# *Butea monosperma* L. Seed Extract Attenuates Methotrexate-Induced Hepatotoxicity in Wistar Albino Rats

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**Abstract:** Hepatotoxicity is one of the major side effects of methotrexate (MTX), which restricts the clinical use of this drug. *Butea monosperma* L. is a natural compound with multiple pharmacological activities such as antioxidant, antiapoptotic and anti-inflammatory effects. In this study, the effects of *Butea monosperma* L. Seeds extract against MTX-induced biochemical changes were studied. A total number of 30 male Wistar rats were randomly divided into five experimental groups. Rats were pretreated with *Butea monosperma* L. seeds extract (BMSE) orally with dose of (200 mg/kg) & (400 mg/ kg) for 21 consecutive days and MTX (20 mg/kg, i.p.) was administrated single dose on day 18th. Then on day 21, blood samples were collected to determine serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) and Total Cholesterol , Triglycerides , LDL and HDL level. The extracted livers were used for histological examination. Results showed that MTX administration significantly increased AST, ALT and ALP levels (all $p < 0.001$). Pre-treatment with BMSE (200 mg/kg) & (400 mg/kg) for 21 days prevented some of these changes. Serum levels of AST, ALT and ALP decreased (all $p < 0.001$) and lipid parameters viz. Total cholesterol, Triglycerides, LDL level is decreased & HDL level is increased. The study results indicated that BMSE might be useful for prevention of the hepatotoxicity induced by MTX via ameliorative effects on liver enzyme and lipid parameters.

**Keywords:** *Butea monosperma*, phytochemistry, pharmacology, Ayurveda palash.

## Introduction

Liver is the largest gland of the human body, situated at the right side of upper abdominal cavity. More than 5,000 separate bodily functions including helping blood to clot, cleansing the blood of toxins to converting food into nutrients to control hormone levels, fighting infections and illness, regenerating back after injury and metabolizing cholesterol, glucose, iron and controlling their levels.[1] Due to the high metabolite biotransformation rate, free radicals can be generated continuously. Most hepatotoxic substances, mainly damage the liver because of the generated oxidative stress; oxygen reactive species induced a rise in lipid peroxidation, a reduction of ATP and oxidative damage in the DNA and proteins. Protecting the liver from the harmful effects of hepatotoxins- which may be ingested- or counteracting the alterations in the antiradical defence mechanisms, is very important; the agents capable of doing this are called hepatoprotective.[2,3]

Various inorganic compounds, such as arsenic, phosphorus, copper, and iron, can cause hepatotoxicity. Organic agents include certain naturally occurring plant toxins like pyrrolizidine alkaloids, mycotoxins, and bacterial toxins.[4] Liver injury caused by hepatotoxins, such as carbon tetrachloride ($CCl_4$), ethanol, and acetaminophen is characterized by varying degrees of hepatocyte degeneration and cell death via apoptosis or necrosis.[1]

Methotrexate is a folic acid antagonist, widely used as a chemotherapeutic agent in the treatment of various cancerous stages such as acute lymphoblastic leukaemia and in the treatment of various inflammatory diseases.[5] It is also used for the treatment of multiple sclerosis, dermatomyositis, sarcoidosis, psoriasis, and rheumatoid arthritis, disorders causing inflammation. Its side effects include hypersensitivity pneumonia, central and peripheral nervous system toxicity, liver and gastrointestinal system dysfunctions, and hematologic failure. MTX exerts its primary toxic effects against the rapidly replicating cells of the bone marrow and gastrointestinal epithelium producing leucopoenia and thrombocytopenia.[6]

MTX has been shown to lead to a reduction in methionine synthesis, antioxidant enzymes such as catalase, glutathione peroxidase, superoxide dismutase and a decrease of SAM (S-adenosyl methionine) in cerebrospinal fluid of patients on MTX treatment. Due to its antioxidant effects, a deficiency of SAM caused by MTX may be a reason for increased reactive oxygen species (ROS).[7] Effects of MTX are partly due to its direct toxicity by increasing ROS production. Altered balance between ROS production and antioxidant defenses leads to the “oxidative stress” and could be led to various pathological conditions. The mechanisms of MTX hepatotoxicity could be related to the cellular pathway of the drug. Methotrexate enters the stellate cell bound to folate transporter and is pumped out by the ATP-binding cassette (ABC) family of transporters.[8]

Lipid peroxidation plays an essential role in damage to the cell membrane through reactive oxygen radicals. In experimental studies, MTX toxicity has been shown to increase malondialdehyde (MDA), an important index of lipid peroxidation, and this increase has been shown to be suppressed by antioxidant therapies. Studies have demonstrated that various anti-oxidants are protective against methotrexate hepatotoxicity.[9]

In recent years many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support liver function and treat diseases of the liver. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanisms and modes of action of these plants as well as reaffirming the therapeutic effectiveness of certain plants or plant extracts in clinical studies. Several hundred plants have been examined for use in a wide variety of liver disorders. Just a handful has been fairly well researched. The latter category of plants include: *Silybum marianum* (milk thistle), *Picrorhiza kurroa* (kutkin), *Curcuma longa* (turmeric), *Camellia sinensis*

