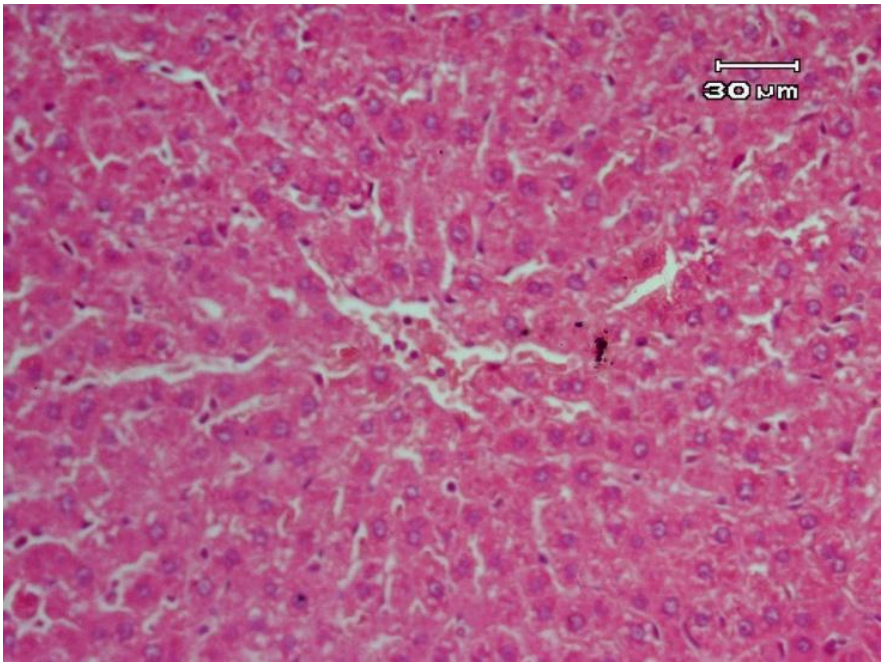

Figure 7: A photomicrograph of a liver tissue section from a normal rat treated with Echinacea purpurea (EP) shows normal structure of tissue, but with very mild dilatation of blood sinusoids.

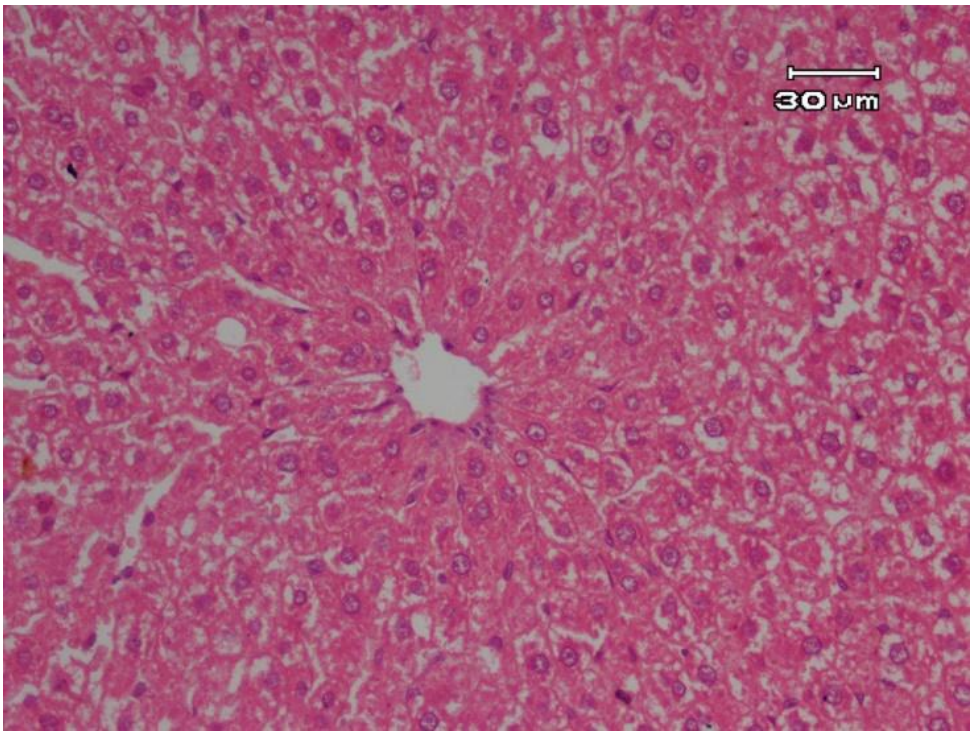

Figure 8: A photomicrograph of a liver tissue section from a normal rat treated with Bee Venom (B) shows normal structure of tissue

