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Anti-Oxidative Effect of a Triherbal Formulation against Ethanol Induced Hepatotoxicity

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ABSTRACT

The hepatoprotective activity of a triherbal formulation (GOV) comprising of 50% ethanolic extract of *Gongronema latifolia, Ocimum gratissimum* and *Vernonia amygdalina* was studied against 50% ethanol (8.5 g/Kg, p.o. on the 24th and 4th hr before sacrificing) induced liver damage in Wistar albino rats. At 8000 mg/Kg, GOV increased PCV, WBC and platelet count compared to Liv 52 and Silymarin. At 8000 mg/Kg, GOV decreased LDH and ALP activities compared to silymarin group, while its ALT activities were lower than that of Liv 52 group. CAT, GPx, GSH, GST, SOD and total protein activities were reduced while the MDA levels were increased in the toxin control group compared to GOV treated groups. At 4000 mg/Kg, GOV attenuated the concentrations of cholesterol and creatinine more than Liv 52 and silymarin, lowered the triglyceride concentration compared to Liv 52 and had almost the same urea concentration as Liv 52. The serum protein concentration of GOV at 8000 mg/Kg was almost equal to that of Liv 52 and silymarin. The effects of GOV on serum marker and antioxidant enzymes were comparable to silymarin and Liv 52. The results of this study support the folkloric claim that GOV possess hepatoprotective activity.

Key words: Ethyl Alcohol, Triherbal Formulation, Hepatic Tissue, Hepatotoxicity

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INTRODUCTION

Alcohol is one of the oldest drugs that humans have used since the beginning of civilization . Alcohol abuse is one of the major healths, social and economic problems facing the world as significant number of people are affected due to severe fatal disease caused by alcohol. Studies show that alcohol is linked to more than 60 disease conditions, including liver disease and mouth, food pipe, bowel and breast cancer [1]. In England in 2006-7, alcohol was estimated to account for £2.7 billion of NHS expenditure, almost double the figure in 2001 [2]. Alcohol is estimated to be the third highest of 26 risk factors for ill health in the European Union, and the World Health Organization (WHO) identifies the need for the formulation of 'effective public health orientated counter-measures in order to minimize the harm caused by alcohol use' [3].

The target organelle for alcohol intoxication is the hepatic mitochondrial fraction. Toxicity of alcohol is shown to be related to its metabolism by alcohol dehydrogenase (ADH) and also to the metabolism by CYP2E1. Also CYP1A2, CYP3A4 and CYP2B families may contribute to ethanol oxidation [4]. There is also a component of metabolism by catalase [5]. In the liver the main pathway of ethanol oxidation is via ADH to acetaldehyde which is associated with the reduction of NAD to NADH which in turn increases Xanthine oxidase activity. This elevates production of superoxide [5]. Enhanced acetaldehyde production after ethanol metabolism decreases hepatic glutathione (GSH) content. Chronic ethanol intake potentially results in serious illness including hypertriglyceridemia, alcohol fatty liver, hypercholesterolemia, cirrhosis, hyperglyceridemia, hyperlipidemia, cardiovascular diseases and inflammation of the pancreas. [6-8]

Plants have been used in traditional medicine for several thousand years. Studies are going on throughout the world for the search of protective molecules that can provide maximum protection of the liver, kidney and other organs which exerts practically very little or no side effect during their function in the body [9; 10]. Plant and plant products have been shown to play an important role in the management of various liver disorders. Plant medicines are most often used in combination in order to get maximum benefits from their combined strength.

Gongronema latifolia, Ocimum gratissimum and *Vernonia amygdalina* are plants cultivated in many countries in the world and belong to the families Asclepiadaceae, Lamiaceae and Compositae respectively. Teas containing *Gongronema latifolium, Vernonia amygdalina* or *Cryptolepsis sanguinolenta* are also used throughout West Africa for the management of diabetes and other metabolic disease associated with the liver [11]. Ocimum gratissimum is used as a febrifuge and as an ingredient in many malaria medicines, for rheumatic pains and lumbago and catarrh remedies [12]. They play an important role in preservation of pharmaceutical products [13] and also inhibit *Staphylococcus aureus* at a concentration of 0.75mg\ml [14]. Phytochemical analysis of the leaves of Vernonia amygdalina yielded 2 known sesquesterpene lactones – Vernolide and Vernodalol [15]. The sesquesterpene lactones have an in vitro cytotoxic action against KB tumour cells and Wilme's myeloma [16]. In this study we investigated the efficacy of a triherbal formulation composed of 50% ethanol extract of *Gongronema latifolia, Ocimum gratissimum* and *Vernonia amygdalina* (GOV) on rats in which acute hepatotoxicity was induced by ethanol.

MATERIALS AND METHODS

PLANTS AND EXTRACTION

Leaves of *Gongronema latifolia, Ocimum gratissimum* and *Vernonia amygdalina* were purchased from the market and voucher specimen (PCGH 444, PCGH 443 and PCGH 432 respectively) was deposited at the Department of Pharmacognosy, College of Medicine of the University of Lagos, Nigeria. Equal quantity of each of the fresh leaves of the plants was blended with ethanol (50% v/v). It was filtered and the solvent was completely removed by rotary vacuum evaporator and was dried in an oven set at 40°C with the yield of 15.69%.

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ANIMALS

Studies were carried out using Wistar albino rats (150-200g), obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos Nigeria. The animals were grouped and maintained under standard laboratory conditions with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Ladokun feeds. The animals were left for 10 days to acclimatize before commencement of experiment. All procedures described were approved by the University of Lagos Animals Ethical Committee.

DRUG TREATMENT PROTOCOL

Animals were randomized and divided into seven groups of seven animals each.

Group 1: serve as normal control received distilled water (10 ml kg^{-1} body weight p.o.) for fourteen days.

Group 2: serve as toxin control received distilled water (10 ml kg^{-1} body weight p.o.) for fourteen days and 50% ethanol (8.5 g/Kgbody weight) on the thirteenth day and four hours before sacrificing on the fourteenth day.

