EVALUATION THE POTENCY OF ETHANOLIC LEAF EXTRACT OF *CASSIA FISTULA* ON FRUCTOSE INDUCED NAFLD IN EXPERIMENTAL RATS

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Abstract:

The aim of the present study was to evaluate the potency ofethanolic extract of *Cassia fistula* against fructose induced Non-alcoholic fatty liver disease in experimental rats. NAFLD is defined as accumulation of fat, triglycerides in hepatocytes. *Cassia fistula* belongs to the family leguminosae. The experimental animals were randomly divided in to 5 groups (n= 6) normal, control, standard, test-1and tet-2 groups and treated for 5 weeks as per the treatment schedule. NAFLD was induced by drinking water containing 10% fructose for 5 weeks. EECF was administered to animals for 5 weeks by oral feeding needle. Studies have shown that there was significant ($p<0.001$) increase in serum parameter levels in control group when compared with normal group. The groups (G-V) receiving EECF (400 mg/kg) showed a significant ($p<0.001$), decrease in serum levels, whereas group (G-IV) receiving EECF (200 mg/kg) also showed significance in decreasing the serum levels when compared to control group (G-II). Hence it is concluded that EECF provides protection against fructose induced NAFLD in male wistar albino rats. Hence, orally applicable, EECF may have great potential as an alternative to the therapeutic agents currently available for treatment of NAFLD.

Key words: induced Non-alcoholic fatty liver disease, *Cassia fistula*, fructose.

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Introduction:

Non-alcoholic fatty liver disease (NAFLD) is defined by macrovesicular steatosis in ≥5% hepatocytes, in the absence of a secondary cause such as alcohol or drugs. It encompasses a spectrum of disease from non-alcoholic fatty liver (NAFL) through to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. [1] NAFLD affects 10–24% of the general population from different countries. The prevalence of NAFLD, however, increases significantly to 57.5–74% in obese individuals. NAFLD affects 2.6% of children10 and this figure increases to 22.5–52.8% in the obese child population.[2] Development of NAFLD is a two-step process. The first step of this process is fat deposition in the liver that will increase insulin resistance. The second part of this process is cellular and molecular changes involving oxidative stress, and oxidation of fatty acids in the liver due to a variety of factors-cytokine injury, hyperinsulinemia, hepatic iron and lipid peroxidation, variation of the extracellular matrix, energy homeostasis, and change in the immune system function. The development of insulin resistance is an intricate process increase in fat mass and adipocyte differentiation plays a key role in developing insulin resistance. [3]. *Cassia fistula* belongs to the family leguminosae. *C. fistula* rich in Alkaloids ,tannins, flavonoids, glycosides, carbohydrates, stearic acids, oleic, oxalic, linoleic, oxyanthraquinones and anthraquinone glycosides like rhein, chrysophanol , Glycosides-Sennosides A & B, carbohydrates, proteins, amino acids, volatile oil like essential oils, Barbaloin [4]. *Cassia fistula* is very important in different traditional medicinal systems because it possesses Distinctive properties valuable in treating the dermal infections, inflammatory conditions, ulcers, rheumatism, jaundice as well as anorexia [5]. *Cassia fistula* exhibits antibacterial, antifungal, antiviral [6], hyplipidemic [7] anti-diabetic,[8] hepatoprotection,[9] anti-pyretic [10] anti-inflammatory,[11] and anti-oxidant activities [12]. Fructose is a monosaccharide, primarily metabolized in the liver. Excessive intake, increased the severity of NAFLD by exacerbating fat deposition, inflammation, oxidative stress, insulin resistance and possibly fibrosis [13]. In the present study, rats was 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 increases the activities of these enzymes as observed in this study confirmed the onset of NAFLD [14].

Materials and methods

Chemicals and reagents:

All reagents used in the present study was were of analytical grade.

Collection and authentication of leaves:

Fresh plant leaves of *Cassia fistula* were collected from local areas of Kadapa, Andhra Pradesh and authentified by Dr. A. Madhusudhana Reddy, Professor, Department of Botany, Yogi vemana University, kadapa, Andhra Pradesh, India. Voucher specimens (No: EC- 1419) for these plants has been kept in the P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India.

