Uttar Pradesh Journal of Zoology

Volume 45, Issue 15, Page 110-128, 2024; Article no.UPJOZ.3743
ISSN: 0256-971X (P)

The Protective Activity of Kaempferol Glycosides from Soya Leaf Extract for Experimentally Induced Non-alcoholic Fatty Liver Disease in Wistar Rats

Jyothi Y [a*], Ajmal Sha S [a], Prasanthi Kallepalli [a], Sairashmi Samanta [a], Sushil Gurung [a], Ankit Kumar Mahato [a], Nishanth D R [a], Rohit Chettri [a] and Royan Chhetri [a]

[a] Department of Pharmacology, Krupanidhi College of Pharmacy, #12/1 Chikkabellanduru, Carmelaram Post, Varthur, Bangaluru, Karnataka, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i154226

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3743

Original Research Article

Received: 27/04/2024
Accepted: 03/07/2024
Published: 06/07/2024

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) ranks as one of the most important chronic liver conditions globally, characterized by excessive fat deposition in the liver, central obesity, insulin resistance and in general with characteristics of the metabolic syndrome. Thus, it is vital to treat

**Corresponding author: Email: jokiran05@gmail.com;*

Cite as: *Y, Jyothi, Ajmal Sha S, Prasanthi Kallepalli, Sairashmi Samanta, Sushil Gurung, Ankit Kumar Mahato, Nishanth D R, Rohit Chettri, and Royan Chhetri. 2024. "The Protective Activity of Kaempferol Glycosides from Soya Leaf Extract for Experimentally Induced Non-Alcoholic Fatty Liver Disease in Wistar Rats". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (15):110-28. https://doi.org/10.56557/upjoz/2024/v45i154226.*

NAFLD as well as its accompanying metabolic disorders. The present study is to evaluate Kaempferol glycosides activity from *Glycine.max* leaves extract in experimentally induced NAFLD in rats. The study evaluated the KG activity of *G.max* extract from leaves on a the HFD and Fructose induced rat model, utilizing Albino Wistar rats with daily doses of 200-250g. Parameters included body weight, liver weight, fasting blood glucose, fasting insulin, insulin resistance, lipid profiles, and histopathological investigations. The doses for KGE were 300mg/kg as medium and 400mg/kg as high. From the research results it was determined that medium dose (300mg/kg) and high dose (400mg/kg) demonstrated a substantial effect when compared to HFD and fructose induced positive control. The study demonstrated that Kaempferol glycosides from *Glycine.max* leaves extract can prevent and cure HFD and fructose-induced non-alcoholic fatty liver in Albino Wistar rats. The extract's action was substantial at medium and high doses, compared to HFD and fructose-induced positive controls. This shows that Kaempferol glycosides could be a promising natural therapeutic agent against NAFLD, hyperlipidaemic diseases, and metabolic syndromes with less adverse effects compared to conventional medications.

Keywords: Kaempferol glycosides; high fat diet; fructose; non-alcoholic fatty liver disease; hyperlipidaemic and insulin resistance.

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is caused by excessive adipose tissue in hepatocytes (>5% of liver weight or volume) [1]. The medical condition known as non-alcoholic steatohepatitis is incredibly benign in terms of liver function [2]. That being said, oxidative processes accelerated by slowly oxidizing fat droplets in the liver can lead to inflammation and non-alcoholic steatohepatitis (NASH) [3]. The final stages of NASH that can occur in the absence of treatment include cirrhosis, fibrosis, and hepatocellular cancer. As far as NAFLD and NASH treatment goes, we still lack a gold standard [4]. In order to treat metabolic abnormalities such hyperglycemia, insulin resistance, and hyperlipidemia, patients with non-alcoholic fatty liver disease (NAFLD) currently undergo dietary, lifestyle, and physical activity modifications [5]. Natural substances found in fruits and vegetables have been demonstrated to reduce the symptoms of non-alcoholic fatty liver disease (NAFLD) and the problems that go along with it [6,7]. Flavonoids have attracted increasing attention recently as a possible nutraceutical for treating or preventing NAFLD [8,9,10].

A more comprehensive term, non-alcoholic fatty liver disease (NAFLD), includes a range of liver-related disorders, such as cirrhosis, hepatocellular carcinoma, steatohepatitis, and simple steatosis [1,2]. When obese, diabetic women denied consuming alcohol, Ludwig et al. (1980) identified a particular form of liver damage in these women and coined the name NASH. Alcoholic hepatitis and this injury's histopathology were comparable. In the general population, the estimated prevalence of NAFLD and NASH are 10%–24% and 1%–5%, respectively. Body mass index (BMI) and the frequency and severity of non-alcoholic fatty liver disease (NAFLD) are directly correlated. When an adult is fat, the prevalence of NAFLD rises to 57.5% to 74% and 90% when the individual is very obese [5,4,6]. The general population in India has a prevalence of NAFLD ranging from 9% to 32% [7,11]. There has been a notable surge in research articles on non-alcoholic fatty liver disease (NAFLD) in recent years. More than two thirds of the papers with over 16,000 PubMed citations for the term "NAFLD" in March 2019 are from the previous five years [11]. NAFLD encompasses the full spectrum of diseases [12]. Simple steatosis without inflammation, hepatocyte destruction, or fibrosis is the definition of non-alcoholic fatty liver (NAFL). With or without fibrosis, NASH is characterized by inflammation and signs of hepatic destruction with ballooning. The development of fibrosis in cases with current or past steatosis is referred to as NAFLD-associated fibrosis and cirrhosis. Obesity and type 2 diabetes are unquestionably linked to cryptogenic cirrhosis, or cirrhosis without a known cause despite extensive research. As a result, in most cases, NAFLD is thought to be the underlying cause of cryptogenic cirrhosis [13]. The glycoside derivatives of almost all dietary flavonoids are found in nature. One type of flavonoid commonly found in many fruits, vegetables, and traditional medicine is kaempferol. Glycosides are primarily formed by the combination of glucose, rhamnose, galactose, and rutinose with kaempferol. Only a

few plant species possess the genetic material and enzymes required to produce glycosides [12,14]. Globally, soybeans, or *Glycine max (L.)* Merrill, are an essential source of protein and a staple of many diets [13,15]. There has been research on the physiological activities of soybeans, but minimal attention has been paid to the ingestion of soybean leaves, particularly those of unripe soybeans (Edamame, Glycine max (L.) Merrill). "Jindai" [16,17]. *Glycine max (L.)* leaves contain lots of kaempferol glycosides. Previous studies identified kaempferol glycosides in soybean leaves, showing antidiabetic and antiobesity effects in mice and also possesses hepatoprotective [18] and hypoglycemic activity [19]. Dietary kaempferol glycosides improve obesity, reduce adipose tissue and lipid levels, improve insulin resistance and leptin resistance [20,21]. In the current investigation, we looked at how soybean leaf-isolated kampeferol glycosides could protect rats with HFD-caused NAFLD.

2. MATERIALS AND METHODS

Collection and authentication: The fresh leaves of *Glycine max (L.)* were obtained in area without any pesticides and other contaminants from the region of Bangalore, Karnataka, India. The plant specimen was recognized and authenticated by Prof. Dr. Kuntal Das, Department of Pharmacognosy from KCP with the herbarium reference number-CP-07/KCP /2019-21.

Extraction: The present invention specifics a technique for extracting *G. max* leaves utilizing water, an organic solvent, or a combination of solvent. The extract is then condensed under decreased pressure to obtain dried out powder. The leaves can be cultured or purchased. The organic solvent may include alcohol, ethyl acetate, or acetonitrile, with C-C lower alcohol usually preferable. The solvent is added to the leaves 5-15 times their volume, with more preferably 10 times. Extraction is performed at room temperature and repeated 1-5 times. The concentration under lower pressure is performed using the traditional approach. The invention focuses on preparing fractions from *G. max* leaf extracts through a technique that comprises focusing the ethanol extracts under reduced pressure to obtain an aqueous solution. The fractions are then obtained by partitioning the concentrated suspension with an organic solvent, and the dry powder is formed by concentrating the fraction under reduced pressure. Dose selections were done based to the toxicity studies of OECD 425 test [22].

