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# EVALUATION OF EFFECT OF ETHANOLIC EXTRACT OF *BRASSICA OLERACEA* AGAINST FRUCTOSE INDUCED NON-ALCOHOLIC FATTY LIVER IN WISTAR RATS

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## ABSTRACT

Aim of present study is to explore the potency of Ethanolic extract of *Brassica oleracea* against fructose induced non-alcoholic fatty liver rats in experimental animals. The experimental animals were randomly divided in to 5 groups (n= 6) and treated for 5 weeks. Non-alcoholic fatty liver disease (NAFLD) was induced by drinking water containing 10% fructose for 5 weeks. Excess intake of fructose causes increase in hepatic triglycerides, hepatic cholesterol, hypertension which ultimately leads to the development of micro and macro vesicular fat deposits on liver. Significant changes were recorded in biochemical parameters i.e. decrease in glucose, Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), cholesterol levels, Triglycerides levels,, Low density lipids( LDL), Very low density lipids (VLDL), and increase oxidative stress markers such as, GSH, and CAT in *Brassica oleracea* treated rats on comparison with the control group. Thus, the current study establishes the effectiveness of *Brassica oleracea*in treating non-alcoholic fatty liver in experimental rats.

## INTRODUCTION

Fatty liver also known as fatty liver disease (FLD) is a reversible condition where excessive amounts of triglycerides and other fats accumulate in liver cells. Fatty liver is commonly associated with alcohol or metabolic syndrome (diabetes, hypertension, obesity and dyslipidaemia) (1). Based on etiology fatty liver is classified in to alcoholic fatty liver disease (AFLD) and non-alcoholic fatty liver disease (NAFLD). AFLD occurs due to excess consumption of alcohol (more than 60 g/day or 25 ml/day) (2) NAFLD is a disease in which excess of triglycerides and other fats accumulates in the liver cells. Signs and symptoms of NAFLD include fatigue, pain in the upper right abdomen (3). Fructose is known as the fruit sugar, excess intake of fructose causes hypertriglyceridemia which causes IR, short term feeding of fructose cause IR, long term feeding causes increase in hepatic triglycerides, hepatic cholesterol, hypertension which ultimately leads to the development of micro and macro vesicular fat deposits on liver .(4) *Brassicaoleracea* (*B. oleracea*) belongs to the family Brassicaceae is one of the most common vegetable growing throughout the country. It consists of Vitamins, glucosinolates, anthocyanin's, polyphenols, sulphur containing compounds, minerals, carbohydrates, fats, proteins,

tryptophan, omega 3 fatty acids (5). It is used in treatment of breast engorgement (6), analgesic, anti-inflammatory (7), ulcer (8), asthma, diabetis (9), cancer (10), and acts as hepatoprotective agent (11). It has cholesterol-lowering ability and also contains antioxidants such as Polyphenols and vitamins (12). In this study, the parameters monitored are Glucose levels, Serum glutamate oxaloacetate transferase (SGOT) levels, Serum glutamate pyruvate transferase (SGPT) levels, Total cholesterol levels, Triglycerides levels, High density lipids(HDL), Low density lipids( LDL), Very low density lipids ( VLDL), Oxidative stress markers, Lipid peroxidation, Hepatic reduced glutathione content, catalase, Body weight and liver index, Histopathological examination in rats.

## MATERIALS AND METHODS:

### Preparation of EthanolicExtract of B. *oleracea* (EEBO)

The leaves of Brassica oleracea were collected, sliced into small pieces and shadow dried. Shadow drying minimizes enzymatic degradation of phonetic compounds i+nside plant tissues. Coarse powder is obtained by pulverization of dried leaves. Powder was loosely packed in the thimble of soxhlet apparatus and extracted with ethanol at 55° C for 18 h. The extract was air dried at 25-30° C and weighed .The dried extract was dissolved in distilled water before administration to normal and diabetic rats.

### Experimental setup

Animals were divided into five different groups, each having 6 rats and treated accordingly. Group I: rats fed with a normal standard diet. Group II receives10% fructose serves as control. Group III receive 10%w/v fructose and statins which serve as standard , Group IV receive 10%w/v fructose and EEBO (200mg/kg,p.o) to assess the potency of EEBO ( low dose), Group V receive 10%w/v fructose and EEBO (400mg/kg,p.o), On 35$^{th}$ day, all the animals were sacrificed by mild ether anaesthesia for histopathological studies

### Collection of blood samples

The blood samples were collected from the retrorbital venous plexus of rats without any coagulant for the separation of serum, at the regular intervals of the treatment. After collecting the blood in ependroff tubes they were kept for 1 h at room temperature and serum was separated by centrifugation at 2000 rpm for 15 min and stored until analyzed for various biochemical parameters.

