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# HEPATOPROTECTIVE ACTIVITY OF *BOERHAAVIA DIFFUSA* ROOTS EXTRACTS AGAINST METHOTREXATE INDUCED HEPATOTOXICITY

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ABSTRACT

This study has been undertaken to investigate the hepatic activity of ethanolic roots extract of against Methotrexate induced hepatotoxicity in albino Wistar rats (150-200 gm) . The roots of which source of bioactive compounds including polyphenol, terpenes alkaloids glycoside steroids and various health benefits including antioxidants, anti-microbials agents,anti-cancer agents anti-convulsion agents' radio protective compounds. extraction was carried by Soxhlet apparatus of *Boerhaavia diffusa* roots was performed by using ethanol solvents. Experimental designed was randomly divided in five groups contains Normal control, Disease control, standard control, test 1(LD), and test 2 (HD). The treatment was given for $14^{th}$ days on $15^{th}$ days the blood was collected via retro orbital route and kept for 30 minutes at 25℃ for clotting following by centrifugation at 2000 rpm for 15 minutes. sera will be collected and performed biochemicals analyses (SGOT, SGPT ALP, TB). The animals were sacrificed after anesthesia. The liver was cut into two parts and one part of tissue excused for homogenate that were used for biochemical parameters (Malondialdehyde, Superoxide dismutase, Glutathione, Catalase) and other part of was used for histopathological studies. Acute toxicity study does not show any mortality and behavial changes 2000 mg/kg extracts. Therefore, pharmacological shows 1/10 the of maximum tolerated dose was selected lower dose 200mg/kg and just double of lower dose was considered the higher dose (400mg/kg). Animal treated with ethanolic extracts of *Boerhaavia diffusa* roots significantly altered the disease control groups. The result of this shows that the hepatoprotective activity of ethanolic extracts of *Boerhaavia diffusa* roots. Conclusion of that to be finding of shows scavenges free radicals

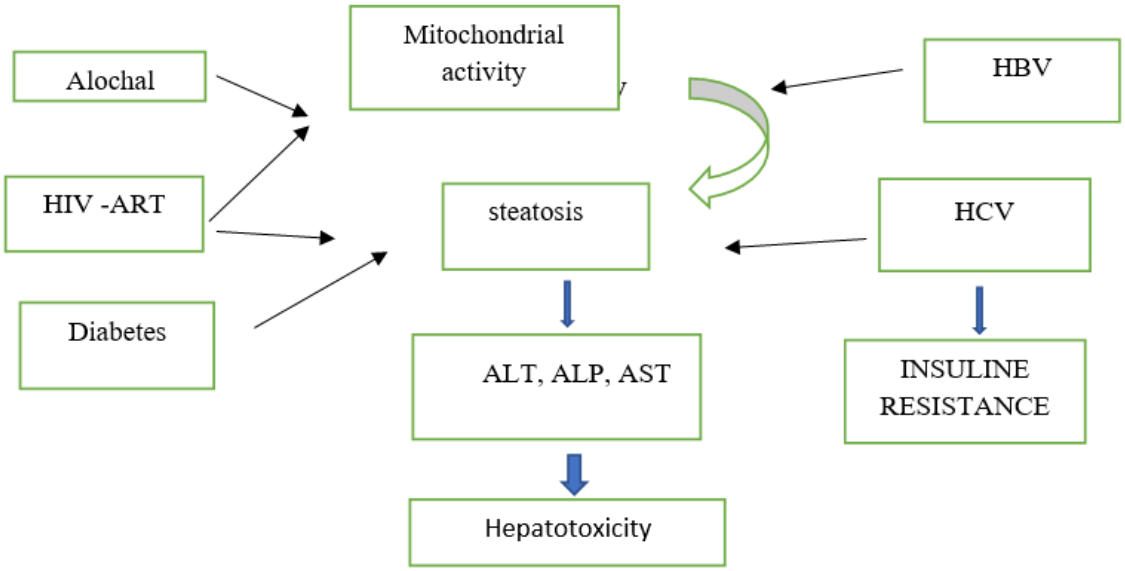
**Keywords:** *Boerhviaa Diffusa,* Methotrexate, Hepatoprotectives, Hepatotoxicity

**Introduction:**

Methotrexate is a chemotherapy drug that works very well to treat cancer. It is also a tried-and-true treatment for bowel problems, such as Crohn's disease, eczema, and inflammatory diseases. Even though it has been successful, that it is harmful to the liver. Through hepatic and cytoplasmic metabolism, MTX is changed into polyglutamated forms. These forms can build up in the liver and cause hepatotoxicity. (*Adikwu. Elias et al. 2019)*