Experimental animals: Female Wistar rats weighing 150-200g [23] were procured from Krupanidhi College of Pharmacy, Bangalore, India. Animal housing with good ventilation was utilized to house and acclimate them. In accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), laboratory conditions were maintained for ten days prior to the experiment [24]. These included a controlled temperature of 25+40C and a relative humidity of 50-60%. The animals were also provided with food and water.

Acute oral toxicity studies: A stepwise technique using a minimum number of animals per stage was used to conduct acute oral toxicity experiments in accordance with OCED 425 standards. Each phase of the experiment involves three animals of the same sex, and the drug is given orally to the animals at prescribed doses. Three more animals are needed to be treated with the same dose or at different dose levels if there are no compound-related deaths [25].

Experimental design: The experimental animals were divided into following groups with six animals in each for high fat diet induced model Group 1 was administered with saline and was served as negative control whereas Group 2 was administered with high fat diet which acted as positive control [26]. Group 3 served as reference control received Metformin [27] (70mg/kg) + High fat diet. Group 4 and 5 received median dose and high dose of Kaempferol glycoside(300mg/kg) + High fat diet and Kaempferol glycoside(400mg/kg) + High fat diet respectively. For high fat diet + fructose induced model Group 1 was administered with saline. Group 2 was administered with high fat diet + fructose acted as positive control [26]. Group 3 served as reference control received Metformin (70mg/kg) + High fat diet+fructose. Group 4 received high dose of Kaempferol glycoside(400mg/kg) + High fat diet+ fructose. The rats were given with a high-fat diet every day for 8 to 16 weeks in place of their regular diet in order to induce non-alcoholic fatty liver disease (NAFLD). For high fat diet and fructose induced Rats to fed with High fat diet and fructose instead of normal diet every day for 8 to 16weeks to induce

NAFLD. 30% fructose water along with high fat diet. Drug treatment was followed for 90 days [28,27].

Evaluation parameters: Physical parameters like body weight, liver weight, liver index was evaluated after 90 days of the drug treatment [28,27].

Biochemical parameters like estimation of blood glucose level by colorimetric method [29]. Estimation of cholesterol, HDL, LDL by CHOD method. estimation of triglycerides [30]. estimation of ALT, AST by mod. IFCC method. estimation of ALP by pNPP kinetic method [29].

Estimation of superoxide dismutase: The measurement of nitrite, which was obtained by oxidizing hydroxylamine hydrochloride and measuring it calorimetrically at 560 nm, served as the basis for the calculation of SOD. At pH 10.2, it can also be produced when hydroxylamine auto-oxidizes. Measured is the reduction of Nitro Blue Tetrazolium (NBT) in a solution containing hydroxylamine hydrochloride, EDTA, and sodium carbonate. Nitro blue tetrazolium (NBT), sodium carbonate, phosphate buffer (0.25 M pH 7.4), 0.2 M sucrose, 5% tissue homogenate, and EDTA were all added to a test tube. A volume of 0.4 ml of hydroxylamine hydrochloride was used to start the reaction. After five minutes of incubation at 25°C, the reaction's NBT decrease was assessed. A parallel control received the same treatment. One SOD enzymatic unit is the amount of proteins in 100 µl of 5% tissue homogenate needed to prevent the 50% reduction of 24 mM NBT [30].

Estimation of Catalase activity: To measure the breakdown of 10 at 240 nm, an assay combination comprising 0.25 M of phosphate buffer having a pH of 7 was used. 100 µl of 5% tissue homogenate in 0.15 M KCl buffer was mixed with 1.9 ml of phosphate buffer (0.25 M, pH 7) and the absorbance at 240 nm was measured. After adding one milliliter of hydrogen peroxide solution to the reaction mixture mentioned above and letting it stand for a inute at 240 nm, the absorbance was measured using phosphate buffer as a blank solution. An international unit of catalase that is used is the quantity that, when expressed in units/mg of protein, catalyzes the breakdown of 1 mM hydrogen peroxide per minute at 37° C [30].

Histopathology: The liver of the animals were removed after they were subjected to sacrifice. Next, the liver was divided into 5 mm pieces and left to cure for at least three days in neutral formalin.

After 12-hour soak in running water, the liver parts were dehydrated with progressively stronger alcohol and then cleaned with xylene. The organ pieces were cleaned and then placed in an automated tissue processing device for paraffin infiltration. The liver chunks were placed into liquid paraffin and allowed to cool after the hard paraffin was melted and heated paraffin was poured into L-shaped blocks. Using microtomes, sections with a 5 mm thickness were cut and placed on micro slides. The sections are stained with eosin and hematoxylin; eosin is used to stain basic cell components and hematoxylin is used to stain acidic cell components. The sections undergo a series of procedures including deparaffinization, dehydration, water washing, hematoxylin staining, and rehydration in both absolute and 95% alcohol. After mounting the sections in DPX mountant, staining indicated that the cytoplasm and nucleus were blue in color, with variations in pink corresponding to alterations in distinct tissue components [31,32,33].

3. RESULTS

Effect of K.G extract, on body weight, Liver weight and liver index: The body weight, liver weight and liver index at different periods of various groups of animals during the study period are given in the below (Table 1), which shows the mean values of the study. HFD and fructose induced NAFLD increased the liver weight significantly in comparison with control whereas, K.G extract significantly prevented the increase in liver weight when compared to control and standard (Figs. 1 and 2).

Table 1. Acute oral toxicity study

Sl.No.	Treatment	Dose mg/kg body weight				Inference
		500	1000	2000	2500	
1.	Kaempferol glycosides extract	**0**	**0**	**0**	**X**	**Stop Dosing**

Table 2. Effect of KG extract on body weight

Groups	Body Weight		Liver Weight	Liver Index (%)
	Initial	Final		
Negative Control (Saline)	205±2.141	226±2.41	6.51±0.10	2.82
Positive Control	210±2.472	245±1.81	10.50±0.177	4.29
HFD+KGMDE(300mg/kg)	208±3.270	228±2.01**	8.02±0.241**	3.50
HFD+KGHDE(400mg/kg)	211±4.011	232±0.75***	7.22±0.118***	3.11
HFD + Fructose KGHDE(400mg/kg)	214±3.410	235±2.07**	7.77±0.094**	3.30
STD(Metformin)70mg/kg + HFD	209±2.141	228±1.91	7.51±0.110	3.29
STD(Metformin)70mg/kg + HFD + fructose	210±2.314	231±1.75	7.89±0.317	3.41