Preparation of extract:

The shade dried leaves of plant *Cassia fistula* were taken, powdered in a grinder-mixer to obtain a coarse powder and then passed through 40 mesh sieves. About 200 gms of powder was extracted by using ethanol by Soxhlet apparatus process up to 24hrs. The solution was filtered through Whatman filter paper and the resultant filtrate was distilled under reduced pressure for recovery of solvent. The dried extract thus obtained was kept in desiccators and used for further experiments.

Experimental animals

Healthy adult male wistar rats weighing between 150-200gm were used for the present study were obtained from Raghavendra enterprises, Bangalore. They were acclimatized for 7 days under standard conditions (27±2ºC, relative humidity 44 - 56% and light and dark cycles of 10 and 14 hours respectively) and fed with standard rat diet obtained from Mysore feeds, Bangalore and purified drinking water *ad libitum* for 1 week before and during the experiments.

All experiments and protocols described in present study were approved by the Institutional Animal Ethical Committee (IAEC) of P. Rami Reddy Memorial College of Pharmacy (1423/Po/a/11/CPCSEA/112/2022).

Experimental schedule

The experimental animals were randomly divided in to 5 groups (n= 6) and treated for 5 weeks as per the treatment schedule. NAFLD was induced by drinking water containing 10% fructose for 5 weeks. EECF was administered to animals for 5 weeks by oral feeding needle.

Table no:1-Treatment Schedule

S.NO	Groups	No. of animals	Treatment (35 days)	Purpose
I	Normal	n = 6	Vehicle (distilled water)	To serve as normal
II	Control	n = 6	10 % w/v fructose	To serve as control
III	Standard	n = 6	10 % w/v fructose + statins (30 mg/kg,p.o)	To serve as standard
IV	Low dose	n = 6	10 % w/v fructose + EECF (200 mg/kg,p.o)	To assess the potency of EECF (at low dose)
V	High dose	n = 6	10 % w/v fructose + EECF (400 mg/kg,p.o)	To assess the activity of EECF (at high dose)

P.O = per oral

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 [16].

Statistical analysis

All the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way analysis of variance ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad 5.0). Statistical significance was set accordingly.

Results and discussion:

a) Effect on glucose, AST &ALT:

Serum glucose, AST&ALT levels were estimated by using commercial erba glucose kit and the results were shown in Graph Table no.2.The result shows the effect of EECF on serum glucose, AST&ALT levels in normal and experimental groups. There was significant ($p<0.001$) increase in serum glucose levels in control group when compared with normal group. The groups (G-V) receiving EECF (400 mg/kg) showed a significant ($p<0.001$), decrease in serum glucose,

AST&ALT levels, whereas group (G-IV) receiving EECF (200 mg/kg) also showed significance in decreasing the serum glucose, AST&ALT levels when compared to control group (G-II).

Table no: 2 Effect of EECF on serum biomarkers

GROUPS	TREATMENT	On 35th Day		
		Glucose (mg/dl)	AST (IU/L)	ALT (IU/L)
I	Normal	32.6 ± 0.52	22.8 ± 2.1	23.6 ± 0.7
II	Control 10 % w/v fructose	220.7 ± 5.2###	59.7 ± 2.6###	59.8 ± 2.3###
III	Standard 10 % w/v fructose + statins 30 mg/kg, p.o	88.5 ± 2.4***	24.3 ± 1.6***	29.3 ± 0.4***
IV	Low dose 10 % w/v fructose + EECF 200 mg/kg, p.o	112.2 ± 1.5**	37.2 ± 2.7**	36.2 ± 1.8**
V	High dose 10 % w/v fructose + EECF 400 mg/kg, p.o	86.4 ± 0.45***	30.4 ± 1.2***	29.3 ± 1.7***

The above values are expressed in Mean ± SEM and n=6. ### indicates P<0.001 when compared to normal group. *** indicates P<0.001, **indicates p<0.01when compared to control group. **AST:** Aspartatetransferase, **ALT:** Alaninetransferase

ii) LIPID PROFILES

a) Effect on serum cholesterol (CH)

Serum cholesterol, TG & HDL levels were estimated by using commercial erba cholesterol kit and the results were shown in Table no.3. The result showed a significant (p<0.001) increase in serum TC, TG & HDL levels in control group when compared with normal group due to fructose. Standard drug significantly reduced (p<0.001) these TC, TG & HDL levels in group-III. Rats receiving EECF at a dose of 200mg/kg also reduced CH levels in group-IV, but high dose i.e., 400 mg/kg significantly reduced TC, TG & HDL levels in group-V.