Group 3: were treated with 2000 mg/Kg body weight of triherbal extract (GOV) for fourteen days. The extract was administered 1hr before 50% ethanol (8.5 g/Kg body weight) was administered on the thirteenth day and four hours before sacrificing on the fourteenth day.

Group 4: were treated with 4000 mg/Kg body weight of triherbal extract (GOV) for fourteen days. The extract was administered 1hr before 50% ethanol (8.5 g/Kg body weight) was administered on the thirteenth day and four hours before sacrificing on the fourteenth day.
Group 5: were treated with 8000 mg/Kg body weight of triherbal extract (GOV) for fourteen days. The extract was administered 1hr before 50% ethanol (8.5 g/Kg body weight) was administered on the thirteenth day and four hours before sacrificing on the fourteenth day.

Group 6: received LIV 52 syrup (300 mg/Kg body weight) for fourteen days. The drug was administered 1hr before 50% ethanol (8.5 g/Kg body weight) was administered on the thirteenth day and four hours before sacrificing on the fourteenth day.

Group 7: received Silymarin (300 mg/Kg body weight in distilled water p.o.) for fourteen days. The drug was administered 1hr before 50% ethanol (8.5 g/Kg body weight) was administered on the thirteenth day and four hours before sacrificing on the fourteenth day.

Biochemical Assays

After the experimental period i.e. 4 hours after the last administration of ethanol, the animals were anesthetized mildly with ether and blood was collected from the retro-orbital plexus. They were sacrificed and more blood samples were collected by cardiac puncture for evaluating the biochemical parameters. The livers were also dissected out for assay of oxidative stress and histology.

About one gram of the perfused liver of each animal was washed with isotonic solution. The liver homogenate was prepared using phosphate buffer solution and centrifuged. The supernatant was collected and used to assay for the effects of oxidative stress.

Serum Total protein (TP) [17), Lactate dehydrogenase (LDH) [18, 19), Gamma-glutamyl transferase (GGT) [20) Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline Phosphatase (ALP) [21) activities were determined on serum. Thiobarbituric acid reactive substances (TBARS) [22), reduced glutathione (GSH) [23), Superoxide dismutase (SOD) [24), glutathione peroxidase (GPX) [25), glutathione-s-transferase (GST) [26) and Catalase (CAT) [27) activities were determined using these marker enzymes on the supernatant and serum samples.

Liver Histopathology [28].

A small chunk of liver was taken from the sacrificed experimental rats used for hepatotoxicity studies and were preserved in 10 % formal saline for histological studies. The tissues were processed and sectioned in paraffin. The paraffin sections of buffered formalin-fixed tissue samples (3 µm thick) were dewaxed and rinsed in alcohol and also water. It was stained with Harris' haematoxylin (Sigma) for 10 minutes, washed in running tap water for 1 minute, differentiated in acid alcohol for 10 seconds and washed again in running tap water for 5 minutes. The tissues were stained with eosin for 4 minutes and washed in running tap water for 10 seconds. It was dehydrated and mounted for photomicroscopic observations of the histological architecture of the different groups. The general structure of the livers of the normal control group (group 1) was compared with those of the treated groups (groups 2-7).

STATISTICAL ANALYSIS

The results were expressed as mean $\pm$ SEM for seven rats. Statistical analysis of the data was performed using ANOVA statistical SPSS package (15.0) version. The significance of differences among all groups was determined by the Tukey HSD test. P – values less than 0.05 ($p \leq 0.05$) were considered to be statistically significant.

(a) = $p < 0.05$ compared with the normal control group (group 1).
(b) = $p < 0.05$ compared to control group (group 2).
(c) = $p < 0.05$ compared with the GOV + toxin control (2000 mg/Kg) group (group 3).
(d) = $p < 0.05$ compared with the GOV + toxin control (4000 mg/Kg) group (group 4).
(e) = $p < 0.05$ compared with the GOV + toxin control (8000 mg/Kg) group (group 5).

RESULTS

Effect of Pre-treatment with GOV on the Hematologic Indices of Rats.

The administration of GOV dose dependently caused a significant ($p<0.05$) increase in PCV, RBC, Hb, WBC, platelet, MCHC, granulocytes and lymphocytes and decrease in MCV and monocytes compared to the ethanol induced toxin control group (tables 1a and 1b) resulting in GOV associated protection of the hematopoietic system. There was an increase in levels of Hb, platelet count and MCHC and granulocytes on administration of GOV at 2000 mg/Kg compared to Liv 52 group, all the other groups and control and silymarin groups respectively. However a decrease in MCV was observed compared to control and silymarin group. There was an increase in PCV at the administration of GOV at 4000 mg/Kg compared to Liv 52 and silymarin groups and it showed an increase in RBC when compared to silymarin treated rats but was almost equal to Liv 52 treated rats. The numbers of monocytes observed in the 4000 mg/Kg group were lowered compared to control and Liv 52 groups and it exhibited the lowest and highest value of MCH and lymphocytes respectively compared to all the groups.

On administration of GOV (8000 mg/Kg), the PCV and WBC levels were elevated compared to Liv 52 and silymarin also, its RBC was high compared to silymarin and almost equal to Liv 52 treated rats. The Hb and platelet counts of GOV (8000 mg/Kg) were elevated compared to Liv 52 though it attenuated the MCH level compared to control and silymarin. It boosted and abated the lymphocyte and granulocytes levels respectively compared to control and Liv 52 experimental groups. There was a significant increase in the PCV and WBC values at administration of GOV at a dose of 8000 mg/Kg, compared to 2000 mg/Kg.