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract*

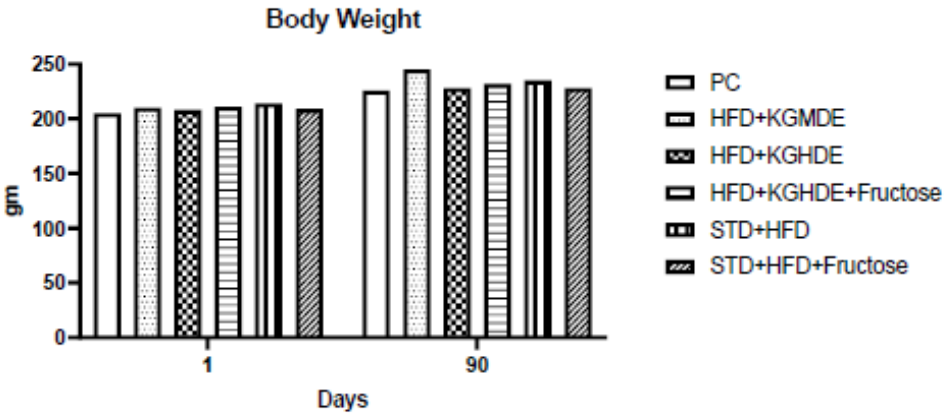


Fig. 1. Effect of KG extract on body weight

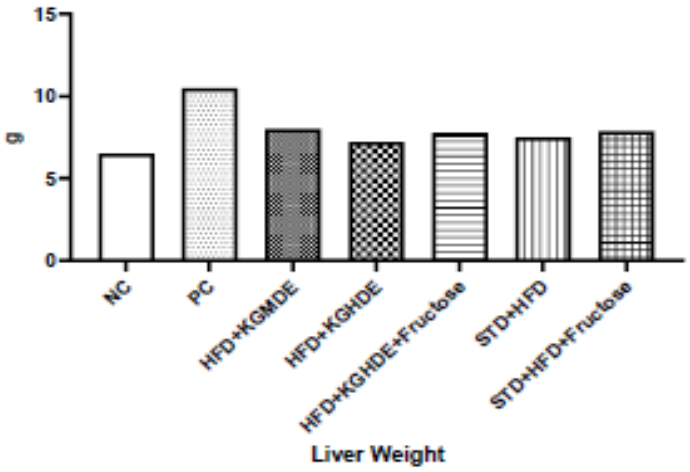


Fig. 2. Effect of KG extract on body weight

Table 3. Effect of KG extract on FBG in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	5.452±0.171	5.497±0.131	5.678±0.091	5.812 ± 0.161
Positive Control	5.312±0.122	6.219±0.102	6.961±0.152	7.521 ± 0.130
HFD+KGMDE(300mg/kg)	5.451±0.183	6.031±0.130	6.574±0.110	6.930 ± 0.101**
HFD+KGHDE(400mg/kg)	5.371±0.190	5.973±0.178	6.209±0.131	6.514 ± 0.091**
HFD + Fructose KGHDE(400mg/kg)	5.410±0.102	6.129±0.143	6.391±0.174**	6.811 ± 0.178**
STD (Metformin)70mg/kg + HFD	5.421±0.171	5.827±0.121	6.107±0.120	6.405±0.143**
STD (Metformin)70mg/kg + HFD + fructose	5.384±0.421	5.913±0.134	6.189±0.115	6.529±0.121**

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract*

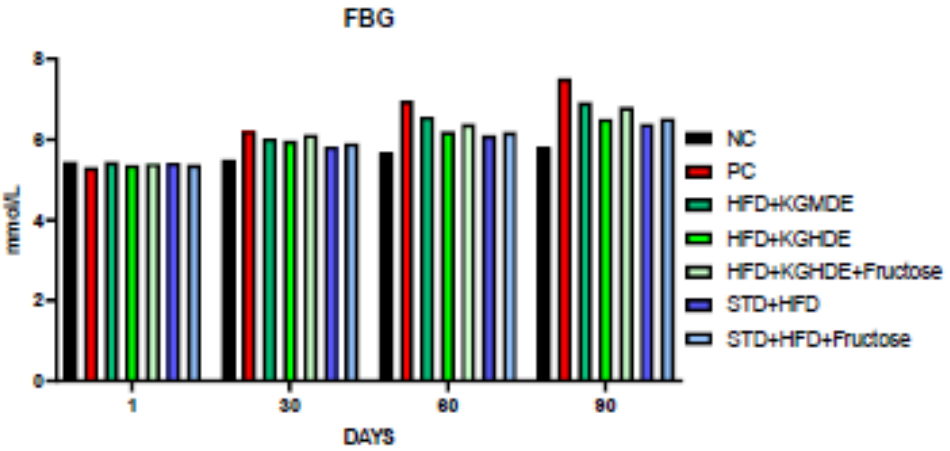


Fig. 3. Effect of KG extract on FBG in NAFLD rats

Table 4. Effect of KG extract on FINS in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	32.21±0.79	33.39±0.84	35.01±0.18	36.10±0.13
Positive Control	33.01±0.91	48.97±1.38	68.17±1.94	75.24±2.83
HFD+KGMDE(300mg/kg)	32.49±0.59	43.78±1.21	62.43±1.36	65.66±2.11**
HFD+KGHDE(400mg/kg)	33.08±0.67	40.38±1.40	59.89±1.29**	56.10±2.81***
HFD + Fructose KGHDE(400mg/kg)	32.71±0.84	41.56±1.12	61.01±1.31	61.29±1.48**
STD (Metformin)70mg/kg + HFD	32.46±0.69	38.96±1.47	57.34±1.52	52.27±1.54**
STD (Metformin)70mg/kg + HFD + fructose	32.51±0.75	39.57±1.83	58.98±1.01	57.32±1.27**

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract.*

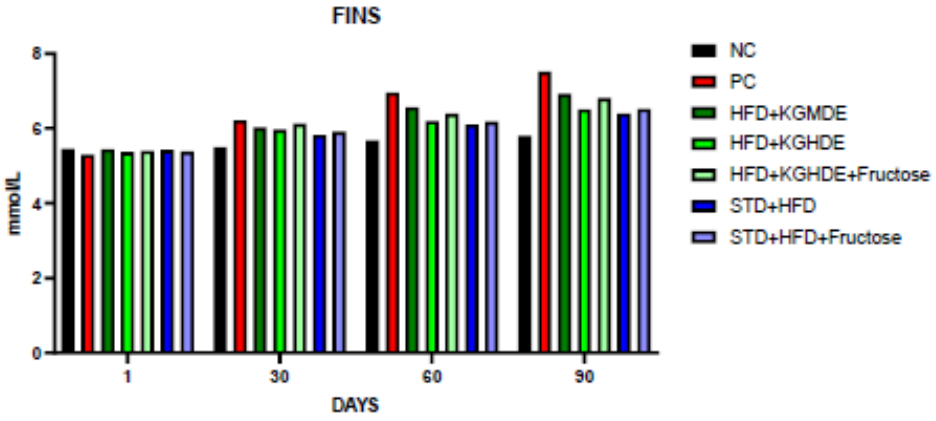


Fig. 4. Effect of *KG* extract on FINS in NAFLD rats

Table 5. Effect of KG extract on HOMA-IR in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	8.17±0.14	8.42±0.12	8.97±0.15	9.20±0.12
Positive Control	8.04±0.09ns	12.69±0.18	18.89±0.17	24.68±0.81
HFD+KGMDE(300mg/kg)	8.16±0.13	11.38±0.15	15.93±0.11	19.76±0.46**
HFD+KGHDE(400mg/kg)	8.23±0.12	10.25±0.19	14.27±0.24**	16.49±0.25***
HFD + Fructose KGHDE(400mg/kg)	8.11±0.11	10.97±0.24	15.14±0.18*	18.07±0.31**
STD (Metformin)70mg/kg + HFD	8.24±0.13	9.92±0.18*	13.87±0.27	15.26±0.84***
STD (Metformin)70mg/kg + HFD + fructose	8.15±0.12	10.19±0.15	14.03±0.26	17.21±0.57**

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract.*

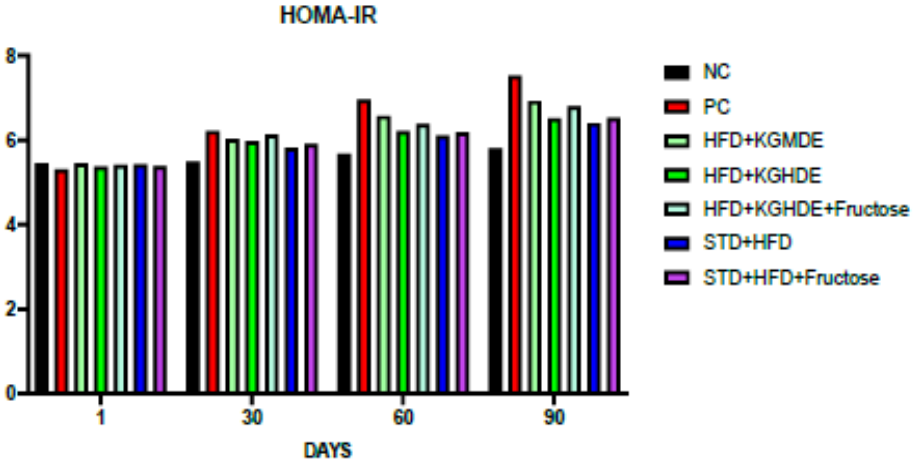


Fig. 5. Effect of KG extract on HOMA-IR in NAFLD rats

Table 6. Effect of KG extract on SOD in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	3.45±0.15	3.42±0.31	3.39±0.24	3.38±0.16
Positive Control	3.47±0.17	3.17±0.24	2.89±0.31	1.72±0.12
HFD+KGMDE(300mg/kg)	3.49±0.11	3.31±0.19	3.08±0.23*	2.47±0.20**
HFD+KGHDE(400mg/kg)	3.43±0.09ns	3.28±0.21	3.17±0.32	2.97±0.43***
HFD + Fructose KGHDE(400mg/kg)	3.42±0.12	3.30±0.14	3.11±0.18*	2.72±0.18
STD (Metformin)70mg/kg + HFD	3.44±0.17	3.29±0.21	3.14±0.21	2.85±0.33*
STD (Metformin)70mg/kg + HFD + fructose	3.41±0.15	3.31±0.17	3.12±0.25	2.61±0.46*