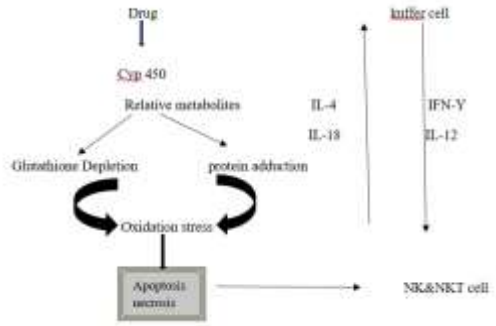
There are many damaging effects of MTX, including effects on the blood, the central and peripheral nerve systems, the lungs, and the stomach, liver, and kidney systems. Hepatotoxicity can happen when a medicine is given in both high and low doses. Even small amounts of methotrexate may affect the liver and change its structure. Also, hepatotoxicity from long-term or high-dose methotrexate treatment (for example, for cancer) can cause fibrosis and cirrhosis to get worse over time.

Hepatotoxicity is the death of liver cells caused by things that are bad for the liver and hepatic gland Hepatotoxicity can be caused by chemical contain agents and over dosage of high risk of medications intake. Chemicals or other toxic substance induce also may affected by laboratory, industries, as well as natural remedies can also damage liver injury called hepatotoxicity. These are participate the whole process caused the liver disease. Hepatotoxicity can get into the human body from a number of places, such as plant products, minerals, and some waste products of how bacteria and fungi break down food. *Premlata Rathore,2014*




**PATHOGENESIS OF HEPATOTOXICITY**

The hepatoxicity of liver can be send oxygenated blood through the portal vein. This means that liver stores a lot of very strong medicines and foreign substances. The liver's main job is to change fat-loving chemicals into water-loving ones, which makes them easier to get rid of. Cytochrome P450 is the main enzyme that breaks down chemicals. It is found in the endoplasmic reticulum of hepatocytes and controls how enzymes work in the liver. Reactive chemicals made from drugs hurt the liver. Because they interact with chemicals in hepatocytes, they could result in oxidative stress, which can damage DNA, lipid breakdown, and proteins that don't work right**.[6]** *Ebtehal Mohammad Fikry et al., 2019**[9]***




***Boerhaavia diffusa***

Herbs from many different kinds of plants have shown promise in stopping illness in many organ systems in people and other animals. Indigenous plants have always been there, so they are the real neighbours. It is important to protect local species because they have gotten used to living in a certain way over time. Researchers have found that some native animals have special traits that can be used to make medicines that save lives. But the natural drug is good for more than just building muscle and dropping weight. Using ancient healing methods as

a starting point, many powerful plant treatments have been made. Plants are the first line of safety against a lot of different diseases. Traditional plant treatments are a hot topic in the field of world health right now. Traditional medicine is the know-how behind many practises and skills.These originate from several places, such as one's own life and one's familiarity with other cultures. They are useful for avoiding illness, fostering health, and curing physical and mental problems. *Boerhaavia diffusa* has been used for a very long time by the native and tribes people of India. People think that the leaves, stem, and roots, among other parts of the plant, can all be used to make medicines. The Charaka Samhita, the Sushruta Samhita, and Ayurveda all say that *Boerhaavia diffusa Linn,* also called punarnava, treating a high range of health issues. Boerhaavia diffusa Linn plant can be eaten, and the juice from the roots can be used to treat meningitis, leukorrhea, arthritis, asthma, and problems with the stomach. Punarnava is the Sanskrit word for it. It is the most important plant for life. The plant is in the family **Nyctaginaceae.** It is a herb. The strong healing plants used in Ayurvedic, Unani, Siddha, and homoeopathic medicine come from the Kumaun Himalaya. The effects of these plants on the body are not all the same. The plant now called *Boerhaavia diffusa* was named after him. In the Atharvaveda, these plants are often called Punarnava, which is made up of the words Punar and Nava.

Punar means "again" and Nava means "**becoming new.**"

**Scientific Name:** Boerhaavia diffusa Linn**.**
**Family Name:** Hog weed, Horse Purslane.
**Useful Parts**: Roots, leaves and seeds
**Other Indian languages**
**Sanskrit:** Punarnava, Raktakanda, Shothaghni, Varshabhu
**Telugu:** ErraGaligeru, Atikamamidi, Punarnava,
**English:** Spreading Hog weed (Red), horse purslane (White)
**Kashmiri:** VanjulaPunarnava;
**Hindi:** Snathikari, Gadapurna, Lalpunarnava
**Gujarati:** Dholiasaturdo, Motosatoda,
**Bengali:** punarnava,Rakta punarnava
**Marathi:** Tmbadivasu, Ghetuli,Vasuchimuli,Satodimuala *(DU Santosha et al.,2020)*