### Drugs and chemicals

Fructose was obtained from Finar Chemicals Limited, Ahmadabad. Statins were obtained from Sigma-Aldrich, Bangalore. Thiobarbituric acids (TBA), trichloro acetic acid, hydrogen peroxide were obtained from SD fine chemicals Ltd Mumbai. Sodium dihydrogen phosphate, potassium dihydrogen phosphate, tris buffer and all other reagents used were of analytical grade. Glucose, SGOT, SGPT, total cholesterol, triglyceride and HDL estimation kits were obtained from erba diagnostic Ltd. Delhi, India.

**Statistical analysis:** Data were expressed as Mean ± SEM (n=10). The results were analyzed using one way ANOVA in graph pad prism ver.5.0. To determine the level of significant difference between each treatment and the control group using Tukey test.

## RESULTS

**Acute toxicity studies:** Acute toxicity study of EEBO was carried out in rats according to OECD 423 guidelines .Different doses of EEBO were administered up to 4000mg/kg b.w. (p.o.) and the rats were observed for a period of 72 hrs for behavioral changes, toxic symptoms and mortality. The dose of 4000mg/kg b.w. (p.o.) did not showed any toxic symptoms in experimental animals hence 1/10$^{th}$ of it was selected as high dose i.e. 400mg/kg and half of the 1/10$^{th}$ dose i.e. 200mg/kg was selected as low dose.

**B) Non-alcoholic fatty liver disease activity**

**i) Serum biomarkers:** The result shows the effect of EEBO on serum glucose, AST, ALT levels in normal and experimental groups. There was significant ($p<0.001$) increase in serum glucose, AST, ALT levels in control group when compared with normal group. Standard drug significantly reduced ($p<0.001$) these levels in group-III The groups (G-V) receiving EEBO(400 mg/kg) showed a significant ($p<0.001$),decrease in serum glucose, AST, ALT levels like standard group, group (G-IV) receiving EEBO (200 mg/kg) also showed significance in decreasing the serum glucose, AST, ALT levels when compared to control group (G-II).

**ii) LIPID PROFILES**

The result showed a significant ($p<0.001$) increase in serum cholesterol levels in control group when compared with normal group due to fructose. Standard drug significantly reduced ($p<0.001$) these CH levels in group-III. Rats receiving EEBO at a dose of 200mg/kg also reduced CH , TG, LDL, VLDL levels in group-IV, but high dose i.e.,400 mg/kg significantly reduced CH levels in group-V.

**Effect on serum HDL-cholesterol:** The result showed a significant ($p<0.001$) increase in serum HDL levels in control group when compared with normal group due to fructose, but these HDL levels are significantly reduced ($p<0.001$) in rats receiving standard drug and EEBO at a dose of 200 and 400 mg/kg.

**III) *IN VIVO* ANTIOXIDANT PARAMETERS**

In the present study, various antioxidant parameters were assessed on 35$^{st}$ day.

**a) Effect on Catalase (CAT):** A significant decrease in the levels of catalase was observed in the control group, when compared to the normal group (G-I). The group-III receiving standard drug only had significant increase in the catalase levels, when compared to the control group (G-II). The groups-IV and V treated with EEBO (200 mg/kg and 400 mg/kg) also exhibited a significant ($p<0.001$) increase in the catalase levels, when compared to the fructose control group (G-II).

**c) Effect on Lipid peroxidation (LPO)**

A significant increase in the levels of MDA was observed in the control group, when compared to the normal group (G-I). The group-III receiving standard drug showed significant decrease in the MDA levels, when compared to the control group (G-II). The groups-IV and V treated with EEBO (200 mg/kg and 400 mg/kg) also exhibited a significant ($p<0.001$) decrease in the MDA levels, when compared to the control group (G-II).

**IV) Effect on liver and body weight:** Rats treated with fructose (G-II) showed increase in liver and body weight on 35$^{th}$day when compared with normal rats. The G-III rats treated with standard drug showed a little decrease in the liver and body weights on 35$^{th}$day.Rats treated with EEBO (200 mg/kg and 400 mg/kg, p.o., once daily) showed a significant ($p<0.001$) decrease in liver and body weights, when compared to the control group

**V) Histopathological results:** Histopathalogical studies of the normal group of liver showed normal cyto architecture of liver (figure no: 1). In fructose induced group of animals showed the fatty change i.e. deposition of fat in liver (figure no: 2). In standard group animals showed normal cyto architecture of liver (figure no: 3). Rats treated with EEBO (200mg/kg p.o and 400mg/kg p.o) showed almost normal cyto architecture of liver (figure no: 4, 5).