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract*

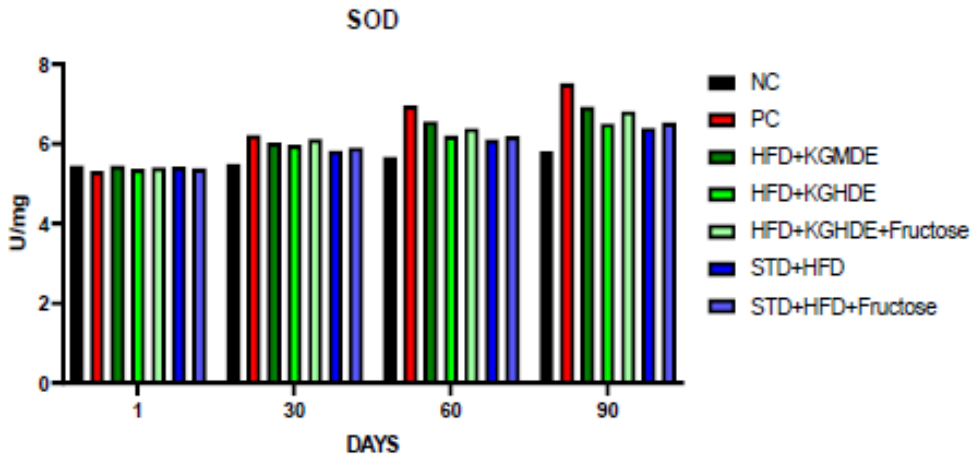


Fig. 6. Effect of KG extract on SOD in NAFLD rats

Table 7. Effect of KG extract on Catalase in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	20.86±0.82	20.37±0.9	19.84±1.10	19.20±0.90
Positive Control	21.06±0.91	17.04±1.10	13.17±0.92	8.92±0.93
HFD+KGMDE(300mg/kg)	19873±1.10	18.86±1.12	15.23±1.23	14.25±1.70**
HFD+KGHDE(400mg/kg)	20.82±1.13	19.47±0.98	16.79±1.15**	17.48±1.17***
HFD + Fructose KGHDE(400mg/kg)	21.91±1.24	17.31±1.14	14.81±1.06	15.17±1.04**
STD (Metformin)70mg/kg + HFD	20.65±1.08	18.92±0.98	16.33±118	16.91±1.18**
STD (Metformin)70mg/kg + HFD + fructose	21.02±1.02	18.37±1.05	15.41±115	14.69±0.97*

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract*

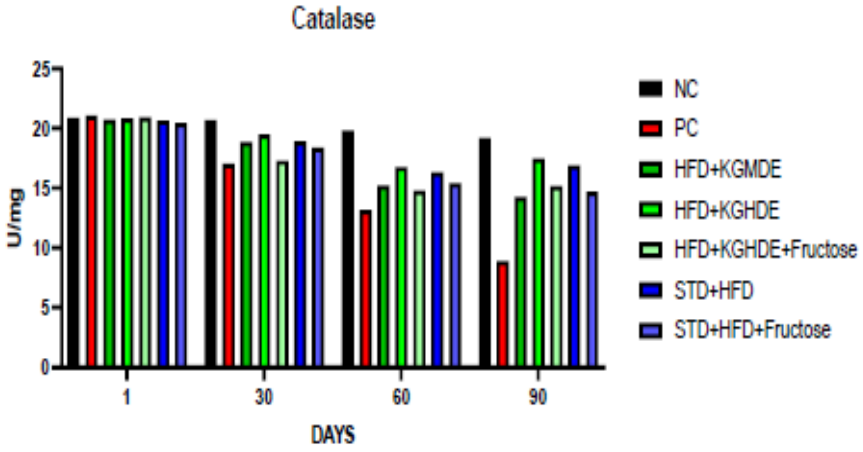


Fig. 7. Effect of KG extract on Catalase in NAFLD rats

Table 8. Effect of KG extract on TC in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	68.71±2.64	69.34±1.89	71.12±2.85	72.90±1.953
Positive Control	68.40±3.02	83.42±2.95	114.59±2.78	158.5 1± 1.65
HFD+KGMDE(300mg/kg)	69.13±2.45	75.78±2.35	96.37±1.87*	110.6 0± 2.41**
HFD+KGHDE(400mg/kg)	68.64±1.95	73.27±2.14	89.52±3.21**	102.60 ± 2.61***
HFD + Fructose KGHDE(400mg/kg)	67.82±3.34	74.98±2.31	93.63±2.81	112.41 ± 3.47**
STD (Metformin)70mg/kg + HFD	69.08±2.96	74.53±1.91*	91.42±2.01	101.52±2.19**
STD (Metformin)70mg/kg + HFD + fructose	68.52±2.56	75.24±2.21	92.72±2.35	110.53±2.87**

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract*

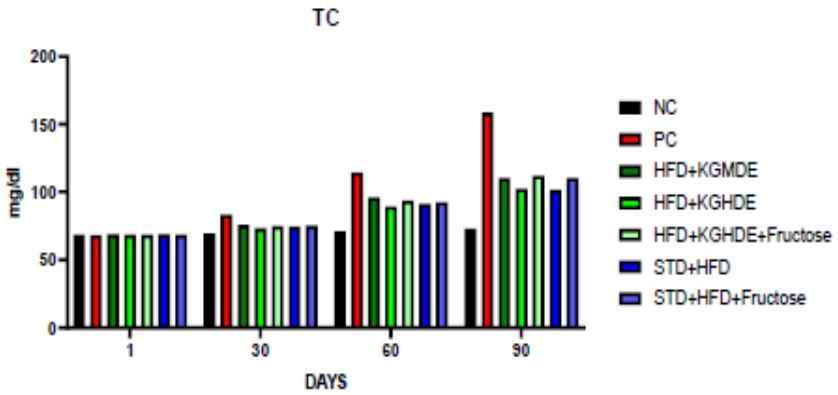


Fig. 8. Effect of KG extract on TC in NAFLD rats

Table 9. Effect of KG extract on TG in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	71.15±0.13	73.45±1.12	78.74±1.56	81.14±2.85
Positive Control	70.89±0.89	91.32±1.67	122.51±2.41	198.6± 3.92
HFD+KGMDE(300mg/kg)	71.24±0.98	84.58±2.12	98.31±1.87**	123.6 ±4.81***
HFD+KGHDE(400mg/kg)	70.27±0.67	81.82±2.35	92.57±2.31**	114.3 ±3.46***
HFD + Fructose KGHDE(400mg/kg)	71.31±0.74	83.45±1.75	94.23±2.28	118.1 ±4.35**
STD (Metformin)70mg/kg + HFD	69.97±0.85	88.32±2.14	99.52±2.19	113.1±3.85**
STD (Metformin)70mg/kg + HFD + fructose	70.17±0.82	89.49±2.29	101.73±2.25	116.2±3.96*

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract*

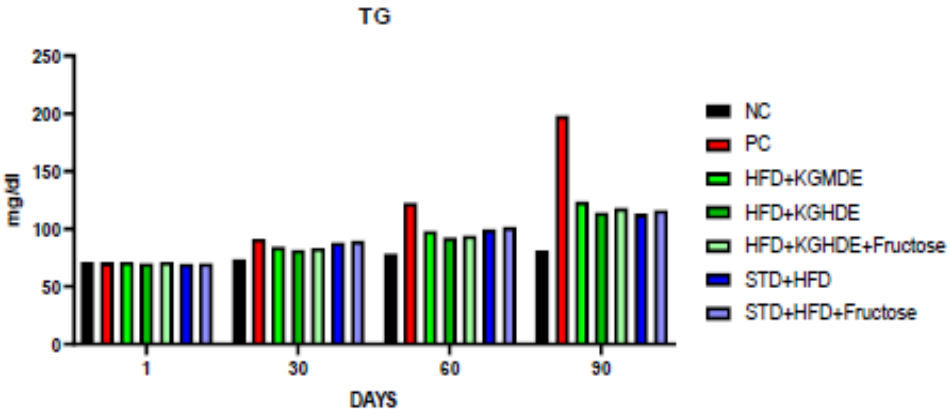


Fig. 9. Effect of KG extract on TG in NAFLD rats

Table 10. Effect of KG extract on HDL-C in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	36.21±0.12	36.88±1.86	37.56±1.76	38.8±1.182
Positive Control	35.89±0.52	29.15±1.54	22.74±2.12	18.23±0.64
HFD+KGMDE(300mg/kg)	35.92±0.97	27.24±1.94	24.14±2.16**	27.09±1.45**
HFD+KGHDE(400mg/kg)	36.13±0.71	29.38±2.17	26.35±1.94**	30.34±1.37***
HFD + Fructose KGHDE(400mg/kg)	36.18±0.84	28.85±2.27	24.82±2.21	28.96±1.17**
STD (Metformin)70mg/kg + HFD	35.97±0.95	28.32±2.10ns	24.34±2.29	31.34±1.25**
STD (Metformin)70mg/kg + HFD + fructose	35.49±0.93	28.49±2.15	23.57±2.41	29.41±1.80**