**Extraction of *Boerhaavia diffusa***

The roots of *Boerhaavia diffusa* were prepared washed and dry in the shade and grind into fine residue. About 500 g of the root powder was extracted. Defatting was performed with n- hexane by percolation method. Extraction was carried by using environmentally benign microwave assisted extraction technique in which application of microwave amplify the kinetics of extraction. It quickly heats a solvent that is in touch with a sample to separate analytes from the solvent. This method has various advantages over conventional methods, including shorter extraction time, a greater extraction rate, and the use of less solvent volume. Ethanol was used as a solvent. After extraction crude extract was filtered with the help of Whatman filter paper and concentrated using vacuum rotatory evaporator after then dried on a dissipating dish at temperature up to 30° C -40° C. The obtained crude extract was found to be dark brown colored sticky (semisolid) material.

**Microwave Assisted Extraction Machine (MAE)**

**Methodology**

**2.8 Experimental Design**

Animals were separated in 6 groups (n=5)

**Group-1:** (N C) The rats were administered normal saline (50mL/kg) once daily for 14 days.

**Group-2: (**D C ) The rats were administered Methotrexate (20 mg/kg, intraperitoneal ) on 12$^{TH}$ and 13$^{th}$ day.

**Group-3**: **(Standard )** The rats were administered Silymarin (20mg/kg, orally) once daily for 14 days and Methotrexate (20 mg/kg intraperitoneal ) on 13$^{TH}$ and 14$^{th}$ day.

**Group-4: (Test I) -** The rats were administered low dose extract (200 mg/kg ,orally) once daily for 14 days and Methotrexate (20mg/kg intraperitoneal ) 13$^{th}$ and 14$^{th}$ day.

**Group-5: (Test II)**-The rats were administered high dose extract (400mg/kg, orally) once daily for 14 days and methotrexate (20mg/kg intraperitoneal ) 13$^{th}$ and 14$^{th}$ day

**BIO CHEMICAL ESTIMATION OF LIVER**

Hepatic markers like ALP, SGOT, SGPT, and total bilirubin in blood were tested with commercial kits from Spin React and Redox in an auto analyzer (Rx Monza, Randox, UK). following what is usually done. To determine SGOT, a kit called SGOT (AST) was employed (AST) activity in serum . The kidney, skeletal muscle, liver cells, and heart muscle were where the SGOT enzymes were most abundant. measurement of serum ALP activity uses alkaline phosphatase kit. The hydrolase class of enzymes include alkaline phosphatase (ALP) , which function in an alkaline media the liver, biliary tract epithelium ,and bones all contain significant amount of it .age -dependent and rising during the development of the brain ,normal levels .Increased level were primarily linked to bone condition .

**Antioxidants enzymes assessments: .**

For the estimation of SOD level tissues there was a need to prepare some listed reagent i.e., (kono et al., 1978).For the assessment of lipid peroxidation, level of malondialdehyde was measured in tissue homogenate using thiobarbituric acid (TBA), 1ml of aliquots of supernatant was taken and add 3ml of TBA reagent 4mM prepared in glacial acetic acid. For the preparation of standard curve of Glutathione (GSH), different concentrations have been prepared from 1mM GSH stock solution by using phosphate buffer 7.6pH (15.37gm of GSH in 50ml of PB). 2mM, 4mM, 6mM, 8mM and 10mM concentrations have been prepared, To the different concentrations of solutions 200µl GSH of 1mM was added with 2.3 ml of phosphate buffer pH 7.6 and 0.5ml of 5, 5-Dithiobis,2-Nitrobenzoic Acid (DTNB) 1mM were mixed and incubate for 5 minutes. The absorbance at 412nm was measured by using spectrophotometer. For the preparation of DTNB blank solution 0.5ml of 1 mM 5, DTNB mixed with 2300 µl of Phosphate buffer pH 7.6. By subtracting DTNB blank absorbance with different concentrations of GSH the real absorbance was obtained.

**HISTOPATHOLOGICAL STUDIES**

For histopathological examination, the rat was sacrificed and the liver was isolated, washed and fixed in 10% neutral buffered formalin. The tissue samples were section at 5 µm thick, slides will be prepared and stained with Haematoxylin & Eosin.