Hepatic Enzymes

The effects of the triherbal formulation on the serum transaminases, ALP, GGT and LDH in alcohol induced toxicity in all the groups are given in table 2. The increase in the levels of serum ALP, AST, GGT, ALT, and LDH in the toxin control group compared to the normal control group indicates liver damage. Pretreatment of rats with GOV, dose dependently decreased the levels of transaminases, ALP, GGT and LDH activities significantly ($p<0.05$) in experimental rats

as compared to the toxin control group. In the 8000 mg/Kg group, ALP activity was lower than that of silymarin and was significantly ($p<0.05$) increased when compared to Liv 52. The ALT, LDH and GGT levels of all the other experimental groups were significantly ($p<0.05$) low compared to toxin control group but were not significant ($p<0.05$) as compared to control. In 8000 mg/Kg group, LDH and ALP activities were decreased when compared to the rats that received silymarin, while its ALT activities were lower than that of Liv 52 group. In the 2000 mg/Kg group, GOV lowered the AST activity compared to Liv 52 and Silymarin. There was a 76%, 67.39%, 66.44%, 52.5% and 63.67% increase in ALP, ALT, AST, GGT and LDH activities respectively in group 2 as compared to group 1 with a 36.8% decrease in total protein level.

The Effects of Alcohol on Antioxidant Defence Enzymes of Albino Rats

The activity of antioxidant defence enzymes e.g. CAT, GPx, GSH, GST and SOD were significantly ($p<0.05$) decreased in the serum, liver and kidney tissues of animals in group 3 -5 as compared to that of animals in group 2 respectively in a dose dependent manner as shown in tables 3, 4 and 5.

Table 3 shows that there was significant ($p<0.05$) difference in serum, CAT, GPx, GSH, GST, SOD and total protein levels after ethyl alcohol administration to rats in group 2 compared to group 1 rats. This was significantly ($p<0.05$) reversed by administration of GOV in a dose dependent manner. The SOD value of GOV at 2000 mg/Kg was elevated more than that of Liv 52 and silymarin and almost equal to that of the control group. At 4000 mg/Kg, the MDA level of GOV was lower than that of silymarin. At 8000 mg/Kg, GOV augmented the levels of GPx, GSH, and GST more than Liv 52, the CAT and GST levels more than silymarin and had almost the same GPx and GSH values as silymarin.

In table 4, the levels of CAT, GPx, GSH, GST, SOD and total protein in the liver were markedly significantly ($p< 0.05$) reduced and that of MDA levels were significantly ($p< 0.05$) increased in the ethyl alcohol treated toxin control group. The thiobarbituric acid reaction showed a significant ($p<0.05$) increase in MDA of the ethyl alcohol treated animals in both hepatic tissues and serum. Treatment with GOV at 2000, 4000 and 8000 mg/Kg) significantly ($p<0.05$) prevented the increase in MDA level which was almost brought to near normal in both hepatic tissues. There was a sign GOV dose dependently resulted in a significant ($p<0.05$) increase of CAT, GSH, GPx, GST, SOD and total protein when compared to the toxin control group hepatic tissues. At 2000 mg/Kg and 4000 mg/Kg, GOV increased the SOD and CAT level more than Liv 52 respectively. The GSH, GST and total protein levels of Liv 52 group was low compared to that of GOV at 8000 mg/Kg while the MDA level was higher.

Table 5 shows the effect of alcohol induced toxicity on rat kidney. At 2000 mg/Kg, GOV exhibited higher concentration of total protein compared to Liv 52 while the GST was almost equal to that of Liv 52 and silymarin. The CAT, GPx and SOD levels of GOV at 4000 mg/Kg were increased while the MDA was lowered compared to Liv 52 and silymarin. When compared to Liv 52, the GSH and GPx activities of GOV at 4000 and 8000 mg/Kg were increased respectively. The CAT activity of GOV at 4000 mg/Kg was significantly ($p < 0.05$) higher than that of 2000 and 8000 mg/Kg. GOV significantly reversed the effects of alcohol on the rats in a dose dependent manner.

Effect of Alcohol Damage on Chemical Analytes of Rat Kidneys

In table 6, the effects of alcohol induced toxicity on serum albumin, cholesterol, creatinine, total protein, triglyceride and urea concentrations of rats fed different doses of GOV are shown. At 4000 mg/Kg, GOV attenuated the concentrations of cholesterol and creatinine more than Liv 52 and silymarin, lowered the triglyceride concentration more than that of Liv 52 and had almost the same urea concentration as Liv 52. The protein concentration of GOV at 8000 mg/Kg was almost equal to that of Liv 52 and silymarin. Silymarin significantly ($p<0.05$) increased the albumin concentration compared to GOV at 2000 and 4000 mg/Kg. The levels of serum albumin and total protein concentrations in alcohol induced toxin control group were

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significantly ($p<0.05$) decreased while increase in cholesterol, creatinine, triglyceride and urea concentrations were observed compared to the three doses of GOV.

Histopathology of Liver of Rats Intoxicated with Alcohol

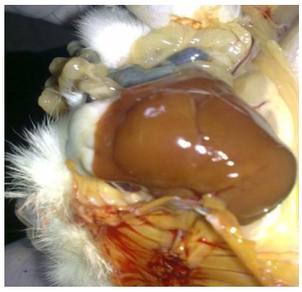
Photographs of the livers of the experimental animals were taken. The photomicrograph of the liver of animals in the control group showed normal histology. Pre-treatment with GOV at 8000 mg/Kg before administration of alcohol showed almost normal liver architecture while at 4000 mg/Kg, it showed almost normal liver with unremarkable central vein and hepatic lobule, with a focus of inflammatory infiltrate but no necrosis. The toxin control group showed loss of architecture, fibrosis, micro- and macrovesicular steatosis and fatty infiltration with extensive necrosis.

DISCUSSION

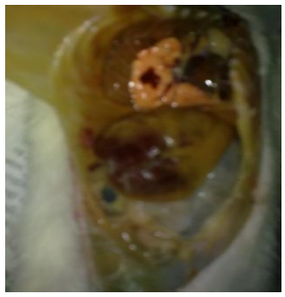
It has been shown that ethanol intake may lead to oxidative damage in several tissues such as liver, erythrocyte or brain [29]. The reactive metabolite [30] formed during ethanol metabolism. In microsomal and peroxisomal pathways e.g. CYP 2E1 mediated ethanol metabolism can modify proteins, lipids and DNA [31; 32]. It is believed that endotoxin initiate a cascade of events that leads to alcohol induced hepatotoxicity causing hypoxia in pericentral regions of the liver lobule where toxic free radicals are formed when oxygen is reintroduced.