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract*

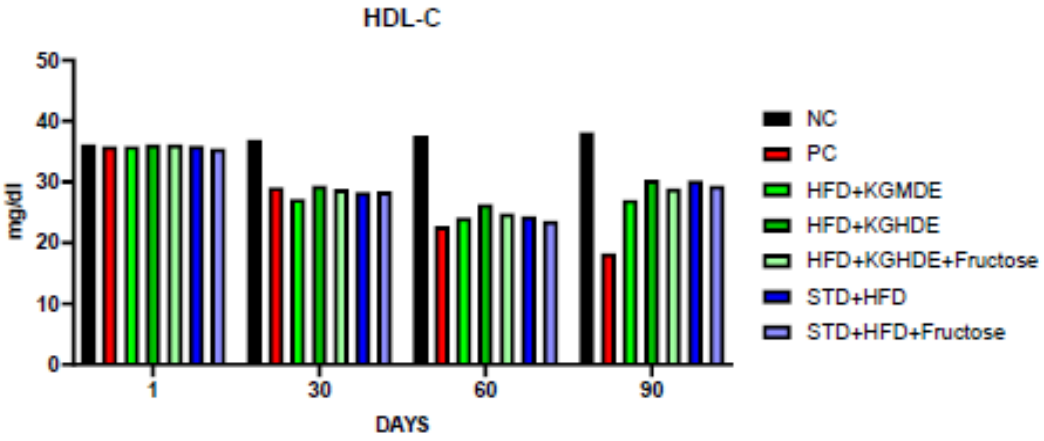


Fig. 10. Effect of KG extract on HDL-C in NAFLD rats

Table 11. Effect of KG extract on LDL-C in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	18.01±0.61	18.37±0.71	19.98±1.21	21.02±0.701
Positive Control	18.12±0.85	65.23±0.69	79.12±1.72	95.57 ± 2.23
HFD+KGMDE(300mg/kg)	17.96±0.93	29.43±1.02	35.88±0.94	42.78 ± 1.35**
HFD+KGHDE(400mg/kg)	18.13±0.79	26.17±1.13*	31.21±1.57**	35.79 ± 1.87***
HFD + Fructose KGHDE(400mg/kg)	18.08±1.07	27.72±1.27	33.23±1.61*	39.85 ± 1.08**
STD (Metformin)70mg/kg + HFD	17.78±1.39	31.23±1.54	37.42±2.06*	43.18±1.68**
STD (Metformin)70mg/kg + HFD + fructose	17.82±0.91	32.01±1.83	38.13±1.86	44.01±1.86*

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract*

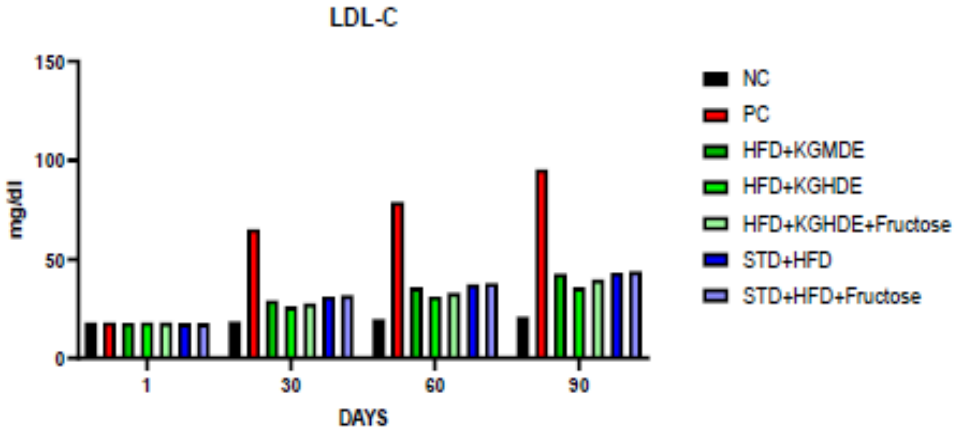


Fig. 11. Effect of KG extract on LDL-C in NAFLD rats

Table 12. Effect of KG extract on ALT in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	20.98±0.91	21.35±1.21	22.72±1.57	24.19±1.27
Positive Control	20.15±1.05	29.81±2.10	53.72±2.21	68.39±2.89
HFD+KGMDE(300mg/kg)	20.53±0.95	27.63±1.85	44.37±2.32*	51.15±2.25**
HFD+KGHDE(400mg/kg)	19.89±1.26	25.42±1.49	36.73±1.76	45.97±1.96***
HFD + Fructose KGHDE(400mg/kg))	21.12±0.87	26.79±1.37	38.29±2.34*	48.16±1.45**
STD (Metformin)70mg/kg + HFD	20.63±1.14	27.24±2.40	36.69±1.53*	47.23±1.87
STD (Metformin)70mg/kg + HFD + fructose	21.21±1.38	28.79±2.64	37.73±2.87	45.25±2.92*

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract*

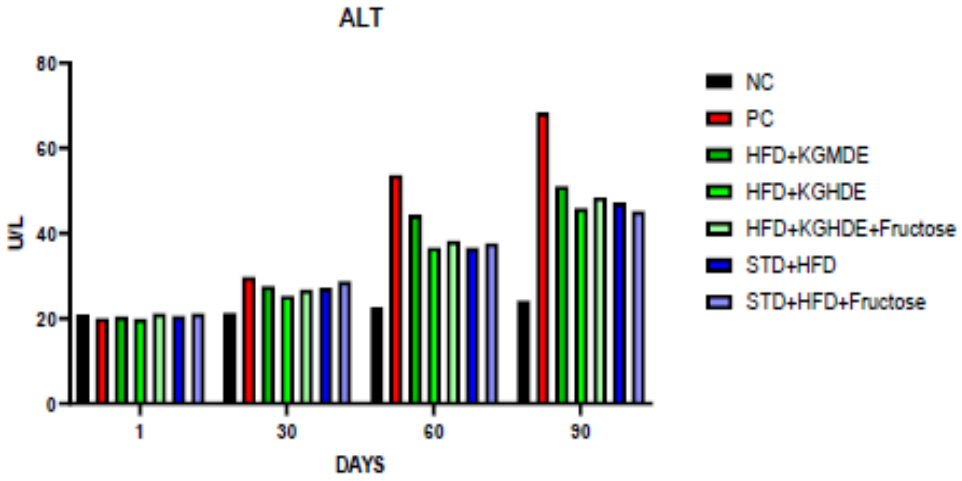


Fig. 12. Effect of KG extract on ALT in NAFLD rats

Table 13. Effect of KG extract on AST in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	40.78±1.26	41.56±1.54	43.31±1.87	45.73±2.64
Positive Control	39.24±1.54	55.63±1.66	63.33±2.24	93.68± 3.21
HFD+KGMDE(300mg/kg)	41.12±1.37	51.21±1.68	56.35±1.89*	65.51± 2.27**
HFD+KGHDE(400mg/kg)	40.31±0.95	49.35±1.37	52.16±1.63**	59.79±2.12***
HFD + Fructose KGHDE(400mg/kg)	40.58±1.28	50.18±1.35	54.93±1.84*	62.06±2.17**
STD (Metformin)70mg/kg + HFD	39.73±1.11	52.37±1.69	59.31±2.24	58.95±2.65*
STD (Metformin)70mg/kg + HFD + fructose	41.20±0.93	53.12±1.47	59.58±1.76*	60.16±2.23*