Results:

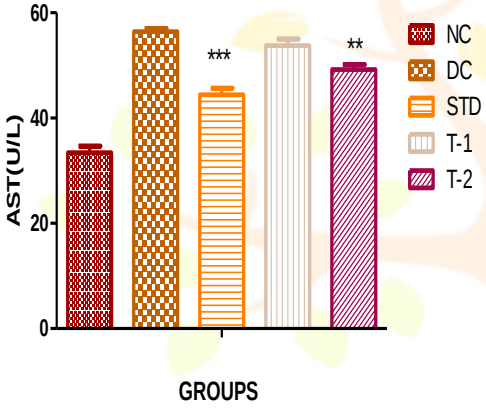
**Effect of EEBD on blood serum SGOT level:**

The effect of EEBD and standard drug silymarin on serum SGOT level are shown in table **no 3.2 and finger no 3.2 .T**he level of SGOT was remarkably reduced in disease control groups in evaluation to normal group . EEBD showed an enhanced in SGOT level in a dose depended manner when equated to disease control. EEBD at 400 mg/kg and standard

dug silymarin at 20 mg/kg significantly (P<0.001 P<0.01) enhanced SGOT level when compared with disease groups.

| S.NO. | Groups & treatment | SGOT (U/L) |
|---|---|---|
| 1. | Normal control | 33.40 ± 1.28 |
| 2 | Disease control (MTX-20 mg/kg) | 56.40± 0.61 |
| 3 | Standard dose (SILY-20 mg/kg) | 44.80 ± 1.27*** |
| 4 | EEBD (200mg/kg) | 53.80 ± 1.22 |
| 5 | EEBD (400mg/kg) | 49.20± 0.95** |

All values are expressed as, mean+ SEM(n=5) and ***P<0.001,**P <0.01 as comparison to disease control group animal by ANOVA( analysis of variation ) followed by Turkey post test




**Effect of EEBD on blood serum SGPT level**:

The effect of EEBD and standard drug silymarin on serum SGPT level are shown in table no 3.3 and finger no 3.3. The level of SGPT was remarkably reduced in disease group in comparison to normal control. EEBD showed an enhanced in SGPT level in a dose depended manner when compared to disease control group. EEBD at 400 mg/kg and standard dug silymarin at 20 mg/kg significantly (P<0.001 P<0.01) improved the SGPT level when associated with disease groups.

**Table no:3.3**

| Sr.no. | Groups & treatment | SGPT (U/L) |
|---|---|---|
| 1. | NC | 31.60 ± 1.11 |
| 2 | DC (MTX-20 mg/kg) | 53.60 ±0..90 |
| 3 | Standard dose (SILY-20 mg/kg) | 41.00± 1.17*** |
| 4 | EEBD (200mg/kg) | 49.20 ± 1.54 |
| 5 | EEBD (400mg/kg) | 46.20 ± 1.33** |

All values are expressed as, mean+ SEM(n=5) and ***P<0.001,**P< 0.01 as comparison to disease control group animal by ANOVA( analysis of variation ) followed by Turkey post test

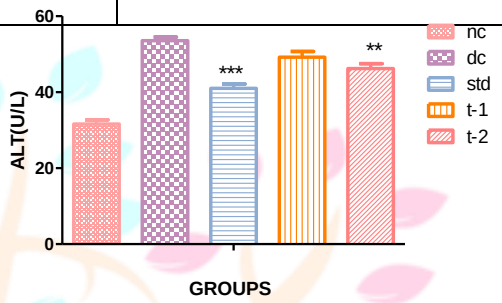



**Fig:3.3** Estimation of SGPT

**3.4.3 Effect of EEBD on blood serum of ALP level** :

The effect of EEBD and standard drug silymarin on serum ALP level are shown in table no 3.4 and finger no 3.4. The level of ALP was remarkably reduced in disease control group in contrast to normal control. EEBD showed an enhanced in ALP level in a dose depended manner when related to disease control. EEBD at 400 mg/kg and standard dug silymarin at 20 mg/kg significantly (P<0.001 P<0.05) increased ALP level when compared with disease group.

**Table no:3.4**

| Sr.no. | Groups & treatment | ALP(U/L) |
|---|---|---|
| **1.** | NC | 27.00 ±1.02 |
| **2** | DC (MTX-20 mg/kg) | 53.40 ±1.12 |
| **3** | Standard dose (SILY-20 mg/kg) | 37.60 ±1.29*** |
| **4** | EEBD (200mg/kg) | 51.02± 1.60 |
| **5** | EEBD (400mg/kg) | 47.25± 1.20* |

All values are expressed as, mean+ SEM(n=5) and ***P<0.001,**P< 0.05 as comparison to disease control group animal by ANOVA( analysis of variation ) followed by Turkey post test

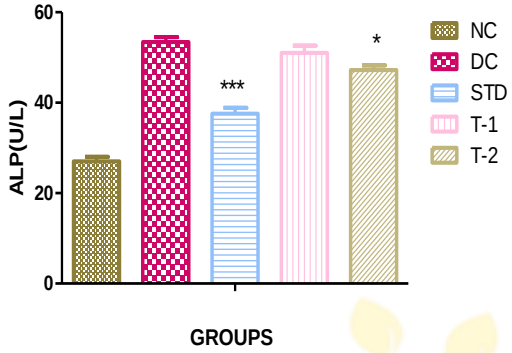



**Fig :3.4** Estimation of ALP

### 3.4.4 EEBD effects on blood serum Total Bilirubin (TB) level

The effect of EEBD and standard drug silymarin on serum TB level are shown in table **no 3.5 and finger no.3.5 T**he level of TB was remarkably increase in disease groups in comparison to normal groups. EEBD treatment reduce the level of TB in a dose dependent manner when compared to disease control groups . EEBD at 400 mg/kg and standard dug silymarin at 20 mg/kg significantly (P<0.001 P<0.01) reduced TB level when compared with disease groups.

