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Comparative Effect of *Gongronema Latifolium* and *Piper Guineense* Ethanol Extract Against Scavaging Enzymes and Marker of Oxidative Stress in Ethanol Induced Liver Injury in Wistar Rats

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Abstract:
Aims: Comparative protective effect of ethanol extracts of Gongronema latifolium and Piper guineense on ethanol-induced toxicity were investigated in male Wistar rats.
Methodology: The acute toxicity test of the extracts was evaluated. The rats were exposed with 70 % ethanol for 7 days to induce liver damage and later treated with ethanol extracts for 21 days. The levels of scavenging enzymes: glutathione peroxidise, superoxide dismutase, catalase; malondialdehyde and cytochrome p4502e1 were monitored in order to evaluate the protective effects of the plant extracts. Hepatic malondialdehyde and reduced glutathione, as well as superoxide dismutase and glutathione-S-transferase activities were determined for the antioxidant status.
Results: Acute toxicity results showed that Gongronema latifolium were safe at doses investigated whereas Piper guineense LD_{50} were 1500mg/kg. Chronic ethanol administration resulted in a significant ($p< 0.05$) decrease in reduced glutathione ,superoxide dismutase,catalase and increased malondialdehyde and cytochrome p4502e1.These were dramatically reversed to normal by the treatment of the plant extract in a dose dependent fashion. Treatment of the plant extracts significantly ($p<0.05$) reduced cytochrome p4502e1 and malondialdehyde, restored the reduced glutathione, enhanced the superoxide dismutase and catalase level. These results highlight the ability of Gongronema latifolium at high doses and Piper guineense at lower doses to ameliorate oxidative damage in the liver and the observed effects are associated with its antioxidant activities.

Keywords: *serum, ethanol, oxidative stress, scavenging enzymes, acute toxicity*

1. Introduction
Practice of herbal medicine is an ancient tradition. Herbal medicines have been used since earliest times to treat illnesses and restore good health, and today, herbalism still remains the most widely practised form of medicine worldwide [1].Globally, medicinal plants are very useful for the treatment and management of diseases or infections. They are mostly particularly useful in countries, where, due to their low income status, they can hardly afford imported and expensive conventional medicine [2]. According to the World Health Organization (WHO) report, it is estimated that 80% of people worldwide rely on herbal medicines for some aspects of their primary health care [3]. The upsurge in the use of herbal remedies in developed countries is due to consumers' preference for products of natural origin [4].
Gongronema latifolium is an edible, perennial climbing vegetable located in many parts of Africa and Nigeria, where it is called "Utazi" in Ibo and "Orokeke" in Yoruba. It contains bitter principle and has many folkloric attributes as a healing herb [5]. Several studies have revealed that its leaves and stem possess anti diabetic properties [6] and intestinal muscle relaxant [6].and anti inflammatory properties [6]. *Piper guineense,* popularly known as African black pepper or hot leave is widely consumed in some part of West Africa especially Nigeria and Ghana on account of its nutritional and medicinal properties [7-12]. It belongs to the family Piperaceae or Sapotaceae [13]. In traditional herbal medicine, the seeds are put into a variety of uses, for instance, in some parts of Nigeria, the seeds are consumed by women after child birth, to enhance uterine contraction for the expulsion of placenta and other remains from the womb, as an adjuvant in the treatment of rheumatic pains and as an anti-asthmatics [14] and also for the control of

weight [15]. It is with these characteristics in mind that this work was designed to compare the efficacy, of these plants extracts in treatment of ethanol induced liver damage in albino rats.

2. Materials and Methods

2.1. Biological Materials

The biological materials used included male albino rats, *Gongronema latifolium* and *Piper guineense.*The male Wistar albino rats of about 8 to 12 weeks old with average weight of 150 to 220g were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka (UNN) and acclimatized for seven days in the Animal House of the Department of Biochemistry Ebonyi State University, Abakaliki.The animals were allowed access to feed (obtained from Safari Feed mill number 5 Zikavenue Abakaliki Nigeria) and water *ad libitum.*The animals were kept in well ventilated cages at room temperature, and under controlled light/dark cycles (12/12h).
Gongronema latifolium and *Piper guineense* leaves were purchased from Abakpa Market Abakaliki, Nigeria and identified by Prof Okafor of the Department of Biology, Ebonyi State University Nigeria, Voucher specimens were deposited in the herbarium unit of the department.