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract*

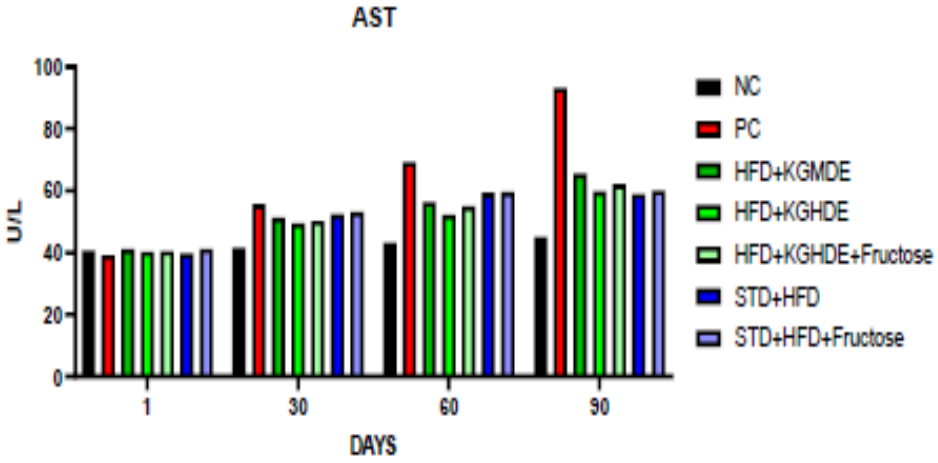


Fig. 13. Effect of KG extract on AST in NAFLD rats

Table 14. Effect of KG extract on ALP in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	74.52±0.86	75.97±0.94	77.31±1.06	78.75±1.21
Positive Control	74.81±1.16	87.83±1.31	94.45±1.87	124.21±2.46
HFD+KGMDE(300mg/kg)	73.98±0.95	82.94±1.15	86.73±1.59*	95.24±1.85**
HFD+KGHDE(400mg/kg)	74.53±1.26	79.25±0.98	82.14±1.67**	88.01±2.51***
HFD + Fructose KGHDE(400mg/kg)	75.11±0.87	81.23±1.21	84.35±1.49	93.51±1.57**
STD (Metformin)70mg/kg + HFD	74.63±0.67	82.45±1.68	87.59±1.98*	86.23±2.14**
STD (Metformin)70mg/kg + HFD + fructose	74.39±1.18	82.98±0.76ns	88.34±1.37	90.12±1.65*

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract*

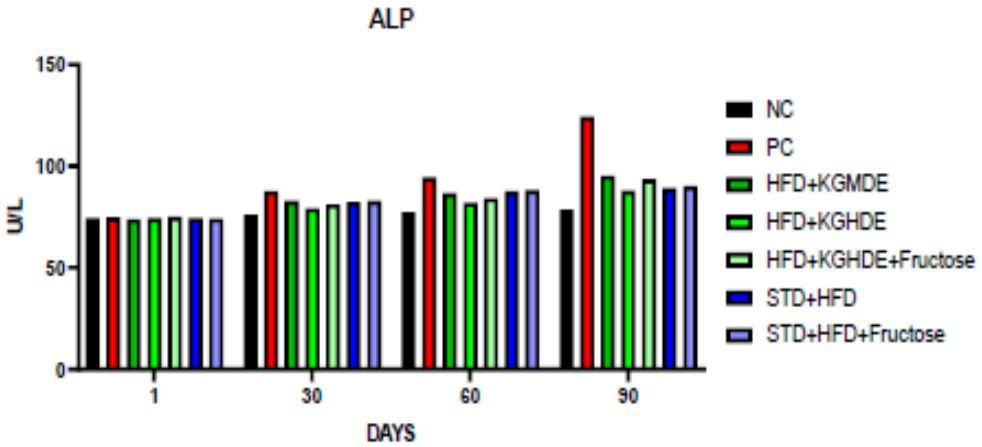


Fig. 14. Effect of KG extract on ALP in NAFLD rats

Table 15. Effect of KG extract on MDA in NAFLD rats

Groups	Day 1	Day 30	Day 60	Day 90
Negative Control (Saline)	3.11±0.14	3.10±0.09	3.22±0.27	3.417±0.36
Positive Control	3.13±0.51	5.64±0.89	6.43±0.64	9.529±0.91
HFD+KGMDE(300mg/kg)	3.09±0.67	4.92±0.77	5.21±0.094	5.91±1.01**
HFD+KGHDE(400mg/kg)	2.97±0.08ns	3.89±0.24	4.53±0.75**	4.91±0.813***
HFD + Fructose KGHDE(400mg/kg)	3.06±0.15	4.43±0.26	5.10±0.51*	5.45±0.69**
STD(Metformin)70mg/kg + HFD	2.98±0.29	4.81±0.16	5.41±0.26*	6.01±0.44
STD(Metformin)70mg/kg + HFD + fructose	3.19±0.19	4.97±0.26	5.58±0.36.	5.89±0.53*

*The notation for P-Values is (Mean ± SEM). n = 6. statistically examined using Dunnett's test after a one-way analysis of variance. A Positive Control Group was used to compare each group. non-significant, or ns-. **significant when compared to the Positive Control Group at P<0.05. NC stands for Normal Control. Positive Control (PC) and High Fat Diet (HFD) STD: Standard, KGHDE: Kaempferol Glycosides High Dose Extract, and KGMDE: Medium Dose Extract*

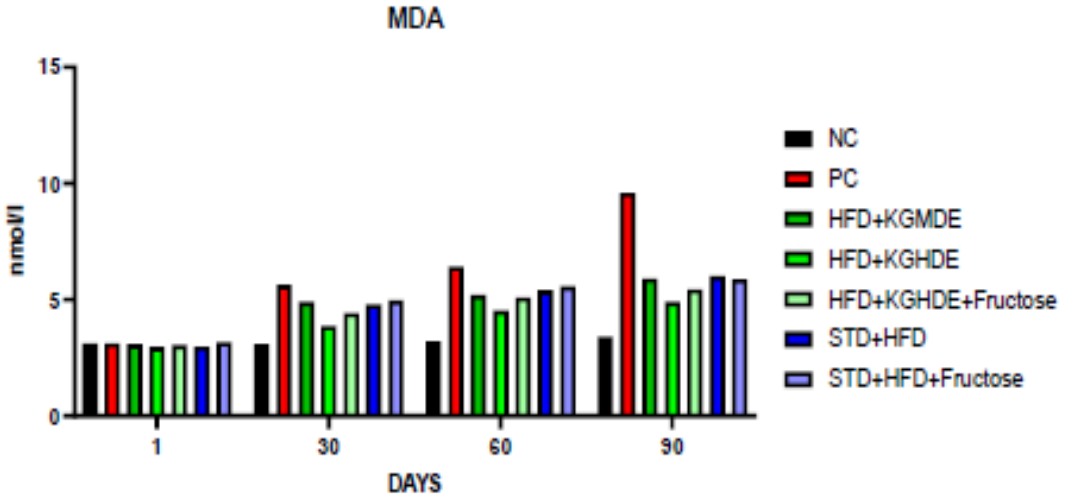
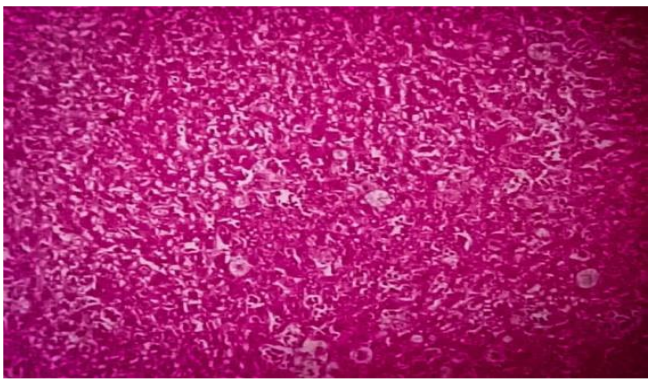
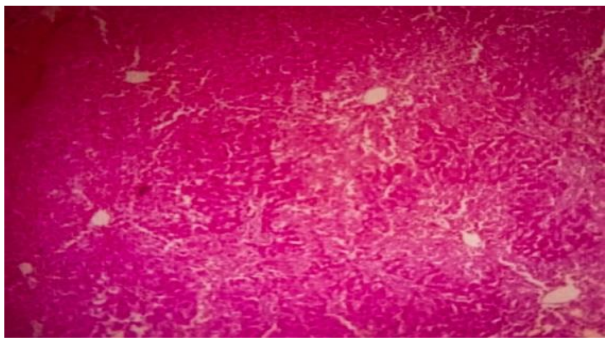