**Table no:3.5**

| Sr.no. | Groups & treatment | Total Bilirubin(mg/dl) |
|---|---|---|
| **1.** | NC | 37.00± 0.72 |
| **2** | DC (MTX-20 mg/kg) | 58.80 ±1.21 |
| **3** | Standard dose (SILY-20 mg/kg) | 46.60 ±1.12*** |
| **4** | EEBD (200mg/kg) | 57.00 ±0.58 |
| **5** | EEBD (400mg/kg) | 52.00± 1.08 ** |

All values are expressed as, mean+ SEM(n=5) and ***P<0.001, **P <0.01 as comparison to disease control group animal by ANOVA( analysis of variation ) followed by Turkey post test

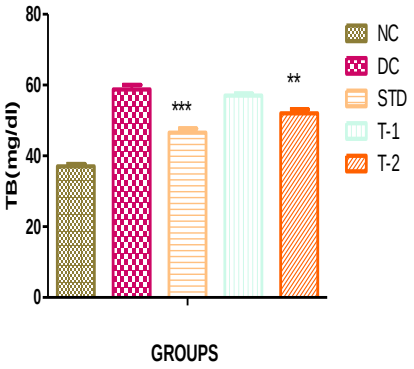



**Fig :3.5** Estimation of Total bilirubin

## 3.5 EX VIVO MEASUREMENT OF ANTIOXIDANT ACTIVITY

### 3.5.1 Effects of EEBD on SOD hepatic tissue

Effect of EEBD and standard drug silymarin on serum SOD level are shown in table no.3.6 and finger no. 3.6 . The level of SOD was remarkably reduced in disease control in comparison to normal control . EEBD showed an increased in SOD level in a dose depended manner when compared to disease control groups. EEBD at 400 mg/kg and standard dug silymarin at 20 mg/kg significantly (P<0.01 P<0.01) increased SOD level when compared with disease groups.

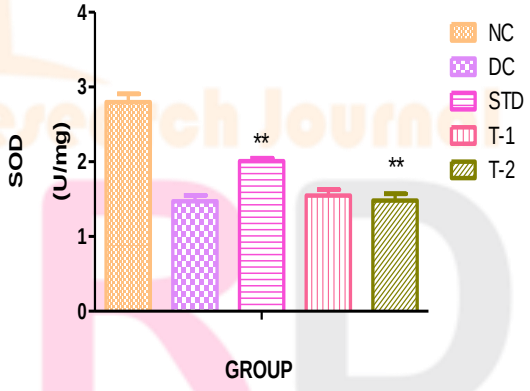



**Fig no 3.6** Estimation of SOD

### 3.5. Effects of EEBD on CAT hepatic tissue

Effect of EEBD and standard drug silymarin on serum CAT level are shown in table **no.3.6 and finger no.3.7 . T**he level of CAT was remarkably reduced in disease groups in comparison to normal control. EEBD pretreatment showed an elevated CAT level in a dose depended manner when compared to disease groups EEBD at 400 mg/kg and standard dug silymarin at 20 mg/kg

significantly (P<0.001 P<0.01) enlarged the CAT level when compared with disease groups.

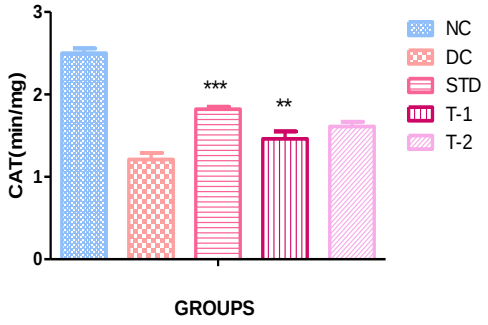



**Fig no:3.7** Estimation of CAT

**Effects of EEBD on GSH hepatic tissue**

In table no. 3.6 and finger no. 3.8, the effects of EEBD and the common medicine silymarin on serum GSH levels are shown. Comparing animals in the illness control group to those in the normal control group, the amount of GSH was noticeably lower. Comparing animals in the illness control group to those that received pretreatment with EEBD revealed a dose-dependent increase in GSH levels. When compared to the disease control group, the doses of EEBD at 400 mg/kg and conventional dosage silymarin at 20 mg/kg considerably (P0.001 P0.01) raise GSH levels.