**DISCUSSION:** In the present study, rats were selected to induce NAFLD because they have close similarities to human NAFLD. The serum enzymes namely glucose, AST and ALT serve as sensitive indices to assess the severity of NAFLD. Fructose increase the activities of these enzymes as observed in this study confirmed the onset of NAFLD.

**Table no: 1 Effect of EEBO on serum biomarkers**

| GROUPS | TREATMENT | On 35th Day | | |
|---|---|---|---|---|
| | | Glucose (mg/dl) | AST(IU/L) | ALT(IU/L) |
| I | Normal | 100.5 ± 1.9 | 17.5 ± 2.1 | 24.5 ± 1.9 |
| II | Control 10%w/v fructose | 211.7 ± 4.2### | 63.2 ± 2.6### | 75.1 ± 2.2### |
| III | Standard 10%w/v fructose + statins 30mg/kg,p.o | 123.1 ± 3.9*** | 29.7 ± 1.4*** | 38.7 ± 0.8*** |
| IV | Low dose 10%w/v fructose + EEBO 200mg/kg,p.o | 192.3 ± 2.5** | 51.9 ± 2.1** | 64.2 ± 2.7** |
| V | High dose 10%w/v fructose + EEBO 400mg/kg,p.o | 137.2 ± 2.7*** | 33.2 ± 1.2*** | 42.3 ± 1.4*** |

**Table no: 2 Effect of EEBO on serum lipid profiles**

| **Groups** | **Treatment** | **On 35th Day** | | | | |
|---|---|---|---|---|---|---|
| | | **CH (mg/dl)** | **TG (mg/dl)** | **HDL(mg/d)** | **LDL (mg/dl)** | **VLDL (mg/dl)** |
| I | Normal | 170 ± 8.5 | 91 ± 9.8 | 44.5 ± 5.4 | 107.3 ± 11.5 | 18.2 ± 1.9 |
| II | Control 10%w/v fructose | 306.7 ± 4.2### | 241.8 ± 10.3### | 8 ± 1.5### | 200.3 ± 4.8### | 48.4 ± 2.1### |
| III | Standard 10%w/v fructose + statins 30mg/kg,p.o | 208.5 ± 7.3*** | 124.7 ± 7.1*** | 32.6 ± 2.7*** | 150.9 ± 8.1*** | 24.9 ± 1.4*** |
| IV | Low dose 10%w/v fructose + EEBO 200mg/kg,p.o | 267.3 ± 4.6** | 196.7 ± 4.8** | 23.5 ± 0.7** | 204.5 ± 5.1** | 39.3 ± 0.96** |
| V | High dose 10%w/v fructose + EEBO 400mg/kg, p.o | 212. ±7.2*** | 137.2 ± 6.3*** | 30.8 ± 2.1*** | 154.4 ± 8.4*** | 27.4 ± 1.3*** |

**Table no: 3Effect of EEBO on tissue antioxidant levels**

| GROUPS | TREATMENT | CAT ($H_2O_2$consumed/gram tissue) | GSH (µg of GSH/mg) | LPO (µM /mg) |
|---|---|---|---|---|
| I | Normal | 55.1 ± 1.1 | 23.5 ± 0.8 | 3.5 ± 0.7 |
| II | Control 10% W/V fructose | 22.6 ± 0.9### | 7.5 ± 0.7### | 13.5 ± 1.2### |
| III | Standard Group 10% W/V fructose + EEBO (400 mg/kg, p.o) | 57.4 ± 0.9*** | 27.5 ± 0.76*** | 5.5 ± 0.8*** |
| IV | Low dose 10% W/V fructose + EEBO (200 mg/kg, p.o) | 28.2 ± 1.6** | 12.17 ± 0.7** | 8.8 ± 0.6** |
| V | High dose 10% W/V fructose + EEBO (400 mg/kg, p.o) | 61.1 ± 0.7*** | 31.1 ± 0.6** | 6.3 ± 0.8*** |