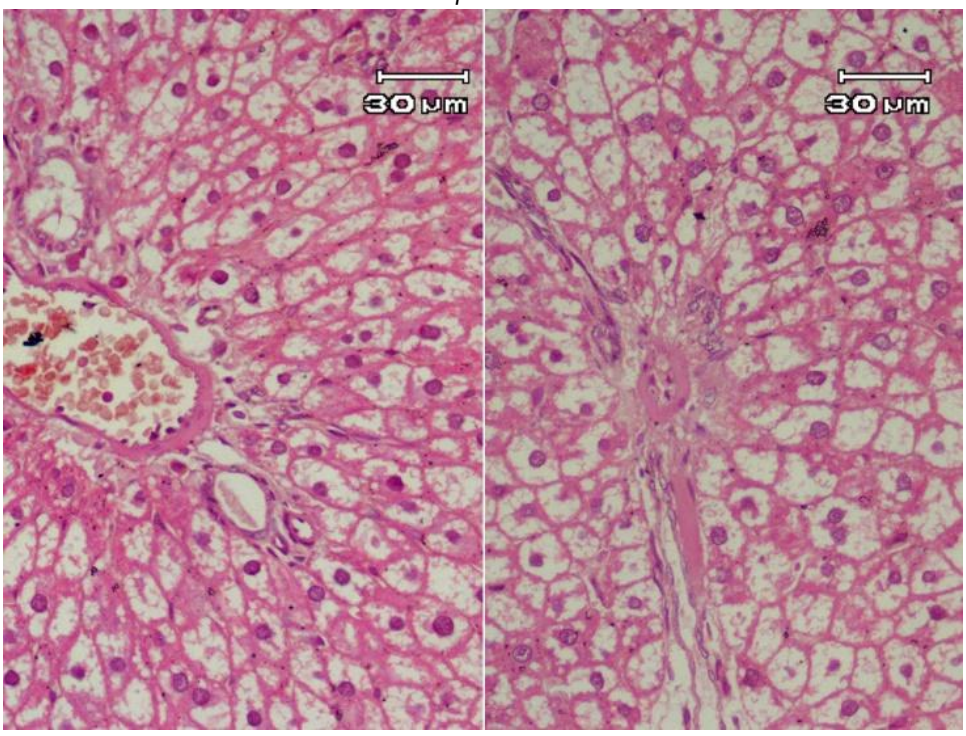

Figure 9: A photomicrograph of a liver tissue section from a DEX treated rat with Echinacea purpurea (EP) shows on the left side slight dilatation of central vein with absence of blood sinusoids dilatation or congestion. On the right side a quite normal portal area is observed.