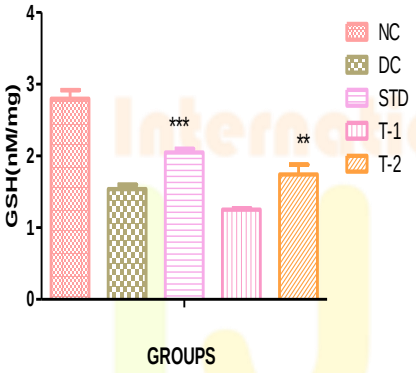



**Fig no: 3.8** Estimation of GSH

**Effects of EEBD on MDA hepatic tissue**

Effect of EEBD and standard drug silymarin on serum MDA level are shown in table no 3.6 and finger no. 3.9. The level of MDA was remarkably reduced in disease control group in comparison to normal groups. EEBD showed an enhanced levels of in MDA as dose dependent manner when compared to DC groups. EEBD at 400 mg/kg and standard dug silymarin at 20 mg/kg significantly (P<0.001 P<0.01) an enhance MDA level when compared with disease groups.

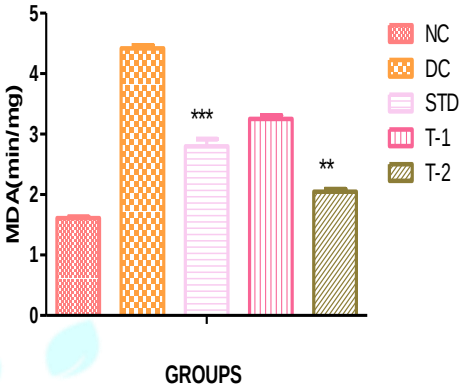



**Fig no :3.9** Estimation of MDA

All values are expressed as, mean+ SEM(n=5) and ***P<0.001 P <0.01 P<0.05 as comparison to disease control group animal by ANOVA ( analysis of variation ) followed by Turkey post test

**Estimation of antioxidants level**

**Table no: 3.6**

| Treatment groups | SOD | CAT | GSH | MDA/LPO |
|---|---|---|---|---|
| Normal group | 0.70± 0.09 | 1.37 ± 0.29 | 1.16 ±0.15 | 1.59 ±0.16 |
| Disease control (MTX-20 mg/kg) | 2.59 ±0.32 | 2.51 ±0.34 | 2.42 ±0.35 | 5.22 ±0.43 |
| Standard dose (20 mg/kg) | 1.24 ±0.16*** | 2.02 ±0.20 | 2.49± 0.28** | 2.81 ±0.21*** |
| EEBD (200mg/kg) | 1.65± 0.23 | 3.31± 0.36** | 2.79± 0.33 | 3.39± 0.41 |
| EEBD (400mg/kg) | 1.72± 0.21** | 4.92 ±0.27 | 1.80± 0.15* | 4.65 ±0.29** |

All values are expressed as, mean+ SEM(n=5) and ***P<0.001 P <0.01 P<0.05 as comparison to disease control group animal by ANOVA (analysis of variation) followed by Turkey post test

**Discussion**

Hepatotoxicity caused by a Large range of chemicals, containing those found in common home goods, lab chemicals, industry chemicals, natural molecules (like macrocystis), and plant drugs. There are several herbal medications and formulations available to improve health, alleviate symptoms, and treat illness. Most of these items, however, don't have any kind of pharmacological proof behind them. When a patient presents with acute liver damage of unknown cause, DILI should be considered as a differential diagnosis. *Pilin Francis;2022*

Clinically, methotrexate is used to treat acute lymphoblastic leukaemia, rheumatoid arthritis, ectopic pregnancy, Crohn's disease, psoriasis, psoriatic arthritis, and ulcerative colitis, just to name a few. A study found that ALT, AST, and ALP were all higher in people who took methotrexate. The main enzymes in the liver, which are often called "hepatic markers for disease indication," show how healthy the organ is by how they work. Enzymes are used as signs to figure out what's wrong and how well treatments are working. So, enzymes are used as testing tools to find out how healthy a person is. Enzymes can also help find damage in the body's tissues..

The terms "alkaline phosphatase" refers to a group of enzymes that work best in a pH 9–10 setting to dephosphorylate molecules like proteins and RNAs. There is less ALP in the kidney tubules, intestine epithelium, lungs, and uterus than in the liver and bones. Young animals' ALP levels go up because their bones are getting bigger. Cholestasis, digestion, or damage to the intestine or bile cells can all raise ALP levels, but the way this happens varies

**3.6 Histopathological studies**

In the microscope examination, all groups except the disease control groups liver tissue exhibited normal hepatocyte cell function.