ILLUSTRATIONS

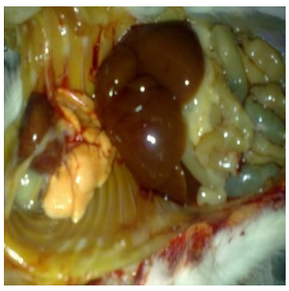
PHOTOGRAPHS OF THE LIVEROF RATS INTOXICATED WITH ALCOHOL



Photograph 1: Control group showing normal liver



Photograph 2: Toxin control group showing Alcohol damaged liver



Photograph 3: Alcohol + GOV 8000 mg kg^{-1}group showing almost normal liver architecture

Photograph 4: Alcohol + GOV 4000 mg kg^{-1}group showing liver damaged with alcohol

HISTOPATHOLOGY OF LIVER OF RATS INTOXICATED WITH ALCOHOL

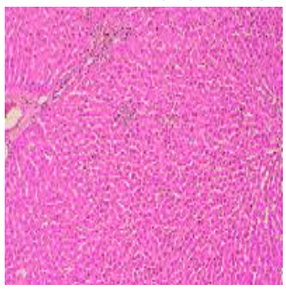


Photo micrograph 1: Control showing normal liver

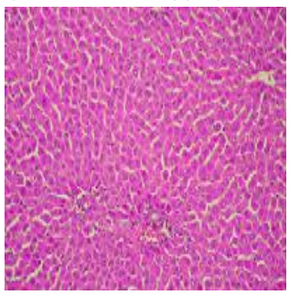


Photo micrograph 2: Alcohol + GOV 8000 mg kg^{-1}showing liver damaged with alcohol

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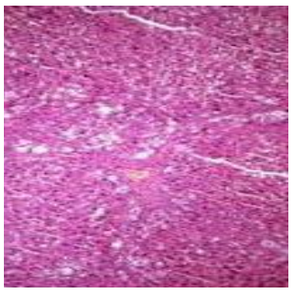

Photo micrograph 3: Toxin control showing alcohol damaged liver

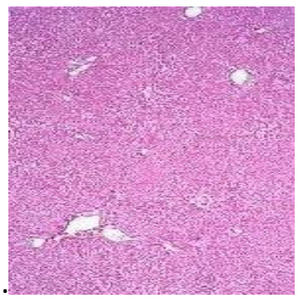

Photo micrograph 4: Alcohol + GOV 4000 mg kg^{-1} showing almost normal liver.

This causes cell death. When the amount of alcohol is high in the body, imbalances are created which can lead to hypoglycemia, hyperuricemia, fatty liver, and or hyperlipemia.

Table 1a: Effect of pretreatment with GOV on the blood hematologic indices in rats with alcohol induced hepatotoxicity.

Dose (mg kg^{-1})	PCV (%)	RBC (10^6/µl)	Hb (g/dl)	WBC (10^3/µl)	Platelet (10^3/µl)
Control	42.86 ± 0.91 (b, c)	7.09 ± 0.27 (b, c)	12.67 ± 0.41 (b, c, d, e)	8.2 ± 0.32 (b, c, d)	46.79 ± 3.18 (b)
Toxin Control	25.96 ± 1.46 (a, c, d, e)	3.09 ± 0.11 (a, c, d, e)	6.13 ± 0.31 (a, c, d, e)	1.95 ± 0.28 (a, c, d, e)	28.46 ± 1.48 (a, c, d, e)
GOV + Alcohol					
2000	34.76 ± 1.52 (a, b, e)	5.71 ± 0.17 (a, b)	10.87 ± 0.42 (a, b)	4.74 ± 0.3 3 (a, b, e)	49.56± 1.3 (b)
4000	39.7 ± 1.2 (b)	6.29 ± 0.19 (b)	10.03 ± 0.25 (a, b)	5.44 ± 0.51 (a, b)	43.34 ± 5.95 (b)
8000	42.21 ± 1.12 (b, c)	6.29 ± 0.15 (b)	10.67 ± 0.54 (a, b)	7.46 ± 0.7 (b, c)	43.72 ± 1.78 (b)
LIV 52 + Alcohol	37.21 ± 2.14 (b)	6.52 ± 0.6 (b)	10.33 ± 0.41 (a, b)	6.57 ± 0.81 (b)	40.07 ± 2.64
Silymarin + Alcohol	39.16 ± 1.71 (b)	6.14 ± 0.22 (b)	11 ± 0.37 (b)	6.2 ± 0.27 (b)	43.75 ± 2.64 (b)

Values are expressed as mean ± SEM for seven rats. The mean difference is significant at the .05 level. (a) = $p < 0.05$ as compared with the normal control group. (b) = $p < 0.05$ as compared to Alcohol control group. (c) = $p < 0.05$ as compared with theGOV + Alcohol(2000mg kg^{-1}) group. (d) = $p < 0.05$ as compared with theGOV + Alcohol (4000mg kg^{-1}) group. (e) = $p < 0.05$ as compared with theGOV + Alcohol (8000mg kg^{-1}) group. Thesignificance of differences among all groups was determined by the Tukey HSD test.

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Table 1b: Effect of pretreatment with GOV on the blood hematologic indices in rats with alcohol induced hepatotoxicity.