Table no: 3 Effect of EECF on serum biomarkers

GROUPS	TREATMENT	On 35th Day

		TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)
I	Normal	117.8 ± 0.83	22.5 ± 2.1	68.4 ± 1.9
II	Control 10 % w/v fructose	263.3 ± 0.2###	67.5 ± 2.6###	19.2 ± 2.3###
III	Standard 10 % w/v fructose + statins 30 mg/kg, p.o	133.2 ± 3.6***	25.6 ± 1.6***	59.7 ± 0.6***
IV	Low dose 10 % w/v fructose + EECF 200 mg/kg, p.o	156.4 ± 8.7**	33.9 ± 2.7**	40.5 ± 1.8**
V	High dose 10 % w/v fructose + EECF 400 mg/kg, p.o	137.5 ± 0.35***	25.2 ± 1.2***	53.4 ± 1.7***

b) Effect on serum LDL&VLDL cholesterol

Serum LDL & VLDL levels estimated were shown in Table no.4. It was observed that fructose significantly ($p<0.001$) increased serum LDL&VLDL levels in control group when compared with normal group. Standard drug significantly reduced ($p<0.001$) these LDL&VLDL levels in group-III. Rats receiving EECF at a dose of 200 mg/kg also reduced LDL-CH levels in group-IV, but high dose i.e.,400 mg/kg significantly reduced LDL&VLDL levels in group-V.

Table no: 4 Effect of EECF on serum biomarkers

GROUPS	TREATMENT	On 35th Day	
		LDL (mg/dl)	VLDL (mg/dl)
I	Normal	85.8 ± 0.83	15.6 ± 1.1
II	Control 10%w/v fructose	125.5 ± 6.2###	45.2 ± 2.6###
III	Standard 10 % w/v fructose + statins 30 mg/kg, p.o	58.4 ± 0.3***	17.3 ± 1.6***
IV	Low dose 10 % w/v fructose + EECF 200 mg/kg, p.o	73.6 ± 1.6**	21.8 ± 2.7**

V	High dose 10 % w/v fructose + EECF 400 mg/kg, p.o	61.2 ± 0.35***	20.4 ± 1.2***

IN VIVO ANTIOXIDANT PARAMETERS

a) Effect on Catalase (CAT) & Reduced glutathione (GSH)

A significant decrease in the levels of catalase & GSH 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 & GSH levels, when compared to the control group (G-II). The groups-IV and V treated with EECF (200 mg/kg and 400 mg/kg) also exhibited a significant ($p<0.001$) increase in the catalase & GSH levels, when compared to the fructose control group (G-II) and results were shown in Table no 5.

Table no: 5 Effect of EECF on tissue antioxidant levels

GROUPS	TREATMENT	CAT (H_2O_2 consumed/ gram tissue)	GSH (µg of GSH/mg)
I	Normal	55.3 ± 0.3	29.5 ± 0.8
II	Control 10% W/V fructose	30.4 ± 0.6$^{###}$	12.6 ± 0.7$^{###}$
III	Standard Group 10% W/V fructose + EECF (400 mg/kg, p.o)	64.4 ± 0.9***	31.8 ± 0.76***
IV	Low dose 10% W/V fructose + EECF (200 mg/kg, p.o)	44.2 ± 1.6**	23.2 ± 0.7**
V	High dose 10% W/V fructose + EECF (400 mg/kg, p.o)	59.3 ± 0.7***	32.3 ± 0.5**

The above values are expressed in Mean ±SEM and n=6. ### indicates $P<0.001$ when compared to normal group. *** indicates $P<0.001$, **indicates $p<0.01$ when compared to control group

CAT: Catalase, **GSH:** Reduced glutathione.

Conclusion:

EECF had shown a significant protection against fructose induced NAFLD that was confirmed by observing the decrease in serum glucose, SGOT, SGPT, cholesterol, triglycerides, HDL, LDL, VLDL and Rat liver weights. Significant increase in HDL was 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 EECF.

Hence the results obtained in this present study indicate that EECF have shown a good protective activity against fructose induced NAFLD by decreasing serum levels. Thus, it may be concluded that the EECF exerts its activity by enhancing the synthesis of endogenous antioxidants.

Hence, orally applicable, EECF may have great potential as an alternative to the therapeutic agents currently available for treatment of NAFLD.

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