Figure 1: Gongronema latifolium

Figure 2: Piper guineense

2.1.1. Equipment

The equipments used in the course of this study are of analytical standard.

2.1.2. Chemicals/Reagents/Samples

All chemicals used in this study were of analytical grade and products of Sigma USA, May and Baker, England; BDII, England and Merck, Darmstadt, Germany. Reagents used for all the assays were commercial kits and products of Randox, USA; QCA, Spain; Tcco (TC), USA; and Biosystem Reagents and Instruments, Spain.

2.1.3. Experimental Design

One hundred and twenty eight male rats were housed in separate cages, and then divided into four groups and four subgroups of eight rats each. The route of administration (exposure) was oral intubation (o.i).Group A represented the normal control, which was fed with the normal rat diet and water *ad libitum.* Group B represented the negative control, which was exposed to 70% ethanol (kg body weight) only for seven days.

Group C and D were subdivided into C_1,C_2,C_3 , C_4, D1, D2, D3 and D4 exposed to 70% ethanol for seven days and later treated (o.i) daily, separately with 200,400,600 and 800 mg/kg body weight of *Gongronema latifolium* and *Piper guineense* extracts for twenty one (21) days [16].

2.2. Methods

2.2.1. Extraction of *Gongronema Latifolium* and *Piper Guineense*

The leaves of *Gongronema latifolium* and *Piper guineense* plants were purchased from Abakpa Market Abakaliki and brought to Department of Biochemistry Laboratory Ebonyi State University and dried under room temperature (25ºC to 27 ºC) for two weeks. After which the leaves were pulverized into coarse form with a milling machine [17]. The coarse form was sieved using standard sieve of 1mm size, and then 200 g was macerated in absolute ethanol (95%), which was contained in a 4-litre percolator. The system was left to stand for 48 hours and then filtered out with the help of a white filter cloth. The resulting ethanol extract was allowed to evaporate to dryness at room temperature. A known weight of the dry extract was determined. This was made into aqueous (saline) solution and ready for biochemical assays [18].

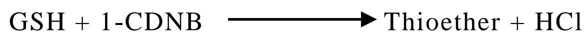
2.2.2. Acute Toxicity Test

The acute toxicity test was carried out according to [19].

2.2.3. Determination of Glutathione S-Transferase (GST) Activity

The glutathione S-transferase (GST) activity was monitored according to the method of [20] as described below:

The serum samples (0.lml) were added to a reaction mixture containing 2.3ml of 0.1M potassium phosphate buffer of pH of 6.5; 0.3ml of 1mM reduced glutathione (GSH) and 0.3ml of 1-CDNB to start the reaction. The contents were quickly shaken and the increase in absorbance measured at 340 nm for 4 minutes in a spectrophotometer. The basic equation for the reaction is given thus:



GSH + 1-CDNB → Thioether + HCl

2.2.4. Erythrocyte Glutathione Peroxidase Assay

Fifty microliters of RBC were mixed with 1ml cyanodilution mixture and then shaken. To 0.02 ml of diluted RBC are added five hundred microliters of potassium buffer, 0.2 ml of glutathione, 0.05ml of GR, 0.48 ml of distilled water, and 0.2ml of NADPH. After 10 minutes, 0.55ml of tertiary butylhydroperoxide was added into the mixture. Following, mixing, the absorbance of the solution was immediately monitored at 340nm for 100 seconds. The activity of glutathione peroxidase was determined by the change of absorbance and calculated as follows: mmole/1/s = 2813* (A sample-A blank).

2.2.5. Erythrocyte Superoxide Dismutase (SOD) Assay

One hundred microliters of RBC were mixed with 900 microliters of distilled water. Then, 60 microliters of RBC lysate is used for the superoxide dismutase assay. Ninety-four microliters of superoxide dismutase standard or diluted RBC lysate sample and 1.27ml of carbonate buffer were added into a cuvette. Just before reading the change of absorbance, 75ml of xanthine oxidase was added; absorbance was taken every 20 seconds continuously for 3 minutes on a spectrophotometer at 500 nm at room temperature. The changing rate of absorbance was used to determine superoxide dismuase activity. Concentrqation of superoxide dismutase in samples was determined by the comparison with the calibration curve from SOD standards [21].