(green tea), *Chelidonium majus* (greater celandine), *Glycyrrhiza glabra* (liquorice), and *Allium sativum* (garlic).[10]

*Butea monosperma* L. is a medium deciduous tree belonging to the Family Fabaceae.[11] It grows maximum up to 40- 50 ft high with cluster of flowers. It is a slow growing tree, grows in full sun and the growth rate is few feet per year. The leaves of this plant are pinnate, compound with three leaflets, obliquely ovate and broadly elliptic in shape. The orange and scarlet blossoms create a gorgeous canopy on the tree's upper section. The blooming of the flowers starts in February and lasts until the end of April. The fruits of *B. monosperma* have a stalked pods and flat legumes and thicken structure containing a single seed. Two substantial cotyledons in big, wrinkled, reddish-brown colour make up the seed coat.[12]

## MATERIALS AND METHOD

### Materials

### Chemicals And Reagents

Methanol (SDFCL), liquid paraffin (Nice), Methotrexate (Micro labs), Chemical Kits – Cholesterol (CH), Triglyceride (TG), Low-density lipoprotein (LDL), and High-density lipoprotein (HDL) (Ambika diagnostics).

## METHOD

### Collection, Identification and Authentication of Plant Material

*Butea monosperma* L. seeds were collected from Chandrapur, Maharashtra, India. The Plant material was identified and authenticated by Vasantrao Naik College of Agriculture Biotechnology, Yavatmal, Maharashtra, India. (Ref. No. VNCABT/Ytl/Hort/1900/2024)

### Extraction of *Butea monosperma* L. Seeds

Seeds of *Butea monosperma* L. plant were collected, dried in shade and coarsely powdered. The seeds were extracted by soxhlet apparatus by using methanol as a solvent.

### Experimental Animals

Wistar albino rats (150-200g) were used in study. The animals were fed with standard pellet diet and water. All the animals were acclimatized for a week before use. The protocol for the animal study was approved by Institutional Animal Ethics Committee with the research project number 650/Po/Re/5/2002/2024/CCSEA/08. Animals received the treatment by oral gavage tube. All the animals were used under ethical consideration as per the CPCSEA guidelines with regular inspections of rats.

### Experimental Design

### Animal Groups

For this study, rats were divided into following groups (n = 6)

**Group I (Normal Control): -** Rats received only normal saline solution

**Group II (Negative Control): -** Hepatotoxicity was induced in rats by using Methotrexate (20mg/kg) i.p. on day 18.

**Group III (BMSE 200 mg/kg): -** Hepatotoxicity was induced by using MXT in rats and rats were treated with methanolic extract of BMSE (200

mg/kg) p.o. for 21 days.

**Group IV (BMSE 400 mg/kg): -** Hepatotoxicity was induced by using MXT in rats & rats were treated with high dose of BMSE (400mg/kg) p.o. for 21 days.

**Group V (Standard Group):** Hepatotoxicity was induced by using Methotrexate in rats & rats were treated by Silymarin (100mg/kg) p.o. for 21 days.

The rats were weighed after the adaptation period and marked with serial numbers and divided randomly into 5 groups, 6 rats each, and then the doses were calculated according to individual body weights. Daily dose of *Butea monosperma* L. Seed Extract was given to group of III (BMSE 200mg/kg) and group IV (400mg/kg) for the duration of 21 days. Hepatotoxicity was induced in rats by using Methotrexate (20mg/kg) i.p. on day 18.

**Samples Collection from Rats**

At the end of treatment period (21 consecutive days), rats were fasted overnight. Whole blood was collected and centrifuged to obtain serum under mild diethyl ether anaesthesia.

**Evaluation of Biochemical Parameters**

These tests include:

1. Liver enzyme parameters: ALT (SGPT), AST (SGOT), ALP
2. Lipid parameters: TC, TG, LDL & HDL.

**Liver Histopathology Study**

Portion of the liver tissues (left medial lobe) from rats of all groups were used for the histopathological analysis. The tissues were transferred in 10% neutral buffered formalin solution for fixation and later on processed for histopathological studies following the standard procedure. The sections were cut on microtome (Microm, HM 315), processed, and stained with Mayer's haematoxylin and eosin (H&E staining) (Mayer, 1891) for examination.

The stained tissues were observed under a light microscope at 20X magnifying power and photographed using Image Pro Plus.

## STATISTICAL ANALYSIS

Results were expressed as Mean ± SD for statistical analysis of the data. Results were compared by one way ANOVA followed by Dunnet's test. The data was statistically analyzed by using Graphpad instat Software. $p<0.01$ was considered to be statistically significant.

