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TOXICOLOGICAL IMPLICATIONS OF COMBINED CONSUMPTION OF HIGH FAT DIET AND ALCOHOL IN RATS

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ABSTRACT

The aim of the present study was to study the combined toxic effect of high fat diet and alcohol consumption on serological and oxidative stress markers in rats. Rats in control group received regular diet and water for 8 weeks, toxicant group were administered with 30% HFD and 10% alcohol for 2, 4, 6 and 8 weeks respectively.Combined consumption of high fat diet along with alcohol induced hepato-renal toxicity as evident by elevation of serum transaminases, alkaline phosphatase, bilirubin, cholesterol and triglycerides with significant decrease in glucose and albumin. Increased urea and uric acid was also seen in serum. Boosted lipid peroxidation and tissue biochemical parameters and decline in glutathione contents confirmed oxidative stress induced hepatorenal toxicity. Present study showed that 2, 4, 6 and 8 weeks administration of high fat diet along with alcohol significantly disturbed physiological integrity of liver and kidney.

Figure : 01 References : 24 Tables : 02

KEY WORDS : Genotypes, Green gram, Jassid, Screening, Thrips, Whitefly

Introduction

Consumption of high fat diet (HFD) alongwith alcohol is a common societal practice around the world. Increase intake of high fat diet (HFD) causes obesity, a prompting factor to nonalcoholic fatty liver disease (NAFLD), it is also one of the most common chronic liver diseases throughout the world[5]. NAFLD pathological condition characterized by the accumulation of triglycerides (TG) in hepatocytes in the absence of alcohol abuse. It can progress to nonalcoholic steatohepatitis (NASH) and in certain cases to fibrosis and cirrhosis[17]. High fat diet leads to hypertension, hyperlipidemia, diabetes and cardiovascular disease[7]. Alcohol intake also causes range of hepatic disorders starting from simple steatosis (fatty liver) to cirrhosis/liver failure[14]. Alcohol consumption increases the ratio of NADH/NAD in hepatocytes which causes disruption of b-oxidation of fatty acids in mitochondria leading to steatosis. Alcohol also rises the lipid transport to the liver from the small intestine leading to enhanced mobilization of fatty acids from adipose tissue which is taken up by the liver[3]. This causes impairment to cell membrane of hepatocytes leading to augmented levels of transaminases (Alanine aminotransferase (ALT) and AST (Aspartate aminotransferase) in blood stream. Alkaline phosphatase (ALP) is also present in hepatocytes which comes into the circulation representative hepatic damage. Glutathione (GSH) is a powerful antioxidant in our body which avoids damage by oxidative stress which is lowered by alcohol. Alcohol intake causes accumulation of oxidative stress markers (Glutathione reductase (GR) and malondialdehyde (MDA) which causes additional damage to liver[19]. In the present investigation, we aimed to study combined toxic effect of high fat diet and alcohol consumption on serological and oxidative stress markers at different duration in rats.

Materials and Methods

Animals and chemical

Female albino Wistar rats (160±10 g) were obtained

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TABLE -1: Effect of combined consumption of HFD and Alcohol on liver function test

	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)	Albumin (mg/dL)
Group 1	39.2±3.58	65.3±4.61	251±18.77	0.26±0.02	4.54±0.29
Group 2	84.3±6.62**	80.2±6.75	521±34.76**	0.52±0.04**	4.50±0.31
Group 3	122±8.31**	90.1±6.60*	706±41.99**	0.83±0.06**	3.66±0.23*
Group 4	128±10.00**	98.1±6.32**	860±55.67**	1.06±0.08**	3.41±0.20*
Group 5	132±10.14**	103±7.22**	930±57.33**	1.39±0.09**	3.12±0.20**
ANOVA	28.4ω	6.70ω	49.6ω	59.5ω	5.04ω

Data are expressed as mean ± SE; n=6; Significant P value Toxicants vs control * Pd"0.05 and ** Pd"0.01. For students test significant ANOVA at Pd"0.05 ù.