2.2.6. Erythrocyte Catalase Assay

Red blood cell lysate was prepared by adding 1.2ml of distilled water to 0.2 ml of RBC. Then five hundred-fold dilution of RBC lysate by phosphate buffer was made before the determination of catalase activity. Immediately following the addition of 1ml phosphate buffer (blank) or hydroxide peroxide solution into 2ml RBC diluted lysate, the change of absorbance of RBC against blank at 240nm was recorded every 15 seconds for 1minute on a spectrophotometer. The activity of catalase was calculated by using the following equation: catalytic concentration (unit/l) = (0.23*log Al/A2)/0.00693, where Al is A240 at t = 0; A2 is A240 at t = 15 seconds.

2.2.7. Malondialdehyde (MDA)

Malondialdehyde(MDA) was assayed using the method of [22] . 0.5ml plasma was precipitated with 10% phosphotungstic acid. The mixture was centrifuged at 3000rpm for 10 minutes, and then suspended the sediment with 4ml distilled water. 0.5 ml of glacial acid and 0.5ml of 0.33% thiobabituric acid (TBA) were added Kept in water bath at 97 degree for 45 minutes. 0.05ml of 5 M HCl was added and extracted with 4ml of butanol and then read the absorbance at 535nm.

2.2.8. Enzymatic Assay of Cytochrome P450 2E1 Reductase

Cytochrome p4502e1 were carried out using method of [23].

2.3. Statistical Analysis

The results were expressed as mean ± SD and tests of statistical significance were carried out-using one-way ANOVA. The statistical package used was Statistical Package for Social Sciences (SPSS for windows, version 20). Data were expressed as mean± standard deviation. Values of $p<0.05$ were considered significant.

3. Results

Medicinal Plants	Dose mg/kg	Lethargy	Apathy	Noisy breathing	Sneezing	Body Weight	Sleeping
G latifolium	Control	N	N	N	N	N	N
	1000	N	N	N	N	N	N
	1500	N	N	N	N	N	N
	2000	N	N	N	N	N	N
	2500	N	N	N	N	N	N
	3000	N	N	N	N	N	N
Piper guineense	Control	N	N	N	N	N	N
	1000	N	N	N	N	N	N
	1500	+	+	+	+	+	+
	2000	+	+	+	+	+	+
	2500	+	+	+	+	+	+
	3000	+	+	+	+	+	+

Table 1: Acute Toxicity study in rats after 48 hours administration of ethanol extract of Piper guineense and G.latifolium leaves (n= 8) OBSERVATIONS (h)
+ =Significant changes
N = Normal

	Group	Dose mg/kg	T/D	Period of observation	% Mortalitties	% Survival
Gongronema latifolium	A1	Distillate water	4/0	48	0	100
	B1	1000	4/0	48	0	100
	B2	1500	4/0	48	0	100
	B3	2000	4/0	48	0	100
	B4	2500	4/0	48	0	100
	B5	3000	4/0	48	0	100
Piper guineense	A2	Distillate water	4/0	48	0	100
	C1	1000	4/0	48	0	100
	C2	1500	4/1	48	25	75
	C3	2000	4/3	48	**75**	25
	C4	2500	4/4	48	100	0
	C5	3000	4/4	48	100	0

Table 2: Percentages mortalities and survival of acute Toxicity study in rats after 48 hours administration of ethanol extract of Piper guineense and G.latifolium leaves (n=8)
T/D =Number of Wistar rats treated / number of deaths