Fig. 15. Effect of KG extract on MDA in NAFLD rats



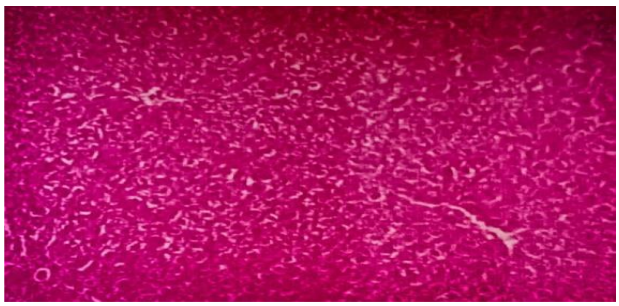
Liver cells arranged regularly
Liver lobules distinct

Fig. 16. Normal Control



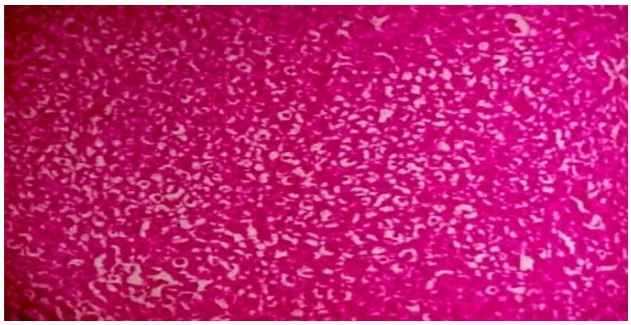
Moderate Lobular inflammation
Macrovesicular steatosis
Ballooning and cloudy swelling of hepatocytes

Fig. 17. Positive Control



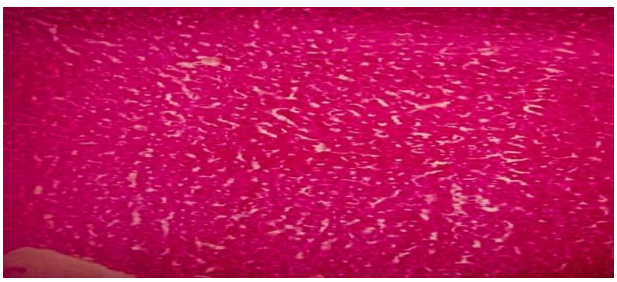
Mild lobular inflammation
No macrovesicular steatosis

Fig. 18. KGMDE



No lobular inflammation
No macrovesicular steatosis
No hepatocyte ballooning or swelling

Fig. 19. KGHDE



Sign of mild lobular inflammation
No hepatocyte ballooning or swelling

Fig. 20. STD+KGHDE

Figs. 16-20. Histopathological changes of liver tissue stained with H&E and oil red O staining (16-19) Representative image of liver tissue of normal control animal (ND) stained with Oil red O. Representative images of the liver tissues of animals treated with (16) Normal Control (17) Positive Control,(18) KGMDE,(19)KGHDE,(20) STD+KGHDE.Pathophysiological examination of the tissue sections was performed under a light microscopy at 200X (H&E) magnification

4. DISCUSSION

Non-alcoholic fatty liver disease (NAFLD) is a common liver condition that has been linked with insulin resistance and type 2 diabetes [34,35]. It is rising as the number of overweight and obese people increases. Insulin resistance-related increases in free fatty acids and decreased lipid oxidation in the liver are probably linked to the development of NAFLD [36,37]. The development of non-alcoholic fatty liver disease (NAFLD) can be significantly influenced by dietary fat, as excess dietary fat can cause hepatic steatosis, which in turn can lead to insulin resistance [38]. Rat models for NAFLD research that are well-established include the High Fat Diet (HFD) model. Overconsumption of fructose, a monosaccharide that is mostly metabolized by the liver, has been linked to the onset and aggravation of non-alcoholic fatty liver disease (NAFLD) by elevating oxidative stress, fat deposition, insulin resistance, inflammation, and maybe fibrosis [39,40,41]. The potential of a kaempferol glycoside derived from soybean leaves to avert fructose-induced NAFLD in rats was investigated and the markers for fatty liver, serum and lipid levels, insulin resistance were determined. The study investigated the impact of kaempferol glycosides extract on the body weight, liver weight, and liver index of rats over a period of ninety days (Table 2). Comparing the KG extract-treated group to the normal and positive control group, (Fig. 2) the results demonstrated that the extract significantly reduced liver weight and liver index of KG extract-treated group over positive control group. Additionally, the extract lowered the levels of HOMA-IR, FINS, and FBG in the treatment group of NAFLD rats as compared to positive control group (Figs. 3,4,5). Due to insulin's inability to inhibit hepatic gluconeogenesis and glycogenolysis, which cause fasting hyperglycemia and persistent stimulation of pancreatic B-cells' production of insulin, the glucose and insulin levels in the NAFLD rat group may have increased. The TC levels were lower in the normal group when the KG extract was administered at both the median and high doses, compared to the HFD and fructose treated groups. Similar to the HFD and fructose therapy groups, the normal group had higher HDL values (Table 10). Comparing the dietary kaempferol glycoside (KG) group to the high-fat diet (HFD) group, the study showed a significant reduction in triglyceride levels and total cholesterol in both the liver and serum(Tables 8 and 9). There was an improvement in cholesterol accumulation in the KG group as seen by higher HDL-C and lower LDL-C values (Tables 10 and 11) (Figs. 10 and 11). Hepatic indicators in the serum and liver were analyzed. The results show that serum ALT, AST, ALP activities, superoxide dismutase, catalase, and MDA activities were normalized in rats treated with KGE which improved the liver function (Figs. 6,7,12,13,14,15). The KG extract at middle dosage (300 mg/kg) and high dose (400 mg/kg) groups normalized these markers, while hepatic indicators showed better results in the fructose and HFD treated groups. Because KG has anti-hyperlipidaemic, anti-obesity, and antioxidant properties, it has also been shown to improve liver functioning levels, suggesting that it may be used to treat liver disease. The study suggests that unripe soy leaves could be utilized as functional food ingredients to treat non-alcoholic fatty liver disease (NAFLD) by reducing liver weight gain, blood glucose level, and showing antihyperlipidaemic and antiobese activity in different doses.

5. CONCLUSION

The leaves of *G.max* contain flavonoids, glycosides, terpenoids, alkaloids, saponins, tannins and reducing sugars. The study was set up to assess KG extract of leaves the plant G.max for protection of NAFLD. Treatment with KG extract has restored back the normal levels of AST, ALP, ALT, MDH in the serum of HFD and fructose induced rodents. Furthermore treatment with KG extract exhibited dosage dependent influence in MDA, SOD and Catalase levels in the liver tissue of rats. Histopathological evaluation demonstrated the treatment with KG extract has reversed the liver injury. The experiment done indicates that high dose and median dose of Kaempferol glycosides extract have appreciable hepatoprotective action for HFD and Fructose caused NAFLD in rats.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