Abbreviations: Group 1= Control; Group 2= High Fat Diet + Alcohol (2 weeks); Group 3= High Fat Diet + Alcohol (4 weeks); Group 4= High Fat Diet + Alcohol (6 weeks); Group 5= High Fat Diet + Alcohol + (8 weeks). AST= Aspartate Transaminases; ALT= Aspartate Transaminases; ALP= Alkaline Phosphatase.

from DRDE, Gwalior and housed in animal facility under standard husbandry conditions (25±2°C temp., 60-70% relative humidity and 12 h photoperiod) with standard rat feed (Pranav Agro Industries, India) and water *ad libitum*. Experiments were conducted in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and experimental protocols were approved by the institutional animal ethics committee (994/ERe/GO/06/CPCSEA). HFD (30%) was procured from VRK Nutritional Solutions, Pune (India).

Experimental design

Thirty animals were divided into five groups of six each as follows: Group 1: received regular diet and water for 8 weeks. Group 2-5: received 30% HFD and 10% alcohol for 2, 4, 6 and 8 weeks respectively. Animals of all the groups were euthanized; blood was collected, serum was isolated to assess various biochemical variables. Tissue samples from liver and kidney were immediately processed for the biochemical analysis.

Isolation of serum and homogenate preparation

After keeping the blood for 1 h at room temperature, serum was isolated by centrifugation at 1000×*g* for 15 min and stored at "20ÚC until analyzed. Tissue samples of liver and kidney were homogenized with ice cold 150 mM KCl solution for determination of TBARS activity. For GSH determination, tissues were homogenized in 1% sucrose solution. The homogenates (10%, w/v) of liver and kidney were prepared in chilled hypotonic solution for proteins, cholesterol and triglyceride.

Serological analysis

Serum transaminases (AST and ALT), alkaline phosphatase (ALP), bilirubin, albumin, glucose, triglycerides, cholesterol, urea and uric acid were determined using diagnostic kits according to manufacturer's instructions (Erba).

Tissue biochemical analyses

Tissues of liver and kidney were immediately processed for determination of total cholesterol[24] and triglycerides[13].

Glutathione and TBARS assay in liver and kidney

The GSH was determined using dithionitrobenzoic acid (DTNB)[7] and optical density was recorded immediately at ë 412 nm. Thio barbituric acid reactive substances was assayed for lipid peroxidation[18] and optical density was recorded immediately at ë 535 nm.

Statistical analysis

Data were expressed as mean±SE of six animals used in each group. Statistical analysis was carried out using one way analysis of variance (ANOVA) considering significant at 5% level and 1% level followed by student's t-test considering significant at pd"0.05 and pd"0.01[20].

Result and Discussion

High fat diet consumption plays a central role to develop obesity which further leads to insulin resistance and non-alcoholic fatty liver disease (NAFLD)[11]. Excessive

TABLE -2: Effect of combined consumption of HFD and Alcohol on serum biochemistry

	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Glucose (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Control	28.9±2.27	23.4±1.95	99.1±7.41	24.8±2.03	2.23±0.17
HFD+Alc 2W	118±8.48**	47.6±3.66**	92.3±7.23	31.6±1.84*	3.81±0.25**
HFD+Alc 4W	296±19.20**	66.2±4.87**	71.7±6.78*	45.9±3.89**	4.11±0.25**
HFD+Alc 6W	328±25.61**	72.3±5.66**	66.8±5.33**	52.1±4.03**	4.86±0.30**
HFD+Alc 8W	356±25.21**	80.1±6.13**	63.3±5.35**	58.6±4.68**	5.13±0.30**
ANOVA	71.4ω	28.0ω	7.34ω	19.6ω	24.0ω

Data are expressed as mean ± SE; n=6; Significant P value Toxicants *vs* control * Pd"0.05 and ** Pd"0.01. For students test significant ANOVA at Pd"0.05 ù.

Abbreviations: Group 1= Control; Group 2= High Fat Diet + Alcohol (2 weeks); Group 3= High Fat Diet + Alcohol (4 weeks); Group 4= High Fat Diet + Alcohol (6 weeks); Group 5= High Fat Diet + Alcohol + (8 weeks).

alcohol consumption is a major public health problem that contributes substantially to the global burden of mortality and morbidity. Alcoholic liver diseases (ALD) can be induced by excessive consumption of alcohol, which is encompassed a spectrum of clinical signs and morphological changes with 5.9% of deaths worldwide[10].