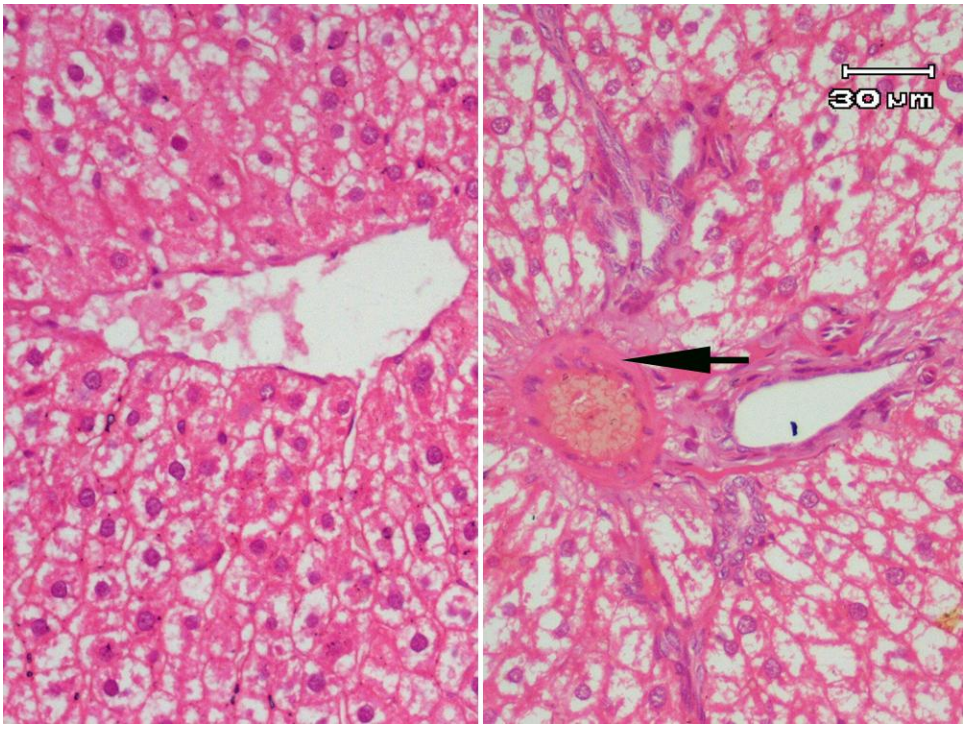

Figure 10: A photomicrograph of a liver tissue section from a DEX rat treated rats with Bee Venom (BV) shows on the left side noticeable dilatation of central vein but with no dilatation nor congestion of blood sinusoids. On the right side, slight thickening of blood vessel walls is observed in portal area (arrow).

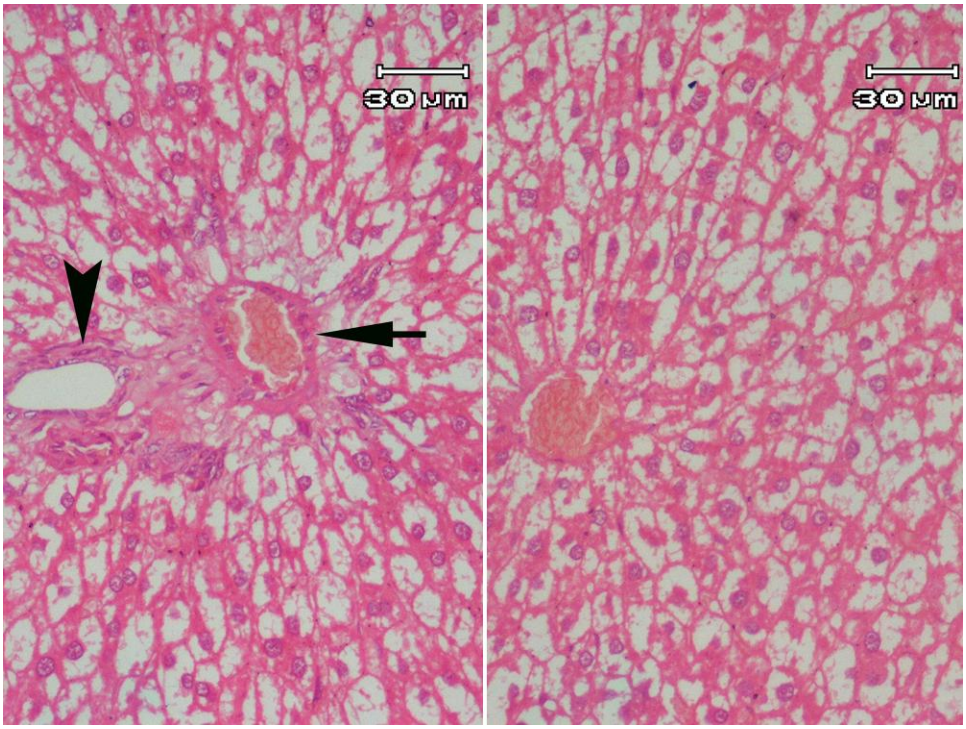

Figure 11: A photomicrograph of a liver tissue section from a DEX treated rat with a combination of the BV & EP shows on the left side slight thickening of blood vessels wall in portal area (arrow) with normal bile duct (arrowhead). On the right side a normal central vein is noticed

Discussion

The liver is structurally heterogeneous organ that performs various important functions, such as detoxification [25].

In present study, administration of DEX led to a significant elevation in the level of total protein, albumin, globulin and A/G ratio in DEX group as compared to the control group. These results are in accordance with Sulaiman *et al* [26] and Isabelle *et al* [27] that attributed this elevation due to DEX damaging effect on the liver protein synthesis due to stress induction.

On the other hand, BV showed normalization and a great refinement of total protein, albumin, globulin and A/G ratio in (B group) and (D+B) group as compared to DEX group. The results are in agreement with Hyunmin *et al* [28] that attributed this refinement to the presence of PLA2 which considered the second most abundant component of BV that acts as antioxidant, anti-inflammatory agent and has a hepatoprotective effect [29]. Additionally, administration of EPshowed a great amelioration in the levels of total protein, albumin, and globulin and A/G ratio in both of (E) and (D+ E) groups as compared to the DEX group (D group).These results are in a harmony with Hosakatte *et al* [30] and Nadia *et al* [31].