## RESULTS

**Estimation of Lipid Parameters**

**1. Total Cholesterol**

**Table No 1: Effect of BMSE on Total Cholesterol level on day 0 & day 21 in rats**

| Sr.No. | Groups | TC level (mg/dl) on day 0 | TC level (mg/dl) on day 21 |
|---|---|---|---|
| 1 | Normal Control | 35.17±5.37 | 33.96±3.63 |
| 2 | Negative Control | 36.7±7.09 | 56.64±2.54@ |
| 3 | BMSE (200mg/kg) | 35.72±5.00 | 46±1.81** |
| 4 | BMSE (400mg/kg) | 34.3±7.76 | 40.91±2.16** |
| 5 | Silymarin (100mg/kg) | 33.95±3.91 | 37.14±2.81** |

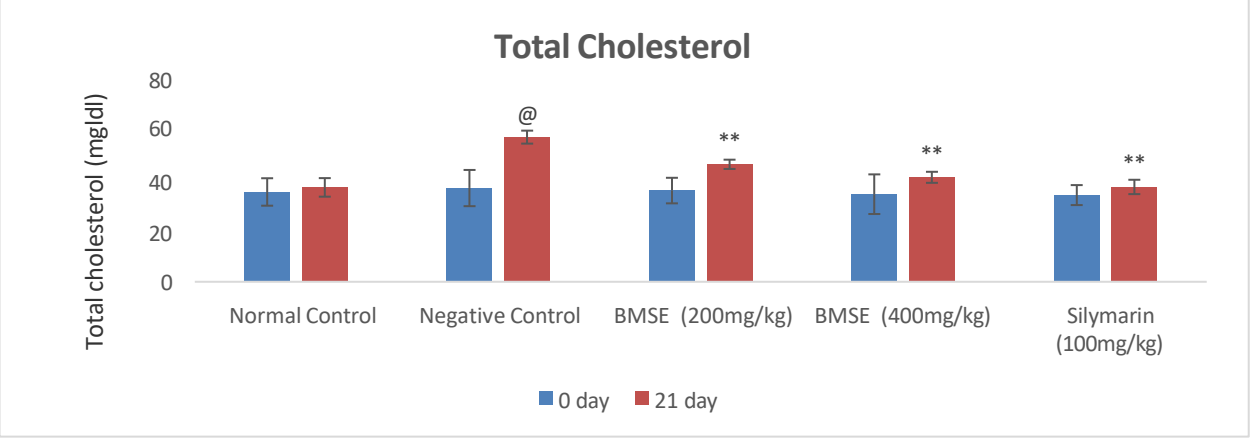


**Figure No. 1: Effect of BMSE on Total Cholesterol level on day 0 & day 21 in rats**

***Values are expressed in Mean±sem (n=6); Significant increase (@P<0.001) in Total Cholesterol level was observed when compared to normal control group. Significant decrease (**P<0. 001) was observed in Total Cholesterol level when compared to negative control group.***

Table 1 and figure 1 Shows the effect of BMSE on Total Cholesterol level on day 0 & 21 in rats. There was a significant ($p<0.001$) increase in Total Cholesterol level in Negative Control group as compared to normal control group. There was significant ($p<0.001$) decrease in Total Cholesterol level in BMSE 200mg/kg, BMSE 400 mg/kg and Silymarin treated groups when compared to negative control group on day 21.

**2. Triglyceride (TG)**

**Table No.2: Effect of BMSE on Triglyceride level on day 0 & day 21 in rats**

| Sr.No. | Groups | TG level (mg/dl) on day 0 | TG level (mg/dl) on day 21 |
|---|---|---|---|
| 1 | Normal Control | 63.02±1.5 | 62.05±1.2 |
| 2 | Negative Control | 64.82±1.1 | 106.3±1.1@ |
| 3 | BMSE (200mg/kg) | 62.43±1.2 | 86.02±1.1** |
| 4 | BMSE (400mg/kg) | 60.21±1.3 | 76.12±1.3** |
| 5 | Silymarin (100mg/kg) | 61.02±1.2 | 70.12±1.2** |

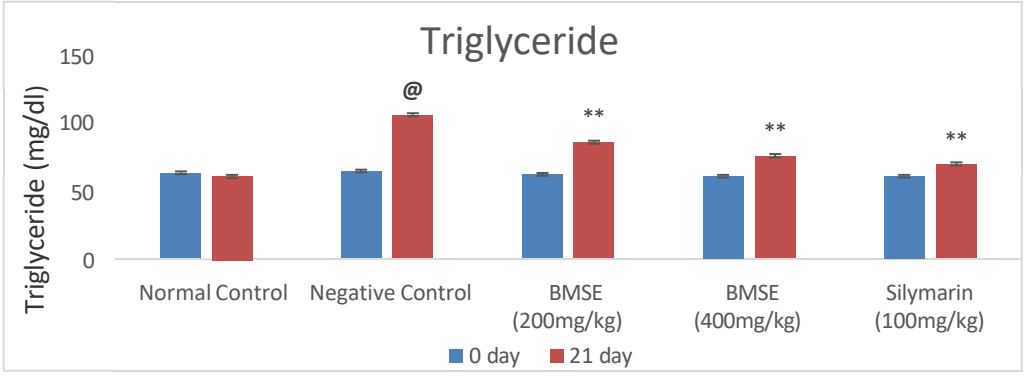


**Figure No.2: Effect of BMSE on Triglyceride level on day 0 & day 21 in rats**

Values are expressed in Mean±sem (n=6); Significant increase (@P<0.001) in Triglyceride level was observed when compared to normal control group. Significant decrease (**P<0. 001) was observed in Triglyceride level when compared to negative control group.