Serum AST, ALT and ALP are major markers for the assessment of hepatocellular damage. In present study, combined consumption of HFD and alcohol caused sharp elevation in ALT, AST, ALP and bilirubin with decline in albumin after 2, 4, 6 and 8 weeks intoxication as compared to control group (*P*d"0.05; *P*d"0.01). However, no significant alteration was noted in AST and albumin in 2 weeks intoxicated rats (Table-1). AST and ALT activities increase significantly in obese diabetic animals[2,9]. Alcohol intake damages plasma membrane of hepatocytes and increases liver markers enzymes in circulation[19]. Increased activities of ALT, AST and ALP in the present study strongly supported significant hepatotoxic effects of high fat diet[21] and alcohol[19] in rats.

Consumption of high fat diet for 10 weeks resulted in development of insulin resistance, hepatic lipid accumulation, and increased oxidative hepatocellular damage[21]. In present study, combined consumption of HFD and alcohol significantly increased serum triglycerides, cholesterol in all toxic duration (*P*d"0.05; *P*d"0.01[15])[22]. Glucose level was significantly decreased after HFD and alcohol consumption for 4, 6 and 8 weeks, may be due to hampered metabolic activity of hepatic cells (*P*d"0.01) (Table-2). Urea is the main nitrogen containing metabolic products of protein metabolism, uric acid is the major product of purine bases, adenine and guanine. Creatinine is endogenously produced and released into body fluid and its clearance is measured as an indicator of glomerular filtration rate[8]. Present study showed high levels of urea and uric acid after consumption of HFD and alcohol which indicated hampered excretion due to severe glomerular injury[6 23].

Liver plays a major role in increasing the cholesterol production during obesity. NAFLD and ALD is characterized by the accumulation of lipids in the liver which aggravates normal liver functions. HFD and alcohol consumption significantly increased triglycerides and cholesterol level in liver for all toxic duration (*P*d"0.01) and kidney for 2 weeks at 5% and 4, 6 and 8 weeks at 1% (Fig. 1 E-H). Hepatic lipid accumulation in high fat consuming mice may be due to the increased production of endogenous fatty acids and increased supply of free fatty acids to the liver.

Oxidative stress can exert destructive effects on the membrane lipids, cellular proteins and nucleic acids by inducing direct lipid peroxidation. Cellular injuries by oxidative stress have been confirmed by measurement of lipid peroxidation in term of TBARS[16]. In present study, combined consumption of HFD and alcohol increased oxidative stress and cellular damage in liver and kidney, as indicated by enhanced TBARS production and diminished GSH contents in 2, 4, 6 and 8 weeks duration as compared to control (*P*d"0.01; Fig. 1 A-D). GSH plays a major role in antioxidant defense by inhibiting cellular oxidative damage through the removal of free radicals and helps to prevent numerous diseases including obesity,