The liver enzymes are the most sensitive markers employed in the diagnosis of hepatic damage because they are cytoplasmic in location and are released into the circulation after cellular damage [32-33]. The serum transaminases levels (AST and ALT) are the common markers for hepatic toxicity that usually elevated in the manifestation of acute hepatotoxicity or mild hepatocellular injury [34].

In the current study, the levels of AST and ALT were significantly elevated in DEX group as compared to the control group. These elevations may be attributed to the damaged structural integrity of the liver induced by this corticosteroid Meanwhile, these results are in accordance with Nkono *et al* [35] and Nabil *et al* [36].

Contrastingly, BV decreased and normalized the liver enzymes in (B) and (D+B) groups as compared to DEX group that reflect the hepato-protective effect of BV. These results are in consistent with other studies [29, 37] that showed the potent hepato-protective effect of BV by inhibiting the elevated serum amino-transferase enzymes in different models of induced hepatic injury due to the presence of PLA2 which considered the second most abundant component of BV that has a potent hepatoprotective effect [29]

Furthermore, *Echinacea purpurea* showed a significant reduction in the liver enzymes ALT, AST in both of (E) (D+E) groups. This improvement might be reflected the ability of EP to stopping the damage effect induced by DEX in the cellular tissues of the liver. These findings are in agreement with Rezaie *et al* [38] as a result to the Echinacea extract antioxidant properties.

In current results a decrease in ATP release was observed after DEX treatment, while ADP and AMP levels were elevated compared with control group. The treatment with BV and EP reversed these changes in the levels of purinegic mediators to the normal values. ATP is a neurotransmitter released by neurons. It has multiple functions such as regulating the formation and development of synaptic vesicles and regulation of the ionic gradients; also controlling the metabolism, structural plasticity and ageing [39]. The alteration in the level of adenosine is associated with a disturbance in cell activity [40]. Furthermore, ATP regulates cytoplasmic Ca^{2+} and cyclic adenosine monophosphate [10, 41]. The levels of the hepatic AMP and ADP were significantly up regulated whereas, hepatic level of ATP was significantly down regulated in the liver DEX treated rats supplemented with BV and EP compared to DEX treated group.

In accordance with the present results, co-administration of BV and EP to DEX treated rats have a potential effect of their livers restored the lowered-levels of ATP, ADP and AMP to the levels near to normal control rats with respect to DEX treated group. This demonstrates the stimulatory effect of BV and EP on ATP utilization. Our findings are in consistent with Kim *et al* [37] who found that administration of BV raises energy consumption and stimulates cellular oxygen consumption as well as ATP synthesis in several organs, including the liver. Based on these results, patients should be monitored periodically liver functions during DEX therapy. These findings suggested that both BV and EP may exert beneficial protective effects on the negative deteriorative influences during DEX treatment through ameliorating liver status and liver cell energy prominence. In conclusion, treatment with DEX resulted in mild liver injuriousness, whose certain manifestations were alleviated on co-treatment with BV and EP.

As regards to the histopathological examinations undertaken on liver tissues of female rats in this study, they confirmed biochemical ones where showed slightly degenerative changes observed in the hepatocytes of DEX treated rats represented in proliferation of bile duct lining epithelium, dilatation and congestion of blood sinusoids which were clearly observed, in agreement with HemanthKumar *et al* [42].

On the other hand, liver hepatocytes treated with both DEX plus BV showed noticeable dilatation of central vein but with no dilatation nor congestion of blood sinusoids, these are in parallel with Park *et al* [43].

In addition, liver tissues treated with both DEX plus EP showed dilatation of central vein with absence of blood sinusoids dilatation or congestion, a quite normal portal area is observed, these are in matching with Bayramoglu *et al* [44] and Ahmed *et al* [41].

Also, liver tissue section from a DEX treated rat with a combination of the BV & EP showed slight thickening of blood vessels wall in portal area with normal bile duct and a normal central vein is noticed which agreed with Kim *et al* [37].

These data confirmed the observed biochemical results, showing marked pathological changes in liver tissues, indicating hepatic injury due to the effect of DEX treatment providing an evidence for obvious hepatoprotection with both BV and EP usage.

Conclusion

The present study suggested that BV and EP treatment effectively reversed the effect of DEX through improving selected hepatic parameters. So, current findings recommended that BV and EP treatment is a powerful protectivestrategy may prove beneficial defending effects against DEX treated cases and represent a novel approach to fast recovery particularly in case of co-treatment with Glucocorticoids, suggesting a potential therapeutic use of both BV and EP in the treatment of liver injury.

Conflict of Interest

The authors declare that there is no financial interests and/or no conflict of interest disclosure associated with this manuscript.

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