Table 2 and figure 2 shows the effect of BMSE on Triglyceride level on day 0 & 21 in rats. There was a significant (p<0.001) increase in Triglyceride level in Negative Control group as compared to normal control group. There was significant (p<0.001) decrease in Triglyceride level in BMSE 200 mg/kg, BMSE 400 mg/kg and Silymarin treated groups when compared to negative control group on day 21.

**3. High Density Lipoprotein**

**Table No. 3: Effect of BMSE on High Density Lipoprotein level on day 0 & day 21 in rats**

| Sr.No. | Groups | HDL level (mg/dl) on day 0 | HDL level (mg/dl) on day 21 |
|---|---|---|---|
| 1 | Normal Control | 49.53±4.03 | 47.9±5.30 |
| 2 | Negative Control | 49.99±5.20 | 29.7±6.00@ |
| 3 | BMSE (200mg/kg) | 48.37±3.63 | 40.8±0.84** |
| 4 | BMSE (400mg/kg) | 50.62±3.72 | 43.3±3.46** |
| 5 | Silymarin (100mg/kg) | 48.44±3.91 | 45.5±3.53** |

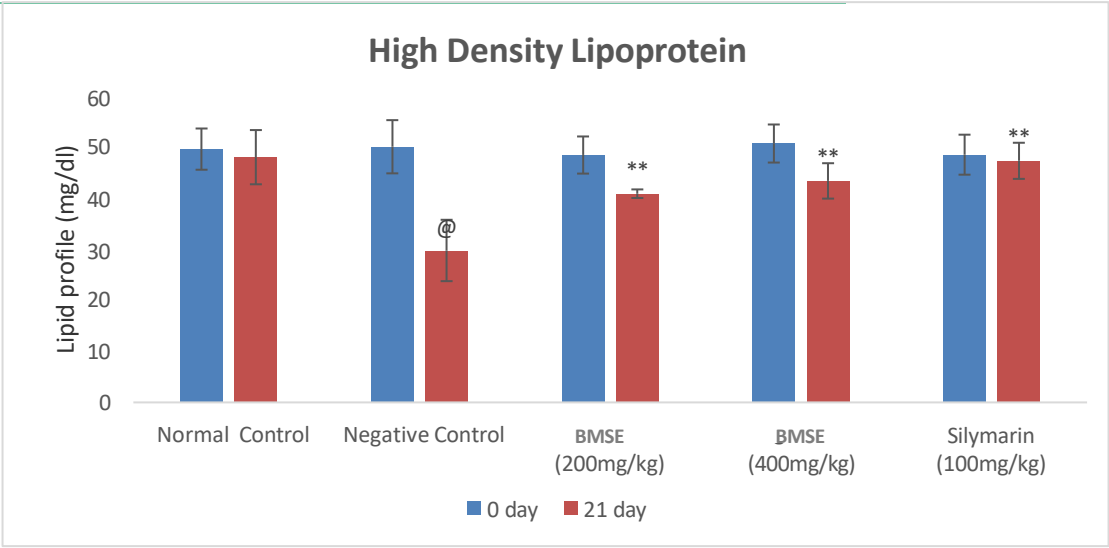


**Figure No. 3: Effect of BMSE on High Density Lipoprotein level on day 0 & day 21 in rats.**

Values are expressed in Mean±sem (n=6); Significant increase (@P<0.001) in High Density Lipoprotein level was observed when compared to normal control group. Significant decrease (**P<0. 001) was observed in High Density Lipoprotein level when compared to negative control group

Table 3 and figure 3 Shows the effect of BMSE on High Density Lipoprotein level on day 0 & 21 in rats. There was a significant (p<0.001) decrease in High Density Lipoprotein level in Negative Control group as compared to normal control group. There was significant (p<0.001) increase in High Density Lipoprotein level in BMSE 200 mg/kg, BMSE 400 mg/kg and Silymarin treated groups when compared to negative control group on day 21.

**4. Low Density Lipoprotein**

**Table No. 4: Effect of BMSE on Low Density Lipoprotein level on day 0 & day 21 in rats**

| Sr.No. | Groups | LDL level (mg/dl) on day 0 | LDL level (mg/dl) on day 21 |
|---|---|---|---|
| 1 | Normal Control | 50.35±4.23 | 52.27±6.02 |
| 2 | Negative Control | 52.03±2.26 | 77.48±6.08@ |
| 3 | BMSE (200mg/kg) | 50.72±0.62 | 59.91±6.0** |
| 4 | BMSE (400mg/kg) | 52.44±2.34 | 56.37±6.32** |
| 5 | Silymarin (100mg/kg) | 53.00±1.61 | 50.11±1.06** |