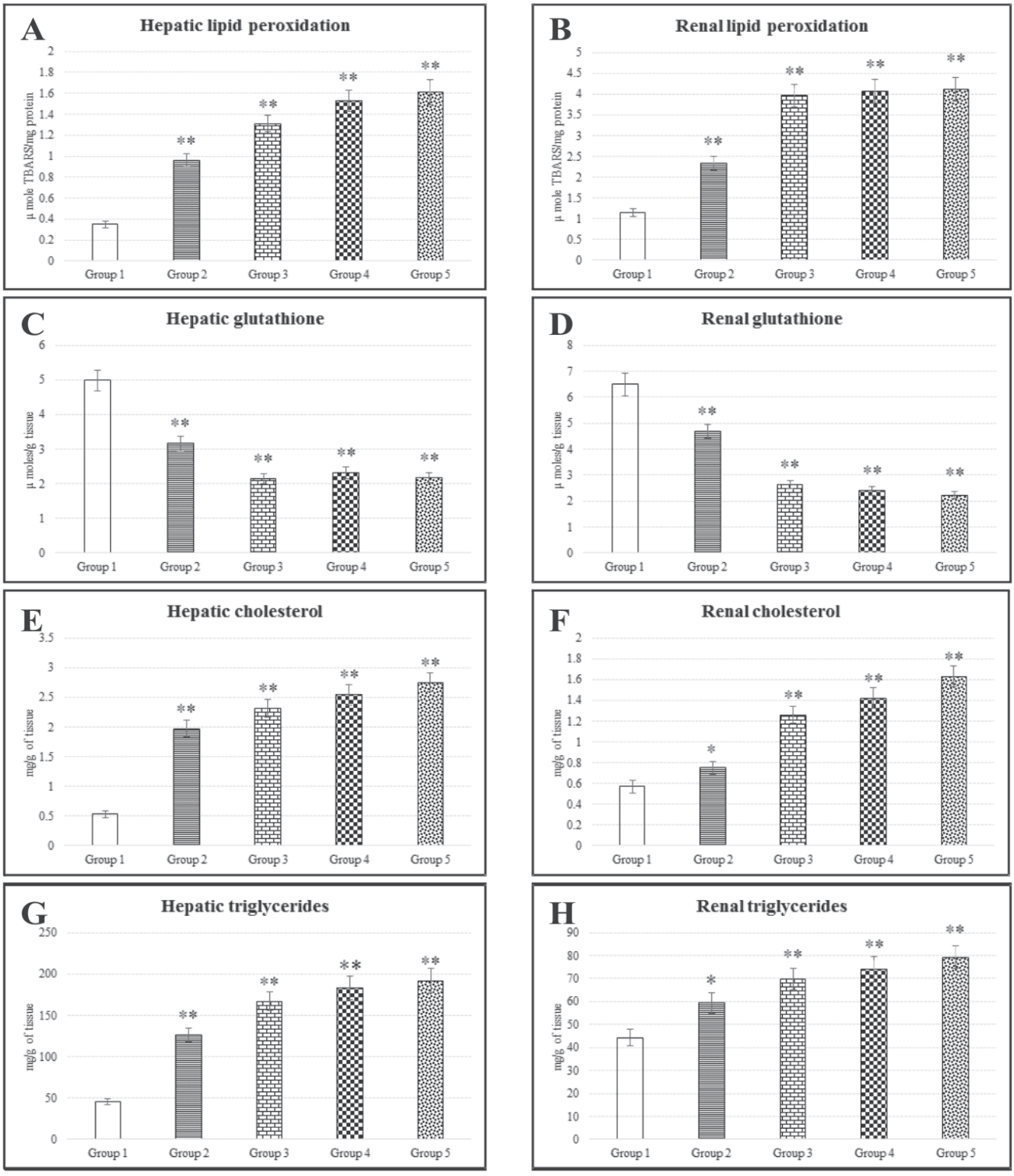

Fig.1: Effect of combined consumption of high fat diet and alcohol on oxidative stress and lipid metabolism

Data are expressed as mean ± SE; n=6; Significant P value HFD+alcohol *vs* control * Pd"0.05 and ** Pd"0.01; for student's test significant ANOVA at Pd"0.05 ù.

	Lipid peroxidation		Glutathione		Triglycerides		Cholesterol	
	Hepatic	Renal	Hepatic	Renal	Hepatic	Renal	Hepatic	Renal
ANOVA	44.5ω	30.0ω	40.0ω	59.4ω	31.5ω	8.27ω	36.4ω	24.1ω

Abbreviations: Group 1= Control; Group 2= High Fat Diet + Alcohol (2 weeks); Group 3= High Fat Diet + Alcohol (4 weeks); Group 4= High Fat Diet + Alcohol (6 weeks); Group 5= High Fat Diet + Alcohol (8 weeks).

type 1 and 2 diabetes, hypertension and cancer. Increased oxidative stress after high fat diet[22] and alcohol[19] consumption might be due to excessive production of oxygen free radicals and impaired cellular antioxidant defenses[1,12,19]. Hyperlipidemia is a major culprit for promoting oxidative stress by induction of lipid peroxidation and diminishing antioxidant defense system. Numerous studies revealed that antioxidants can protect from lipid peroxidation through the removal of free radicals in high fat diet and alcohol intoxicated animals[19,22]. Result of the present study confirms that combined consumption of high fat diet and alcohol caused serious damage in liver as well as kidney.

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