**Table no: 4: Effect of EEBO on rat liver and body weight**

| GROUPS | TREATMENT | 35th Day | |
|---|---|---|---|
| | | Liver weight | Body weight |
| I | Normal | 3.8 ± 0.23 | 15 ± 1.16 |
| II | Control 10% W/V fructose | 5.6 ± 0.14 ### | 37.7 ± 0.65### |
| III | Standard 10% W/V fructose + statins (30 mg/kg, p.o) | 3.9 ± 0.26*** | 23.4 ± 0.89 *** |
| IV | Low dose 10% W/V fructose + EEBO (200 mg/kg, p.o) | 4.1 ± 0.21** | 32.5 ± 0.73 ** |
| V | High dose 10% W/V fructose + EEBO (400 mg/kg, p.o) | 3.9 ± 0.23*** | 26.6 ± 0.64 *** |

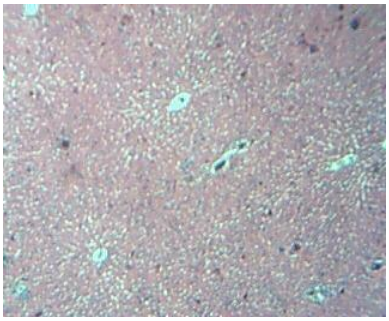

Fig 1: Photo micrograph (PM) of the liver of Normal group

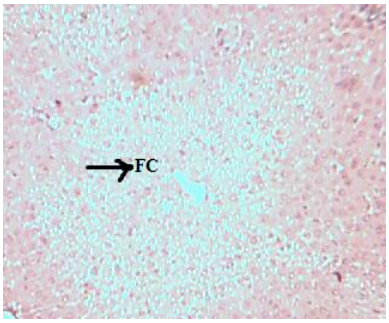


Fig 2: P M of the liver of control group

FC- Fatty Change

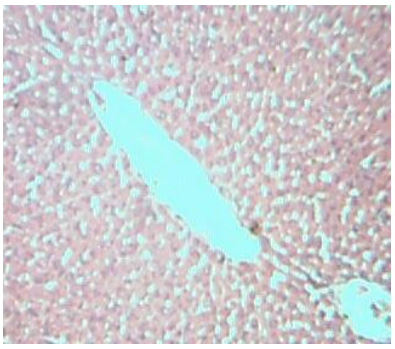
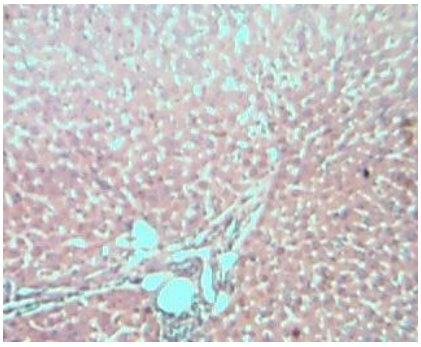
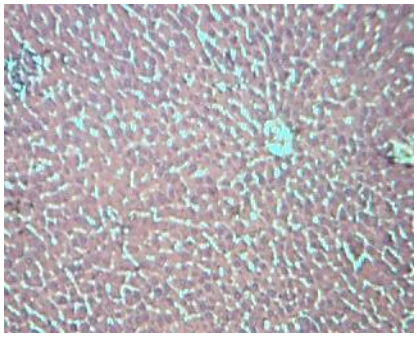

Fig.3: P M of the liver of standard group, Fig.4: P M of the liver of test-1 group
Fig.no:5 P M of the liver of test-2 group

In the present study, serum markers such as serum cholesterol, triglycerides, LDL and VLDL levels were estimated at the end of the study. The results were showed a significant increase in serum cholesterol, triglycerides, LDL and VLDL. The results were showed a significant increase in serum glucose, AST, ALT levels in control group. Standard group has shown significant decrease serum levels. Different doses (200mg/kg and 400mg/kg) of FJBO treated groups have shown significant and dose dependent decrease in serum glucose, AST, ALT levels. In the present study, there is a significant increase in CAT and GSH levels in standard and FJBO treated groups due to its potent free radical scavenging ability. GSH is an important antioxidant which plays the role of an intracellular radical scavenger and is a substrate for many xenobiotic elimination reactions. Microscopic evaluation of liver tissue in fructose group showed the presence of fat accumulation in liver. Fat is not accumulated in the liver of standard group and different doses of FJBO i.e., 200mg/kg and 400mg/kg treated groups have shown significant and dose dependent action when compared with control group.

## CONCLUSION

EEBO had shown a significant protection against fructose induced NAFLD that is confirmed by observing the decrease in serum glucose, SGOT, SGPT, cholesterol, triglycerides, HDL, LDL, VLDL and Rat liver weights. Significant increase in HDL is observed. It is also observed from the present study that significant protection against fructose induced oxidative stress, noted by decrease in LPO and increase in CAT and GSH levels and histopathology of the liver also supports the protective action of EEBO.

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