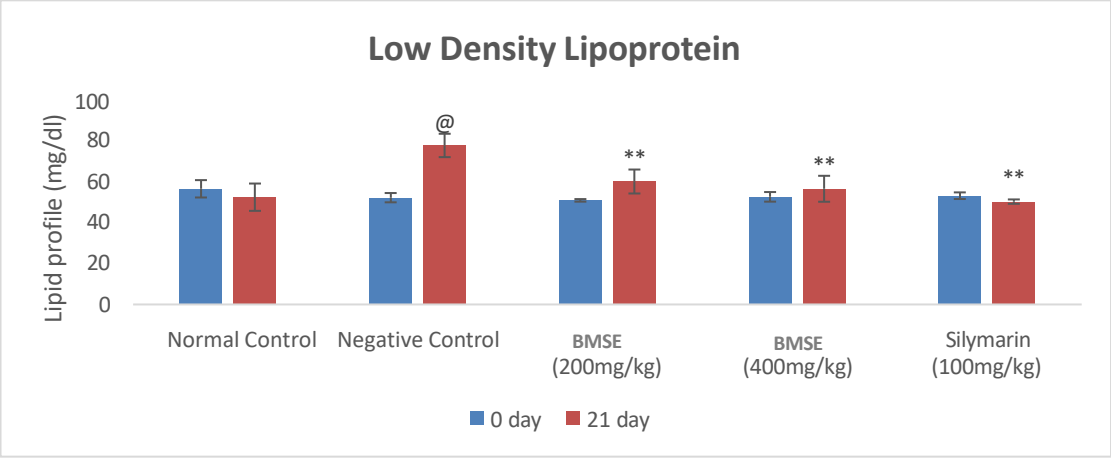


**Figure No: 4 Effect of BMSE on Low Density Lipoprotein level on day 0 & day 21 in rats**

Values are expressed in Mean±sem (n=6); Significant increase (@P<0.001) in Low Density Lipoprotein level was observed when compared to normal control group. Significant decrease (**P<0. 001) was observed in Low Density Lipoprotein level when compared to negative control group Table 4 and figure 4 Shows the effect of BMSE on Low Density Lipoprotein (LDL) level on day 0 & 21 in rats. There was a significant (p<0.001) increase in Low Density Lipoprotein level in Negative Control group as compared to normal control group. There was significant (p<0.001) decrease in Low Density Lipoprotein in BMSE 200mg/kg, BMSE 400 mg/kg and silymarin treated groups when compared to negative control group on day 21.

**Estimation of Liver Parameters**

### 1. Alkaline Phosphate (ALP)

**Table No. 5: Effect of BMSE on Alkaline Phosphate level on day 0 & day 21 in rats**

| Sr.No. | Groups | ALP level (u/l) on day 0 | ALP level (u/l) on day 21 |
|---|---|---|---|
| 1 | Normal Control | 50.70±4.23 | 45.41±6.02 |
| 2 | Negative Control | 52.79±5.58 | 85.69±6.08@ |
| 3 | BMSE (200mg/kg) | 53.14±2.31 | 74.13±7.12** |
| 4 | BMSE (400mg/kg) | 52.02±5.65 | 66.27±4.92** |
| 5 | Silymarin (100mg/kg) | 52.84±1.92 | 58.65±3.32** |

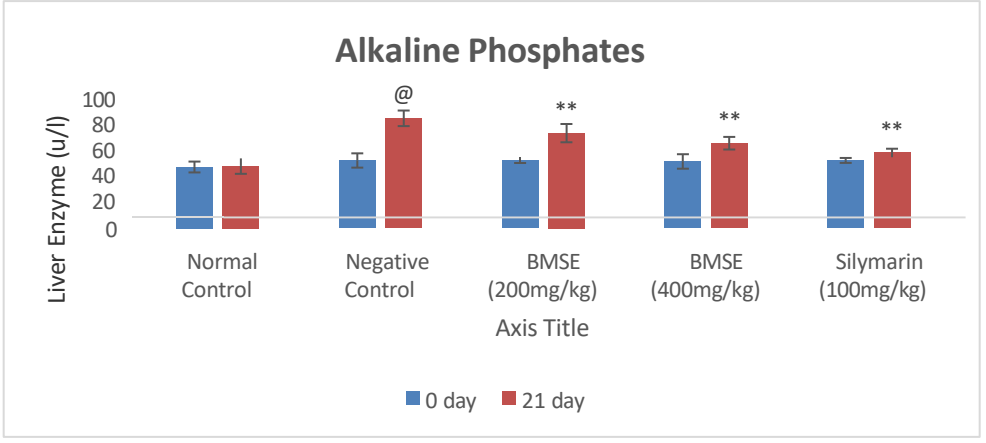


**Figure No. 5: Effect of BMSE on Alkaline Phosphate level on day 0 & day 21 in rats**

Values are expressed in Mean±sem (n=6); Significant increase (@P<0.001) in Alkaline Phosphate level was observed when compared to normal

Alkaline Phosphate level in Negative Control as compared to normal control group. There was significant ($p<0.001$) decrease in Alkaline Phosphate level in BMSE 200mg/kg, BMSE 400 mg/kg and silymarin treated groups when compared to negative control group on day 21.