Dose (mg kg^{-1})	MCHC (%)	MCV (fl)	MCH (pg)	Granulocytes (%)	Lymphocyte (%)	Monocyte (%)
Control	29.71 ± 1.22	60.91 ± 2.03$^{(b)}$	18.1 ± 0.99	10.01 ± 0.84	83.61 ± 1.28$^{(b)}$	6.37 ± 0.8$^{(b)}$
Toxin Control	24.34 ± 2.28$^{(c)}$	83.97 ± 3.89$^{(a, c, d, e)}$	19.97 ± 1.25	5.67 ± 0.26$^{(c)}$	65.01 ± 2.11$^{(a, c, d, e)}$	29.31 ± 2.05$^{(a, c, d, e)}$
GOV + Alcohol						
2000	31.33 ± 0.44$^{(b)}$	60.9 ± 2.04$^{(b)}$	19.04 ± 0.51	11.36 ± 1.46$^{(b)}$	81.47 ± 2.15$^{(b)}$	7.17 ± 0.82$^{(b)}$
4000	25.39 ± 0.95	63.54 ± 3.14$^{(b)}$	15.96 ± 0.77	8.24 ± 0.59	86.66 ± 0.85$^{(b)}$	5.1 ± 0.5$^{(b)}$
8000	25.45 ± 1.64	67.39 ± 2.47$^{(b)}$	17.05 ± 1.01	9.86 ± 0.67	84.36 ± 0.78$^{(b)}$	5.79 ± 0.34$^{(b)}$
LIV 52 + Alcohol	28.18 ± 1.53	58.53 ± 3.17$^{(b)}$	16.73 ± 1.72	11.9 ± 1.14$^{(b)}$	81.43 ± 1.69$^{(b)}$	6.67 ± 0.69$^{(b)}$
Silymarin + Alcohol	28.3 ± 1.06	64.52 ± 4.16$^{(b)}$	18.09 ± 0.97	10.64 ± 1.39$^{(b)}$	84.41 ± 1.74$^{(b)}$	4.94 ± 0.62$^{(b)}$

Values are expressed as mean ± SEM for seven rats. The mean difference is significant at the .05 level. (a) = $p < 0.05$ as compared with the normal control group. (b) = $p < 0.05$ as compared to Alcohol control group. (c) = $p < 0.05$ as compared with the GOV + Alcohol(2000mg kg^{-1}) group. (d) = $p < 0.05$ as compared with theGOV + Alcohol (4000mg kg^{-1}) group. (e) = $p < 0.05$ as compared with theGOV + Alcohol (8000mg kg^{-1}) group. Thesignificance of differences among all groups was determined by the Tukey HSD test.

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Table 2: Serum levels of ALT, AST, ALP, LDH and GGT in rats pretreated with GOV before alcohol damage.

Dose (mg kg^{-1})	ALP (U/L)	AST (U/L)	ALT (U/L)	GGT (U/L)	LDH (U/L)
Control	73.93 ± 1.34$^{(b, c, d, e)}$	56.51 ± 2.32$^{(b, c, d, e)}$	29.43 ± 1.29$^{(b)}$	1917.56 ± 137.87$^{(b)}$	12.18 ± 0.91$^{(b)}$
Toxin Control	308 ± 24.57$^{(a, c, d, e)}$	168.36 ± 5.43$^{(a, c, d, e)}$	90.24 ± 5.37$^{(a, c, d, e)}$	4037.15 ± 171.62$^{(a, c, d, e)}$	33.53 ± 0.29$^{(a, c, d, e)}$
GOV + Alcohol					
2000	185.97 ± 4.64$^{(a, b)}$	74.17 ± 2.25$^{(a, b)}$	37.67 ± 6.05$^{(b)}$	2217.04 ± 210.17$^{(b)}$	15.02 ± 1.89$^{(b)}$
4000	188.8 ± 2.9$^{(a, b)}$	79.2 ± 1.52$^{(a, b)}$	39.37 ± 2.27$^{(b)}$	2308.59 ± 118.8$^{(b)}$	15.43 ± 0.93$^{(b)}$
8000	153.6 ± 3.43$^{(a, b)}$	76.84 ± 1.86$^{(a, b)}$	35.6 ± 2.55$^{(b)}$	2268.9 ± 19.08$^{(b)}$	13.56 ± 1.29$^{(b)}$
LIV 52 + Alcohol 300	143.68 ± 5.67$^{(a, b, d)}$	76.82 ± 4.87$^{(a, b)}$	34.97 ± 2.92$^{(b)}$	2196.03 ± 111.8$^{(b)}$	12.66 ± 1.55$^{(b)}$
Silymarin + Alcohol 300	157.25 ± 4.6$^{(a, b)}$	76.26 ± 3.17$^{(a, b)}$	32.9 ± 1.56$^{(b)}$	2127.78 ± 125.21$^{(b)}$	13.84 ± 0.71$^{(b)}$

Values are expressed as mean ± SEM for seven rats. The mean difference is significant at the .05 level. (a) = $p < 0.05$ as compared with the normal control group. (b) = $p < 0.05$ as compared to Alcohol control group. (c) = $p < 0.05$ as compared with theGOV + Alcohol(2000mg kg^{-1}) group. (d) = $p < 0.05$ as compared with theGOV + Alcohol (4000mg kg^{-1}) group. (e) = $p < 0.05$ as compared with theGOV + Alcohol (8000mg kg^{-1}) group. Thesignificance of differences among all groups was determined by the Tukey HSD test.

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Table 3: The effect of alcohol damage on serum antioxidant enzymes in rats pre-treated with GOV