The study protocol for the experiment designated with the number KCP/IAEC/PCOL/ 44/2020 was approved by the Institutional Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Falck-Ytter Y, Younossi ZM, Marchesini G, McCullough AJ. Clinical features and natural history of nonalcoholic steatosis syndromes. InSeminars in liver disease 2001;21(01):017-026.
2. Shimada M, Hashimoto E, Taniai M, Hasegawa K, Okuda H, Hayashi N, Takasaki K, Ludwig J. Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. Journal of hepatology. 2002 Jul 1;37(1):154-60
3. Ludwig J, Viggiano TR, Mcgill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. InMayo Clinic Proceedings 1980 Jul 1;55(7):434-38.
4. Tuong TT, Tran DK, Phu PQ, Hong TN, Chu Dinh T, Chu DT. Non-alcoholic fatty liver disease in patients with type 2 diabetes: Evaluation of hepatic fibrosis and steatosis using fibroscan. Diagnostics. 2020 Mar 14;10(3):159.
5. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: Summary of an AASLD Single Topic Conference. Hepatology. 2003 May 1;37(5): 1202-19.
6. Angulo P. Nonalcoholic fatty liver disease. New England Journal of Medicine. 2002 Apr 18;346(16):1221-31.
7. Gyr KE. Tropical gastroenterology. Baillière's clinical gastroenterology. 1987;1(2):211-471.
8. Marcellin P, Kutala BK. Liver diseases: A major, neglected global public health problem requiring urgent actions and large-scale screening. Liver International. 2018 Feb;38:2-6.
9. Khlifi R, Dhaouefi Z, Toumia IB, Lahmar A, Sioud F, Bouhajeb R, Bellalah A, Chekir-Ghedira L. Erica multiflora extract rich in quercetin-3-O-glucoside and kaempferol-3-O-glucoside alleviates high fat and fructose diet-induced fatty liver disease by modulating metabolic and inflammatory pathways in Wistar rats. The Journal of Nutritional Biochemistry. 2020 Dec 1;86:108490.
10. Yu C, Guo X, Cui X, Su G, Wang H. Functional food chemical ingredient strategies for non-alcoholic fatty liver disease (NAFLD) and hepatic fibrosis: Chemical properties, health benefits, action, and application. Current Nutrition Reports. 2024 Jan 3:1-4.
11. Singh SP, Nayak S, Swain M, Rout N, Mallik RN, Agrawal O, Meher C, Rao MV. Prevalence of nonalcoholic fatty liver disease in coastal eastern India: A preliminary ultrasonographic survey. Tropical gastroenterology: Official Journal of the Digestive Diseases Foundation. 2004 Apr 1;25(2):76-9.
12. Hashimoto E, Tokushige K, Ludwig J. Diagnosis and classification of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis: Current concepts and remaining challenges. Hepatology Research. 2015 Jan;45(1):20-28.
13. Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: Clinical characterization and risk factors for underlying disease. Hepatology. 1999 Mar;29(3):664-69.
14. M Calderon-Montano J, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M. A review on the dietary flavonoid kaempferol. Mini reviews in medicinal chemistry. 2011 Apr 1;11(4):298-344.
15. Zang Y, Zhang L, Igarashi K, Yu C. The anti-obesity and anti-diabetic effects of kaempferol glycosides from unripe soybean leaves in high-fat-diet mice. Food & function. 2015;6(3):834-41.
16. Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology. 2006 Oct;44(4):865-73.
17. Choi MS, Ryu R, Seo YR, Jeong TS, Shin DH, Park YB, Kim SR, Jung UJ. The beneficial effect of soybean (*Glycine max* (L.) Merr.) leaf extracts in adults with prediabetes: A randomized placebo controlled trial. Food & function. 2014;5(7):1621-30.
18. Zang Y, Zhang L, Igarashi K, Yu C. The anti-obesity and anti-diabetic effects of kaempferol glycosides from unripe soybean leaves in high-fat-diet mice. Food & function. 2015;6(3):834-41.
19. Zang Y, Sato H, Igarashi K. Anti-diabetic effects of a kaempferol glycoside-rich fraction from unripe soybean (edamame,

Glycine max L. Merrill.'Jindai') leaves on KK-Ay mice. Bioscience, biotechnology, and biochemistry. 2011 Sep 23;75(9):1677-84.

20. M Calderon-Montano J, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M. A review on the dietary flavonoid kaempferol. Mini Reviews in Medicinal Chemistry. 2011 Apr 1;11(4):298-344.
21. Zang Y, Igarashi K, Yu C. Anti-obese and anti-diabetic effects of a mixture of daidzin and glycitin on C57BL/6J mice fed with a high-fat diet. Bioscience, Biotechnology, and Biochemistry. 2015 Jan 2;79(1):117-23.
22. Cid-Ortega S, Monroy-Rivera JA. Extraction of kaempferol and its glycosides using supercritical fluids from plant sources: A review. Food technology and biotechnology. 2018 Dec 31;56(4):480-93.
23. Kucera O, Cervinkova Z. Experimental models of non-alcoholic fatty liver disease in rats. World journal of gastroenterology: WJG. 2014 Jul 7;20(26):8364.
24. Pereira S, Veeraraghavan P, Ghosh S, Gandhi M. Animal experimentation and ethics in India: the CPCSEA makes a difference. Alternatives to laboratory animals. 2004 Jan;32(1_suppl):411-15.
25. Saleem U, Amin S, Ahmad B, Azeem H, Anwar F, Mary S. Acute oral toxicity evaluation of aqueous ethanolic extract of *Saccharum munja* Roxb. roots in albino mice as per OECD 425 TG. Toxicology reports. 2017 Jan 1;4:580-85.
26. Xu P, Zhang XG, Li YM, Yu CH, Xu L, Xu GY. Research on the protection effect of pioglitazone for non-alcoholic fatty liver disease (NAFLD) in rats. Journal of Zhejiang University Science B. 2006 Aug;7:627-33.
27. Asadollahpoor A, Abdollahi M, Rahimi R. Pimpinella anisum L. fruit: Chemical composition and effect on rat model of nonalcoholic fatty liver disease. Journal of Research in Medical Sciences. 2017 Jan 1;22(1):37.
28. Gamede M, Mabuza L, Ngubane P, Khathi A. Plant-derived oleanolic acid ameliorates markers associated with non-alcoholic fatty liver disease in a diet-induced pre-diabetes rat model. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2019 Oct 1:1953-62.
29. Fraulob JC, Ogg-Diamantino R, Fernandes-Santos C, Aguila MB, Mandarim-de-Lacerda CA. A mouse model of metabolic syndrome: Insulin resistance, fatty liver and non-alcoholic fatty pancreas disease (NAFPD) in C57BL/6 mice fed a high fat diet. Journal of clinical biochemistry and nutrition. 2010;46(3):212-23.
30. Azmi NU, Purba AV, Fitriani A, Apriliani AA, Saputri FC. Effect of pioglitazone tablet administration on liver functions, lipid profile, and antioxidant parameters associated with fatty liver in male sprague-dawley rats. Pharmaceutical Sciences Asia. 2020 Oct 1;47(4):340-6.
31. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005 Jun;41(6): 1313-21.
32. Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA, NASH Clinical Research Network (CRN). Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: Distinct clinicopathologic meanings. Hepatology. 2011 Mar;53(3): 810-20.
33. Lomonaco R, Ortiz-Lopez C, Orsak B, Webb A, Hardies J, Darland C, Finch J, Gastaldelli A, Harrison S, Tio F, Cusi K. Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. Hepatology. 2012 May;55(5):1389-97.
34. Musso G, Gambino R, De Michieli F, Cassader M, Rizzetto M, Durazzo M, Fagà E, Silli B, Pagano G. Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. Hepatology. 2003 Apr 1;37(4):909-16.
35. Targher G, Chonchol M, Pichiri I, Zoppini G. Risk of cardiovascular disease and chronic kidney disease in diabetic patients with non-alcoholic fatty liver disease. Journal of endocrinological investigation. 2011 Jul;34:544-51.
36. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. The American Journal of Medicine. 1999 Nov 1;107(5):450-5.

37. Choi SS, Diehl AM. Hepatic triglyceride synthesis and nonalcoholic fatty liver disease. Current Opinion in Lipidology. 2008 Jun 1;19(3):295-300.
38. Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, Bhanot S, Monia BP, Li YX, Diehl AM. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology. 2007 Jun;45(6):1366-74.
39. Lomonaco R, Ortiz-Lopez C, Orsak B, Webb A, Hardies J, Darland C, Finch J, Gastaldelli A, Harrison S, Tio F, Cusi K. Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. Hepatology. 2012 May;55(5):1389-97.
40. Softic S, Gupta MK, Wang GX, Fujisaka S, O'Neill BT, Rao TN, Willoughby J, Harbison C, Fitzgerald K, Ilkayeva O, Newgard CB. Divergent effects of glucose and fructose on hepatic lipogenesis and insulin signaling. The Journal of clinical investigation. 2017 Dec 19;127(11):4059-74.
41. Wada T, Kenmochi H, Miyashita Y, Sasaki M, Ojima M, Sasahara M, Koya D, Tsuneki H, Sasaoka T. Spironolactone improves glucose and lipid metabolism by ameliorating hepatic steatosis and inflammation and suppressing enhanced gluconeogenesis induced by high-fat and high-fructose diet. Endocrinology. 2010 May 1;151(5):2040-49.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3743