**2. Aspartate Aminotransferase (AST)**

**Table No. 6: effect of BMSE on Aspartate Aminotransferase level on day 0 & day 21 in rats**

| Sr.No. | Groups | AST level (u/l) on day 0 | AST level (u/l) on day 21 |
|---|---|---|---|
| 1 | Normal Control | 17.82±5.81 | 18.50±4.42 |
| 2 | Negative Control | 18.84±4.61 | 51.27±7.66@ |
| 3 | BMSE (200mg/kg) | 19.90±2.72 | 36.02±4.64** |
| 4 | BMSE (400mg/kg) | 18.76±1.95 | 29.02±6.39** |
| 5 | Silymarin (100mg/kg) | 19.50±1.06 | 20.67±8.33** |

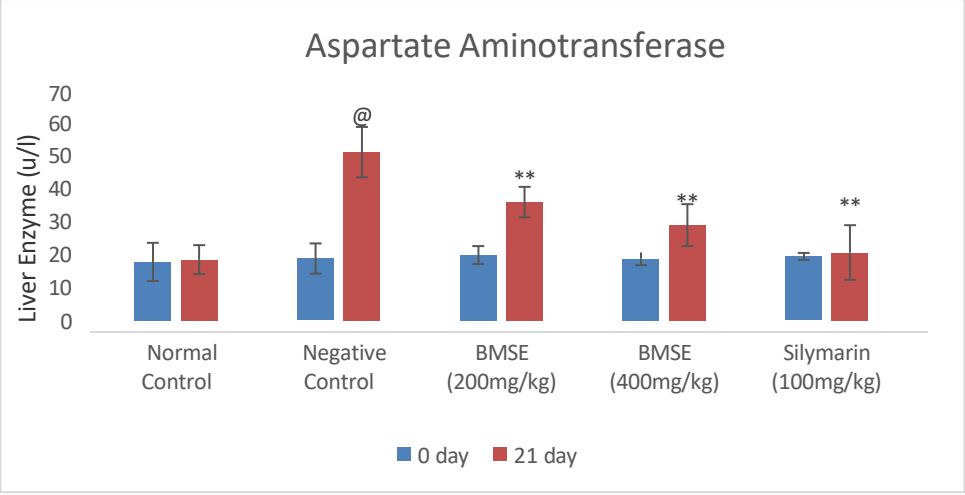


**Figure No: 6 Effect of BMSE on Aspartate Aminotransferase level on day 0 & day 21 in rats**

control group. Significant decrease (**P<0. 001) was observed in Alkaline Phosphate level when compared to negative control group

Table 5 and figure 5 Shows the effect of BMSE on Alkaline Phosphate level on day 0 & 21 in rats. There was a significant (p<0.001) increase in Values are expressed in Mean±sem (n=6); Significant increase (@P<0.001) in Aspartate Aminotransferase level was observed when compared to normal control group. Significant decrease (**P<0. 001) was observed in Aspartate Aminotransferase level when compared to negative control group

Table 6 and figure 6 Shows the effect of BMSE on Aspartate Aminotransferase (AST) level on day 0 & 21 in rats. There was a significant (p<0.001) increase in Aspartate Aminotransferase level in Negative Control as compared to normal control group. There was significant (p<0.001) decrease in Aspartate Aminotransferase level in BMSE 200mg/kg, BMSE 400 mg/kg and silymarin treated group when compared to negative control group on day 21.

**3. Alanine Transaminase (ALT)**

**Table No. 7: Effect of BMSE on Alanine Transaminase level on day 0 & day 21**

| Sr.No. | Groups | ALT level (u/l) on day 0 | ALT level (u/l) on day 21 |
|---|---|---|---|
| 1 | Normal Control | 36.53±6.12 | 35.19±5.77 |
| 2 | Negative Control | 38.30±6.13 | 71.35±8.26@ |
| 3 | BMSE (200mg/kg) | 34.41±8.10 | 63.36±8.61** |
| 4 | BMSE (400mg/kg) | 35.93±7.77 | 54.92±7.95** |
| 5 | Silymarin (100mg/kg) | 31.12±7.22 | 48.28±10.00** |

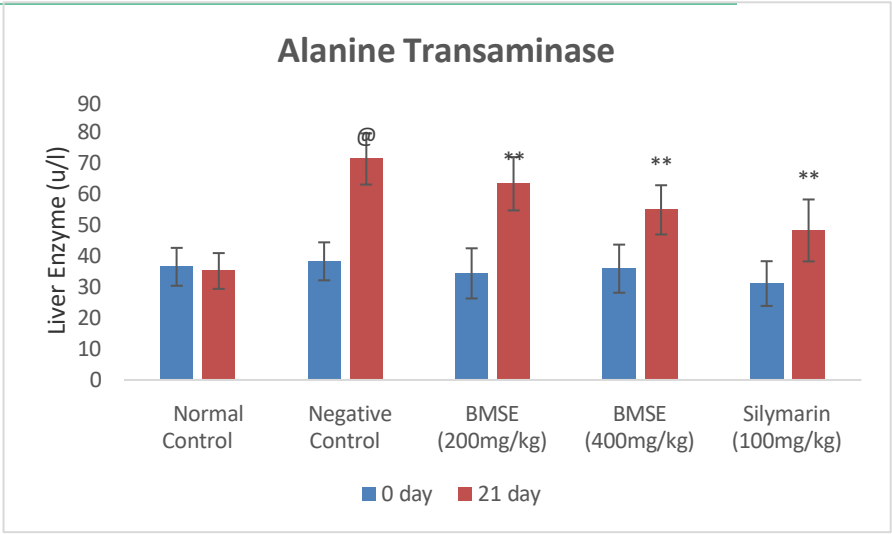


**Figure No. 7: Effect of BMSE on Alanine Transaminase level on day 0 & day 21 in rats**

Values are expressed in Mean±sem (n=6); Significant increase (@P<0.001) in Alanine Transaminase level was observed when compared to normal control group. Significant decrease (**P<0. 001) was observed in Alanine Transaminase level when compared to negative control group