Dose (mg kg^{-1})	CAT (µmol/min/mg protein)	GPx (µmol/ml)	GSH (µmol/ml)	GST (µmol/ml)	MDA (nmol/ml)	SOD (µmol/ml)	Total protein (g/L)
Control	11.55 ±.48$^{(b, c, d, e)}$	0.65 ±.03$^{(b, c, d, e)}$	0.21 ±.01$^{(b, d)}$	1139.07 ± 56.84 $^{(b,c, d, e))}$	4.66 ± 0.25$^{(b, c,d, e)}$	69.32 ± 2.56$^{(b)}$	86.07 ± 2.76$^{(b)}$
Toxin Control	5.7 ±.24$^{(a, c, d, e)}$	0.19±.02$^{(a, c, d, e)}$	0.1 ± 0.01$^{(a, c, e)}$	454.99 ± 10.47$^{(a, c, d, e)}$	14.4 ± 0.42$^{(a, c, d, e)}$	31.65 ± 2.72 $^{(a, c, d, e)}$	54.39 ± 2.64$^{(a, c, d, e)}$
GOV + Alcohol							
2000	9.02 ± 0.22$^{(a, b)}$	0.48 ±.01 $^{(a, b)}$	0.15 ± 0.01 $^{(b)}$	810.76 ± 24. 1$^{(a, b)}$	8.14 ± 0.26$^{(a, b)}$	62.13 ± 1.12$^{(b)}$	77.42 ± 2.69$^{(b)}$
4000	9.43 ±.42$^{(a, b)}$	0.5 ±.01$^{(a, b)}$	0.15 ± 0.01$^{(a)}$	850.06 ± 28.18 $^{(a, b)}$	7.5 ± 0.3$^{(a, b)}$	58.15 ± 2.66$^{(b)}$	78.34 ± 3.83$^{(b)}$
8000	9.68 ±.53$^{(a, b)}$	0.52 ± 0.01$^{(a, b)}$	0.17 ± 0.01$^{(b)}$	899.55 ± 9.87$^{(a, b)}$	7.71 ± 0.32$^{(a, b)}$	59.56 ± 3.92$^{(b)}$	80.3 ± 2.2$^{(b)}$
LIV 52 + Alcohol300	9.73 ±.64 $^{(b)}$	0.51 ± 0.01$^{(a, b)}$	0.16 ±.01 $^{(b)}$	877.02 ± 40.47$^{(a, b)}$	7.38 ± 0.37$^{(a, b)}$	58.05 ± 2.53 $^{(b)}$	81.19 ± 2.99$^{(b)}$
Silymarin + Alcohol300	9.58 ±.27$^{(a, b)}$	0.54 ± 0.03$^{(a, b)}$	0.19 ±.03$^{(b)}$	860.18 ± 57.79$^{(a, b)}$	7.56 ± 0.35$^{(a, b)}$	58.27 ± 2.27$^{(b)}$	81.41 ± 1.12$^{(b)}$

Values are expressed as mean ± SEM for seven rats. The mean difference is significant at the .05 level. (a) = $p < 0.05$ as compared with the normal control group. (b) = $p < 0.05$ as compared to Alcohol control group. (c) = $p < 0.05$ as compared with theGOV + Alcohol(2000mg kg^{-}1) group. (d) = $p < 0.05$ as compared with theGOV + Alcohol (4000mg kg^{-}1) group. (e) = $p < 0.05$ as compared with theGOV + Alcohol (8000mg kg^{-}1) group. Thesignificance of differences among all groups was determined by the Tukey HSD test.

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Table 4: The effect of alcohol damage on liver antioxidant enzymes in rats pre-treated with GOV.

Dose (mg kg^{-1})	CAT (µmol/min/mg protein)	GPx (µmol/ml)	GSH (µmol/ml)	GST (µmol/ml)	MDA (nmol/ml)	SOD (µmol/ml)	Total protein (g/L)
Control	206.68 ± 5.56$^{(b, c)}$	8.82 ± 0.24$^{(b, c, d, e)}$	26.64 ± 1.13$^{(b, c)}$	424.58 ± 17.46$^{(b, c, d, e)}$	10.77 ± 0.56$^{(b)}$	115.81 ± 10.46$^{(b, c, d, e)}$	84.2 ± 2.24$^{(b, c, d)}$
Toxin Control	55.93 ± 2.83$^{(a, c, d, e)}$	3.94 ± 0.18$^{(a, d, e)}$	10.76 ± 0.8$^{(a, c, d, e)}$	90.2 ± 5.96$^{(a, c, d, e)}$	35.65 ± 2.76$^{(a, c, d, e)}$	42.7 ± 2.49$^{(a, c, d, e)}$	53.97 ± 1.94$^{(a, d, e)}$
GOV + Alcohol							
2000	157.16 ± 11.48$^{(a, b)}$	4.43 ± 0.1$^{(a, e)}$	18.75 ± 1.34$^{(a, b)}$	265.38 ± 12.24$^{(a, b)}$	14.46 ± 0.46$^{(b)}$	85.49 ± 4.18$^{(a, b)}$	63.13 ± 1.72$^{(a, e)}$
4000	191.27 ± 4.49$^{(b)}$	5.16 ± 0.43 $^{(a, b, e)}$	21.07 ± 1.36$^{(b)}$	214.23 ± 4.24$^{(a, b, e)}$	15.02 ± 0.89$^{(b)}$	71.45 ± 4.2$^{(a, b)}$	67.05 ± 2.23$^{(a, b, e)}$
8000	176.63 ± 10.2$^{(b)}$	6.66 ± 0.28$^{(a, b, c, d)}$	24.22 ± 2.57$^{(b)}$	292.14 ± 34.04$^{(a, b, d)}$	12.66 ± 1.29$^{(b)}$	82.52 ± 6.49$^{(a, b)}$	77.87 ± 3.32$^{(b, c, d)}$
LIV 52 + Alcohol300	185.08 ± 5.38$^{(b)}$	6.72 ± 0.13$^{(a, b, c, d)}$	22.19 ± 1.44$^{(b)}$	275.33 ± 4.54$^{(a, b)}$	12.95 ± 0.61$^{(b)}$	85.37 ± 3.91$^{(a, b)}$	76.9 ± 1.85$^{(b, c, d)}$
Silymarin + Alcohol300	192.71 ± 12.32$^{(b)}$	7.03 ± 0.4 $^{(a, b, c, d)}$	21.01 ± 1.29$^{(b)}$	293.93 ± 9.31$^{(a, b, d)}$	12.31 ± 0.44$^{(b)}$	93.14 ± 4.15$^{(b)}$	78.27 ± 1.67$^{(b, c, d)}$

Values are expressed as mean ± SEM for seven rats. The mean difference is significant at the .05 level. (a) = $p < 0.05$ as compared with the normal control group. (b) = $p < 0.05$ as compared to Alcohol control group. (c) = $p < 0.05$ as compared with theGOV + Alcohol(2000mg kg^{-}1) group. (d) = $p < 0.05$ as compared with theGOV + Alcohol (4000mg kg^{-}1) group. (e) = $p < 0.05$ as compared with theGOV + Alcohol (8000mg kg^{-}1) group. Thesignificance of differences among all groups was determined by the Tukey HSD test.