**Normal group**

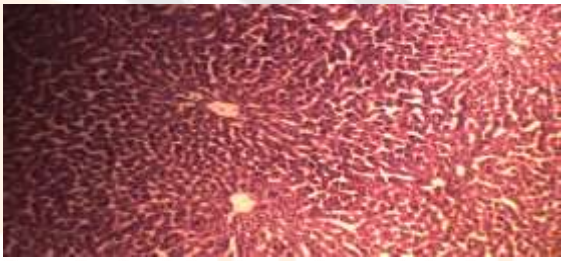


**Figure 3.10** The histopathological study of experimental rat liver in normal control showed that it is composed of lobules that normally hexagonal in shape. It has observed the all normal sinusoidal cells intact to each other, normal appearance of central vein, there are no damage in hepatocyte showed.

**Disease control group ( MTX)**

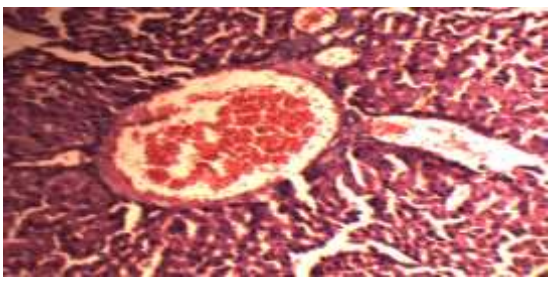


**Figure 3.11** The histopathological study of experimental rat liver in disease control that showed necrosis. Hepatocyte widened sinusoidal spaces, fatty change and dilation of veins.

**Standard dose (silymarin)**

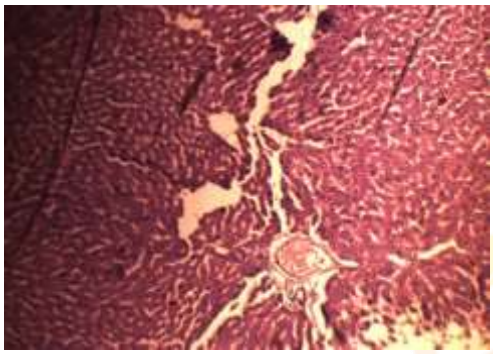


**Figure 3.12** The histopathological study of experimental rat liver in standard drug treated group in comparison to disease control the animals there is a smaller number of necrotic cells, sinusoidal space are reduced and lesser dilation of veins.

**Test group 1(Low dose):**

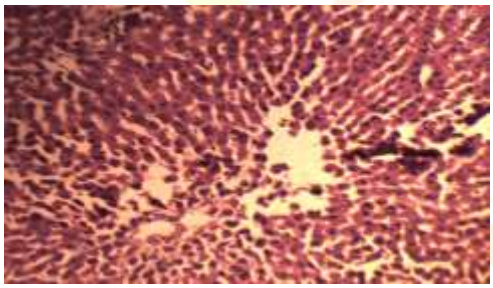


**Figure 3.13**The histopathological study of experimental rat liver in test drug (low dose) treated group after there are a smaller number of necrotic cells, sinusoidal spaces are comparatively reduced with no fatty changes and only few areas dilation of veins.

**Test group 2(high dose):**

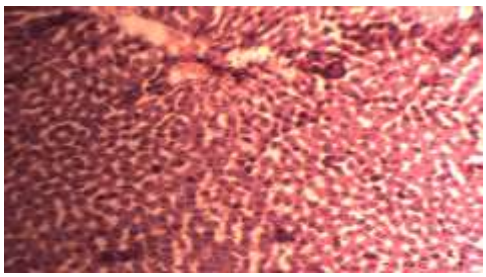


**Figure 3.14** The histopathological study of experiment rat liver in test drug (high dose) showed are better activity on narcotics cells, no fatty changes, very less dilation in veins and sinusoidal space.

Aspartate aminotransferase (AST) is that turns alpha-ketoglutarate into oxaloacetate and glutamate. This enzyme, which used to called (SGOT), is found in all organs except bone. The liver and skeletal muscles have the most of it. When the liver or muscles are hurt by things like bleeding, injuries, death, infection, or cancer, the amount of AST in the blood goes up.

ALT is a transaminase enzyme. It used to be called as (SGPT). The alanine cycle is a process in which the enzyme alanine aminotransferase moves an amino group from alanine to alpha-ketoglutarate. This makes pyruvate and glutamate. The ALT enzyme is found in high amounts in the liver, kidney, skeletal muscle, and heart. However, other organ tissues have much more. The liver, heart, and lungs all have less ALT than they should. When a lot of cells die, the amount of alanine aminotransferase in the blood goes up. This is used as a measure of how well the liver is working. When you have hepatitis, heart failure, liver or bile duct damage, or myopathy, your ALT level may go up. Plasma ALT in rats can be affected by their diet, their living conditions, and the drugs they take.