Table 7 and figure 7 Shows the effect of BMSE on Aspartate Aminotransferase (AST) level on day 0 & 21 in rats. There was a significant (p<0.001) increase in Alanine transaminase level in Negative Control group as compared to normal control group. There was significant (p<0.001) decrease in Alanine transaminase level in BMSE 200mg/kg, BMSE 400 mg/kg and silymarin treated group when compared to negative control group on day 21.

**Histopathological Examination:**

Results of histopathological examination of rat liver on day 21 are depicted figure No. 8.

**Group I:** The histopathological examination of control group showed normal structure and architecture. No evidence of portal inflammation, degeneration or fatty change and necrosis.

**Group II:** Methotrexate intoxicated rats showed clear cellular degeneration and loss of the distinct liver characteristic configuration. Centrilobular necrosis and necrosis in all area of lobule was observed. Congestion in central veins and sinusoids were noted. Vacuolar degeneration and inflammatory changes associated with fatty changes were noted.

**Group III:** The section of liver treated with BMSE 200mg/kg showed less centrilobular necrosis. Central vein and sinusoid congestion were not observed. Mild degree of inflammation and fatty changes were noted.

**Group IV:** Rats treated with BMSE 400mg/kg. showed a pathological protection to liver. The section of liver showed normal architecture of liver, no degeneration, no evidence of congestion in central veins and sinusoids. Necrotic lesions and fatty changes were observed. A dose dependent protection of *Butea monosperma* L. seeds Extract was observed in histopathological examination.
**Group V:** The section of liver treated with Silymarin 100 mg/kg showed almost normal architecture of the liver.

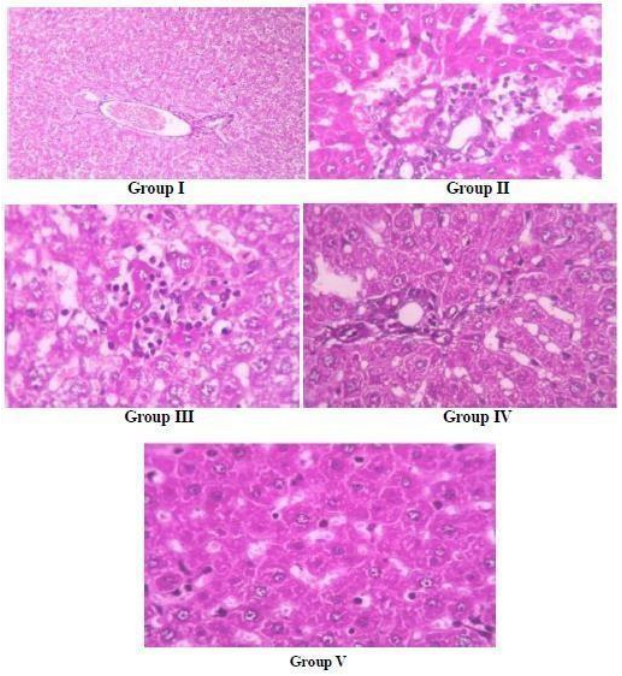


**Figure No. 8: Histopathological Examination of Rat Liver on day 21**

**Group I:** Normal Control Group, Showing normal hepatocytes
**Group II:** Negative Control group shows the hepatic damage and congestion of the liver.
**Group III:** BMSE (200 mg/kg) shows that few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells.
**Group IV:** BMSE (400mg/kg) shows that few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells.
**Group V:** Silymarin (100mg/kg) treated Group shows almost normal architecture of liver.

## DISCUSSION

Hepatotoxin is a toxic chemical substance which damages the liver. Toxic liver injury produced by drugs and chemicals may virtually mimic any

form of naturally occurring liver disease. Hepatoprotective effect was studied against chemicals and drugs induced hepatotoxicity in rats Hepatotoxicity refers to liver damage caused by chemicals, drugs, or other agents. The liver, being the primary site for metabolizing and detoxifying substances, is vulnerable to injury from various compounds. Hepatotoxicity can manifest in different forms, ranging from mild liver enzyme elevations to severe liver failure. Common causes include medications such as acetaminophen and certain antibiotics, herbal supplements, industrial chemicals, and environmental toxins. The mechanisms of hepatotoxicity can involve direct toxicity to liver cells, disruption of cellular functions, immune-mediated responses, or oxidative stress. Early detection and management are crucial to prevent irreversible liver damage and associated complications.[13]

Allopathic medicine offers several approaches to treat hepatotoxicity. Hepatoprotective agents help prevent liver damage and support liver health. Common examples include N-acetylcysteine (NAC), which replenishes glutathione levels and is used for acetaminophen overdose, Ursodeoxycholic acid aids bile flow and treats cholestatic liver diseases, Silybon, Hepamerz demonstrated significant hepatoprotective effects in studies involving liver damage induced by various toxins. These agents work through mechanisms such as antioxidant activity, enhancing detoxification, and reducing inflammation.