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Table 5: The effect of alcohol damage on kidney antioxidant enzymes in rats pretreated with GOV

Dose (mg kg^{-1})	CAT (µmol/min/mg protein)	GPx (µmol/ml)	GSH (µmol/ml)	GST (µmol/ml)	MDA (nmol/ml)	SOD (µmol/ml)	Total protein (g/L)
Control	263.15 ± 5.01$^{(b, c, d, e)}$	3.91 ± 0.2$^{(b, c, d, e)}$	36.53 ± 1.36$^{(b, c, e)}$	481.79 ± 22.81$^{(b, c, d, e)}$	9.57 ± 0.73$^{(b, e)}$	129.53 ± 3.24$^{(b, c, d, e)}$	84.21 ± 3.59$^{(b, e)}$
Toxin Control	74.09 ± 5.15$^{(a, c, d, e)}$	1.45 ± 0.15$^{(a, c, d, e)}$	13.71 ± 0.22$^{(a, c, d, e)}$	77.76 ± 5.1$^{(a, c, d, e)}$	38.04 ±. 5$^{(a, c, d, e)}$	52.08 ± 3.09$^{(a, c, d, e)}$	47.19 ± 1.87$^{(a, c, d, e)}$
GOV + Alcohol							
2000	131.68 ± 12$^{(a, b, d)}$	2.71 ± 0.11$^{(a, b)}$	25.29 ± 0.93$^{(a, b)}$	301.27 ± 17.03$^{(a, b)}$	12.98 ± 1.63$^{(b)}$	73.51 ± 3.11$^{(a, b)}$	77.68 ± 3.41$^{(b)}$
4000	193.71 ± 17.73$^{(a, b, c, e)}$	3.27 ± 0.15$^{(a, b)}$	28.36 ± 1.43$^{(b)}$	292.4 ± 12.24$^{(a, b)}$	12.48 ± 0.57$^{(b)}$	80.57 ± 3.85$^{(a, b)}$	79.72 ± 3.07$^{(b)}$
8000	136.77 ± 5.47$^{(a, b, d)}$	2.97 ± 0.2$^{(a, b)}$	27.15 ± 4.17$^{(a, b)}$	263.64 ± 16.23$^{(a, b)}$	13.22 ± 0.58$^{(a, b)}$	70.51 ± 1.4$^{(a, b)}$	71.49 ± 2.63$^{(a, b)}$
LIV 52 + Alcohol300	163.03 ± 9.03$^{(a, b)}$	2.92 ± 0.14$^{(a, b)}$	28.01 ± 1.41$^{(a, b)}$	309.16 ± 9.96$^{(a, b)}$	12.65 ± 0.63$^{(b)}$	79.18 ± 1.29$^{(a, b)}$	76.3 ± 2.31$^{(b)}$
Silymarin + Alcohol300	157.46 ± 2.37$^{(a, b)}$	3.1 ± 0.14$^{(a, b)}$	29.59 ± 1.19$^{(b)}$	310.05 ± 13.82$^{(a, b)}$	12.54 ± 0.53$^{(b)}$	77.43 ± 1.77$^{(a, b)}$	79.81 ± 1.42$^{(b)}$

Values are expressed as mean ± SEM for seven rats. The mean difference is significant at the .05 level. (a) = $p < 0.05$ as compared with the normal control group. (b) = $p < 0.05$ as compared to Alcohol control group. (c) = $p < 0.05$ as compared with theGOV + Alcohol(2000mg kg^{-}1) group. (d) = $p < 0.05$ as compared with theGOV + Alcohol (4000mg kg^{-}1) group. (e) = $p < 0.05$ as compared with theGOV + Alcohol (8000mg kg^{-}1) group. Thesignificance of differences among all groups was determined by the Tukey HSD test.

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Table 6: Serum levels of albumin, cholesterol, creatinine, total protein, triglyceride and urea in rats pretreated with GOV before alcohol damage.

Dose (mg kg^{-1})	Albumin (g/L)	Cholesterol (mmol/L)	Creatinine (mmol/L)	Total protein(g/L)	Triglyceride (mmol/L)	Urea (mmol/L)
Control	47.05 ± 1.76$^{(b, c, d)}$	1.78 ± 0.11$^{(b, c, e)}$	61.11 ± 3.61$^{(b, c)}$	86.07 ± 2.76$^{(b)}$	1 ± 0.03$^{(b)}$	5.02 ± 0.34$^{(b)}$
Toxin Control	25.28± 1.15$^{(a, c, d, e)}$	4 ± 0.18$^{(a, c, d, e)}$	93.07 ± 4.38$^{(a, c, d, e)}$	54.39 ± 2.64$^{(a, c, d, e)}$	3.28 ± 0.16$^{(a, c, d, e)}$	10.53 ± 1.45$^{(a, c, d, e)}$
GOV + Alcohol						
2000	37.82 ± 0.85$^{(a, b)}$	2.7 ± 0.12$^{(a, b)}$	76.51 ± 0.78$^{(a, b)}$	77.42 ± 2.69$^{(b)}$	1.37 ± 0.16$^{(b)}$	5.87 ± 0.34$^{(b)}$
4000	37.45 ± 0.8$^{(a, b)}$	2.19 ± 0.16$^{(b)}$	63.44 ± 2.57$^{(b)}$	78.34 ± 3.83$^{(b)}$	1.24 ± 0.18$^{(b)}$	5.58 ± 0.32$^{(b)}$
8000	43.29 ± 1.81$^{(b)}$	2.51 ± 0.12$^{(a, b)}$	72.77 ± 2.38$^{(b)}$	80.3 ± 2.2$^{(b)}$	1.45 ± 0.16$^{(b)}$	6.19 ± 0.48$^{(b)}$
LIV 52 + Alcohol 300	42.31 ± 1.52$^{(b)}$	2.28 ± 0.14$^{(b)}$	69.01 ± 2.65$^{(b)}$	81.19 ± 2.99$^{(b)}$	1.3 ± 0.16$^{(b)}$	5.5 ± 0.25$^{(b)}$
Silymarin + Alcohol300	43.95 ± 1.28$^{(b, c, d)}$	2.24 ± 0.05$^{(b)}$	64.89 ± 3.31$^{(b)}$	81.41 ± 1.12$^{(b)}$	1.12 ± 0.06$^{(b)}$	5.15 ±.35$^{(b)}$

Values are expressed as mean ± SEM for seven rats. The mean difference is significant at the .05 level. (a) = $p < 0.05$ as compared with the normal control group. (b) = $p < 0.05$ as compared to Alcohol control group. (c) = $p < 0.05$ as compared with theGOV + Alcohol(2000mg kg^{-}1) group. (d) = $p < 0.05$ as compared with theGOV + Alcohol (4000mg kg^{-}1) group. (e) = $p < 0.05$ as compared with theGOV + Alcohol (8000mg kg^{-}1) group. Thesignificance of differences among all groups was determined by the Tukey HSD test.