So, the amount of bilirubin is used to tell how healthy the liver and bile ducts are. A high amount of total bilirubin, which includes both conjugated and unconjugated (free) types, may be a sign of hemolysis or liver damage. When the plasma turns a noticeable shade of yellow, this can be a sign of an increase in total bilirubin. A diazotization reaction is used in a popular colorimetric test for total bilirubin. The functioning part of the liver is made up of enzyme activity and an increase in the amount of total bilirubin. Both of these things are signs of liver damage in tissues.

*Boerhviaa diifusa* roots extract may have a defensive effect because it has molecular components like alkaloids, glycoside carbohydrates, flavonoids, proteins, and phenolic groups, but not enough saponins, terpenoids, and tannins. Generally, reported that, hepatoprotective activity of the *Boerhviaa difussa* roots were due to the presence of polyphenols and flavonoids. Dose dependant activity of the *Brohaviaa diffusa* roots extract are also carried out by the present study( low dose 200 mg/kg and high dose 400 mg/kg ) doses showed hepatoprotective activity. In the clinical assessment of liver damage, serum SGPT, SGOT, ALP, and bilirubin levels are commonly estimated as these

enzymes are released into the blood by cellular membrane damage or necrosis of hepatocyte .SGPT is a more specific biomarker of liver damage. Elevated levels of serum SGOT indicate damage of liver and also other tissues like muscle and kidney The raise of serum ALP indicates the damage of hepatic cells and bones while raised levels of bilirubin indicates the damage of liver and hemolytic anemia. induced hepatotoxicity was used by several workers as model for screening hepatoprotective activity of different dose depend upon body weight of animals.

To studies treatment including the root extracts at the doses described in treatment schedule produced a dose dependent inhibition in MTX induced rise in the biochemical parameters. Silymarin at 20 mg/kg body weight, used as standard drug produced maximum inhibition compared to root extracts. Silymarin was shown to have higher impacts on liver protection. Silymarin, a polyphenolic flavonoid that may be found in Milk Thistle (Silybum marianun) seeds, has long been used to treat liver disorders.. Its hepatoprotective activity was well established in several hepatotoxicity models and the mechanism involved is thought to be free radical scavenging and membrane stabilizing activities . All the selected root extracts showed hepatoprotective activity in accordance with their both in vitro and in vivo antioxidant activity. Among selected EEBD showed significant hepatoprotective activity however prophylactic treatment was found to be more effective than curative treatment

Oxidative stress and free radical mediated damage are the main reason for drug induced hepatotoxicity. The different extracts of *Boerhaavia diffusa* root extract had been reported for its antioxidant activity Antioxidant status characterized by antioxidants depletion and oxidation and regeneration. It may occur as a result of either depletion of antioxidants or increased generation of free radicals. SOD and catalase are important antioxidant enzymes present in the cell to defend against free radicals. Superoxide dismutase scavenges superoxide anion and forms hydrogen peroxide which is then catalysed by catalase to form water. Thus, it attenuates the toxic effect of free radicals. The study has demonstrated that administration of methotrexate has caused depletion of SOD and catalase. Methotrexate poisoning may happen when enzymes like catalase and SOD are used up. When BD extract was given, superoxide levels went up. This shows that BD reduces the liver damage caused by methotrexate by increasing antioxidant levels. BD has altered the levels of, superoxide dismutase by which it has reduced the effect of oxidative stress. The study suggested the effect could be because antioxidant property of the BD.

When B. diffusa root extract was given, CAT and superoxide levels were higher than when methotrexate was given. This shows that B. diffusa extract makes methotrexate less harmful to the liver by increasing antioxidant levels. has also shown that Boerhaavia diffusa changes the amount of CAT and makes oxidative stress less harmful.

GSH and GPX are two other stress-related oxidation factors that change a lot when MTX is given. As a co-factor for GPX, GSH guards against free radicals like hydroxyl radicals and is a very important internal antioxidant. Our results back up earlier study that showed MTX treatment may raise the levels of liver enzymes and cause hepatotoxicity.

**Conclusion:**

The therapeutic potential of the current investigation was the hepatoprotective activity of EEBD was investigated through biochemicals test (total bilirubin, albumin), lipid profile test (SGOT,SGPT) through histopathological study as well as antioxidant potential was also estimated from the above test hepatoprotective potential of EEBD. It has been from to improve lipid profile, after the levels of TB, Albumin and improved hepatotoxicity rat model.it has been observed that antioxidant was reduced level of markers was recover in the therefore it can be conclude that EEBD might have a potentials in the treatment of hepatotoxicity and further research in this record may open a new era of research. If the study standard to the extended to the molecular levels.

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