Herbal treatments for hepatotoxicity focus on leveraging the liver-protective properties of certain plants known for their antioxidative, anti-inflammatory, and regenerative effects. Milk thistle (*Silybum marianum*) is renowned for its active component, silymarin, which helps stabilize liver cell membranes and stimulates protein synthesis. Turmeric (*Curcuma longa*), rich in curcumin, also offers potent anti-inflammatory and antioxidant benefits, aiding in liver detoxification. Dandelion root (*Taraxacum officinale*) enhances bile flow, supporting liver function and detoxification processes. Additionally, licorice root (*Glycyrrhiza glabra*) contains glycyrrhizin, which reduces liver inflammation and helps repair liver tissue. Herbal treatments for hepatotoxicity offer several advantages over allopathic medicine. They often provide a holistic approach, addressing not only the symptoms but also supporting overall liver function and health through their antioxidative, anti-inflammatory, and regenerative properties. Additionally, herbal treatments can be cost-effective and more accessible, particularly in regions where conventional medications are expensive or hard to obtain. Moreover, the multi-faceted benefits of herbs can address other underlying health issues, promoting overall well-being.[14]

There are many factors which are responsible for the liver damage or injuries such as chemicals and drugs. In the present study Methotrexate was used to induce Hepatotoxicity, since it is clinically relevant. Methotrexate-like chemotherapeutic agents have been One of the serious adverse effects caused by use of MTX is hepatotoxicity. The majority of Methotrexate is metabolized in the liver and single high dose developing Hepatitis. It has been shown to lead to a reduction in methionine synthesis, antioxidant enzymes such as catalase, glutathione peroxidase, superoxide dismutase and a decrease of SAM (S-adenosyl methionine) in cerebrospinal fluid of patients on MTX treatment. Due to its antioxidant effects, a deficiency of SAM caused by MTX may be a reason for increased reactive oxygen species (ROS). Besides the development of fatty liver (steatosis), another early sign of excessive Methotrexate consumption is liver enlargement and protein accumulation, both of which are common findings.[15]

*Butea monosperma* is commonly used in the native system of medicine. Various parts of the plant like leaves, Seeds and roots are medicinally important. BMSE is used traditionally for hepatoprotective activity thus the current study was undertaken to evaluate hepatoprotective activity of BMSE against Methotrexate induced hepatotoxicity in rats. Preliminary phytochemical analysis of Methanolic extract of *Butea monosperma* L. Seeds showed the presence of phytoconstituents like Fatty acid (linoleic acid), oleic acid, linolenic acid, palmitic acid, stearic acid, arachidic acid,

behenic acid and linoceric acid, palasonin, monospermoside, glycoside. Phenolic and tannin compounds are widely distributed in the plants which have hepatoprotective activity.[16]

On examining the Liver functions in Methotrexate induced hepatotoxicity in rats, the SGOT, SGPT, ALP, Total Cholesterol & Triglycerides level was Significantly increased & HDL level was significantly decreased. After treatment with the *Butea monosperma* L. seeds Extract (200mg/kg and 400mg/kg), there was significant decrease in SGOT, SGPT, ALP, & TC, TG, LDL level & increase in HDL level when compared to negative control group on day 21. Although the Bis more BMSE (400m/kg) is found to be more potent than BMSE (200mg/kg).[17]

The structural integrity of the hepatocellular membrane and liver cell architecture damaged by Methotrexate which was confirmed by histopathological examination. Treatment with BMSE (200mg/kg) & (400mg/kg) showed normal architecture of liver, less centrilobular necrosis, no degeneration, no evidence of congestion in central veins and sinusoids was observed in histopathological examination.

The study found that *Butea monosperma* significantly protected the liver from damage caused by Methotrexate in rats. The protective effects of *Butea monosperma* might be due to its antioxidant properties, which help to reduce harmful oxidative stress in the liver, and its anti-inflammatory effects, which lower harmful inflammation. It may also help liver cells repair themselves and prevent cell death. Importantly, the BMSE (200mg/kg & 400mg/kg) extract was safe and did not cause any side effects. These results suggest that *Butea monosperma* Seeds Extract might be a useful and safe treatment for preventing liver damage.[18]

## CONCLUSION

The study successfully demonstrated the hepatoprotective activity of *Butea monosperma* L. seeds extract against methotrexate-induced hepatotoxicity in rats. The results showed that the BMSE 200mg/kg & 400mg/kg extract significantly improved liver functions by lowering harmful liver enzyme levels, improved antioxidant status, and mitigated histopathological changes compared to the methotrexate-induced group. These findings suggest that *Butea monosperma* L. seeds possess potent hepatoprotective properties, potentially due to their rich phytochemical composition, including flavonoids and Glycoside, Phenolic acid.

This research underscores the therapeutic potential of *Butea monosperma* L. seeds in managing liver damage caused by methotrexate, a common chemotherapeutic agent. The study also opens avenues for further research, particularly in isolating specific active compounds responsible for the hepatoprotective effects and evaluating their mechanisms of action.

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