The significant increase in ALP, ALT, AST, GGT and LDH activity levels shows that there is leakage of cellular enzymes into the serum indicating hepatic injury or damage. GOV (group 3-5) dose dependently decreased the ALP, ALT, AST, GGT and LDH levels and increased the total protein level when compared to the toxin control group (group 1). The observed decrease in the ALP, ALT, AST, GGT and LDH levels and increased total protein level may be attributed to the reduced leakage of these marker enzymes in serum. It is likely that GOV could be acting by modulating the repair of hepatic injury and or restore cellular permeability thereby lowering the toxicity of ethyl alcohol. It could also prevent and or reduce enzyme leakage into blood circulation.

The vital function that blood cells perform (e.g. transport and defence) increases its susceptibility to intoxication by xenobiotics and makes the hematopoeitic system a unique target organ. It ranks with liver and kidney as one of the most important considerations in the risk assessment of potential environmental toxicants or xenobiotics [33]. Hematological parameters namely PCV, WBC and differentials were monitored in this study because of their diagnostic significance and role in providing information concerning hematological changes caused by ethyl alcohol-induced toxicity. The recorded hematotoxicity could be secondary to the deleterious effect of ethyl alcohol on organs of hematopoeisis in the body which include liver and kidneys. Results of this study showed that the extract could contain active biological principle(s) annulling the hematotoxic effect of ethyl alcohol, with ensuing improvement of hematopoiesis. The biological principle(s) could also be arbitrating hematopoietin-like effect or augmenting the release of hematopoietin from hematopoetic organs such as the kidneys or liver.

It is possible that the triherbal formulation dose dependently prevents glutathione depletion induced by ethanol intoxication by up-regulating the biosynthesis of glutathione in the liver. Works in our laboratory showed that GOV contains alkaloids, saponins, phenolics, tannins and other constituents that could actively scavenge free radicals. Many phytochemical constituents e.g. steroidal saponins could play vital roles as anti-inflammatory agents, in the

induction of protein synthesis, and in tissue regeneration and repair. Silymarin has been reported to help stabilize mast cells and inhibit neutrophil migration, Kupffer cells, and the formation of inflammatory prostaglandins and leukotrienes [34]. Therefore GOV is likely to produce the same effects since its activities are similar and in some cases better than silymarin. GOV has been reported to possess anti-inflammatory activity [35] so could act by reducing oxidative stress caused by binding of alcohol oxidation products, e.g. acetaldehyde, to liver cell proteins, forming neoantigens that results in inflammation. Pretreatment with GOV, Liv 52 and silymarin showed increased synthesis of protein suggesting the regeneration of liver.

The reduction in albumin and protein concentrations and increase in triglyceride concentration is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver. Oral administration of GOV dose dependently and significantly ($P<0.05$) attenuated serum cholesterol, triglyceride, urea and creatinine concentrations and increased the albumin and total protein concentrations when compared to the toxin control group.

Inhibition of the generation of free radicals or antioxidation activity is important in the protection against alcohol induced liver damage because it increases free radical induced lipid peroxidative damage, accumulation of neutrophils and other WBCs, which are attracted by lipid peroxidative damage and neoantigens and Inflammatory cytokines secreted by WBCs. The ability of GOV to significantly ($P<0.05$) increase the CAT, GPx, GSH, GST, SOD and total protein activity and significantly ($P<0.05$) deplete the levels of TBARS in both serum and liver tissue after intoxication with ethyl alcohol can be ascribed to abated lipid peroxidation and improvement of the serum and tissue antioxidant defence enzymes activity levels. GOV can be said to be able to attenuate the generation of free radicals and also accrue mechanisms of scavenging free radicals thereby stabilizing the structure of the cell membrane. It significantly ($P<0.05$) prevented the diminution in the level of the protective enzymes CAT, GPx, GSH, GST and SOD, induced by ethyl alcohol when examined in the serum and liver homogenate. It is possible that the mechanism of hepatoprotective action of GOV might be due to its anti-oxidant properties. The significant increase in the level of total protein in GOV, Liv 52 and silymarin groups (groups 3-7) suggests that there may have been a repair of damaged hepatocytes and restoration of normal functions of liver after alcohol induced hepatotoxicity.

The biochemical results obtained agrees with the histological studies of the liver sections. The hepatoprotective property of this triherbal mixture can be attributed to the presence of these active principles which alone or in combination may be responsible for the hepatoprotection demonstrated in this study. This indicates that GOV has hepatoprotective effects against ethyl alcohol induced toxicity

CONCLUSION

The hepatoprotective formulation may have a role in the process of regeneration, thus, our data indicates that treatment with GOV offers protection against free radical-mediated oxidative stress in serum and liver of animals with ethanol-induced liver injury. The antioxidant activity may be by inhibiting the formation of the free radicals or scavenging of the formed radical assisted by the presence of the phenolic compounds. The ability of GOV to reduce the injurious effects caused by ethyl alcohol is the index of its hepatoprotective efficiency.

In conclusion, it can be said that GOV has exhibited liver protective effect against ethyl alcohol-induced hepatotoxicity and possessed antioxidant activities in a dose dependent manner and exhibited significant protection to the liver thus justifying its antihepatotoxic ability. These results support the folkloric claims that GOV possess hepatoprotective activities. Efforts are in progress to isolate and purify the active principle involved in the hepatoprotective efficacy of this triherbal formulation.

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