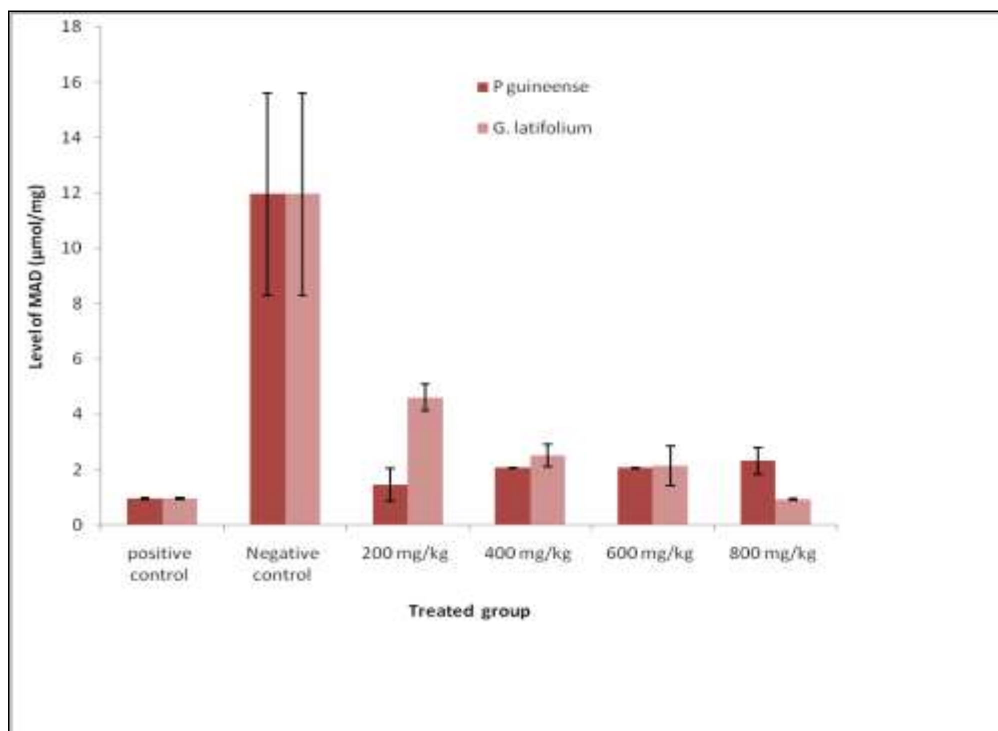


Figure 2: Effect of P. guineense and G. latifolium ethanolic extracts on the level of Malondialdehyde (MDA) in ethanol- exposed albino rats. There were significant (P<0.05) increase in the level of MDA in negative control when compared with normal control and treated groups. The results are mean± SD of eight rats in each group.

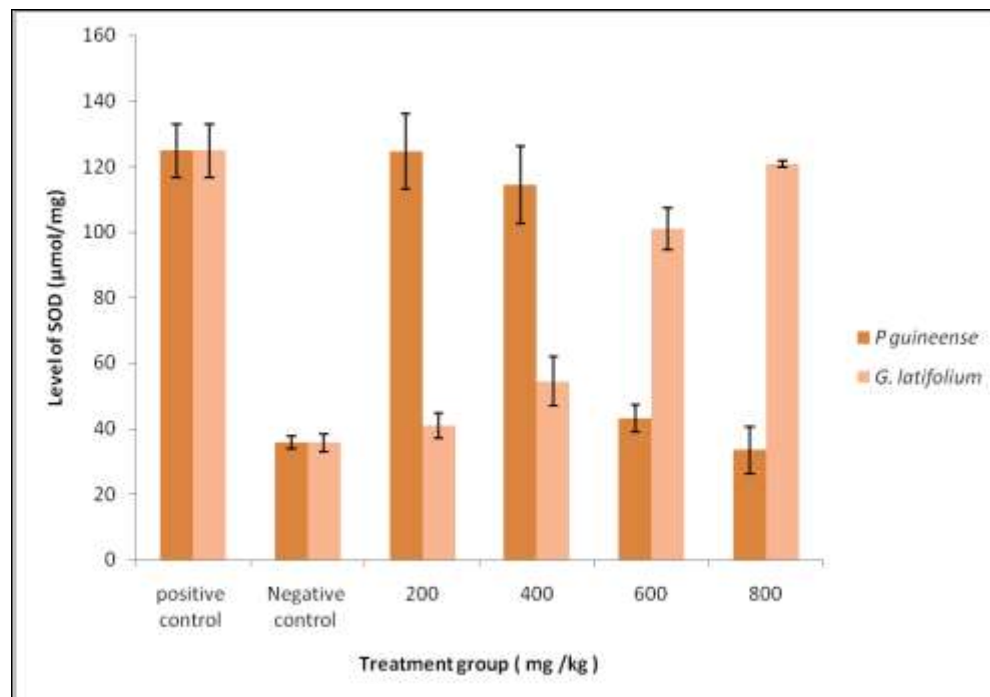


Figure 3: Effect of P. guineense and G. latifolium ethanolic extracts on the level of Superoxide dismutase (SOD) in ethanol-exposed albino rats. There were significant (P<0.05) decrease in the level of SOD in negative control when compared with normal control and treated groups. The results are mean± SD of eight rats in each group

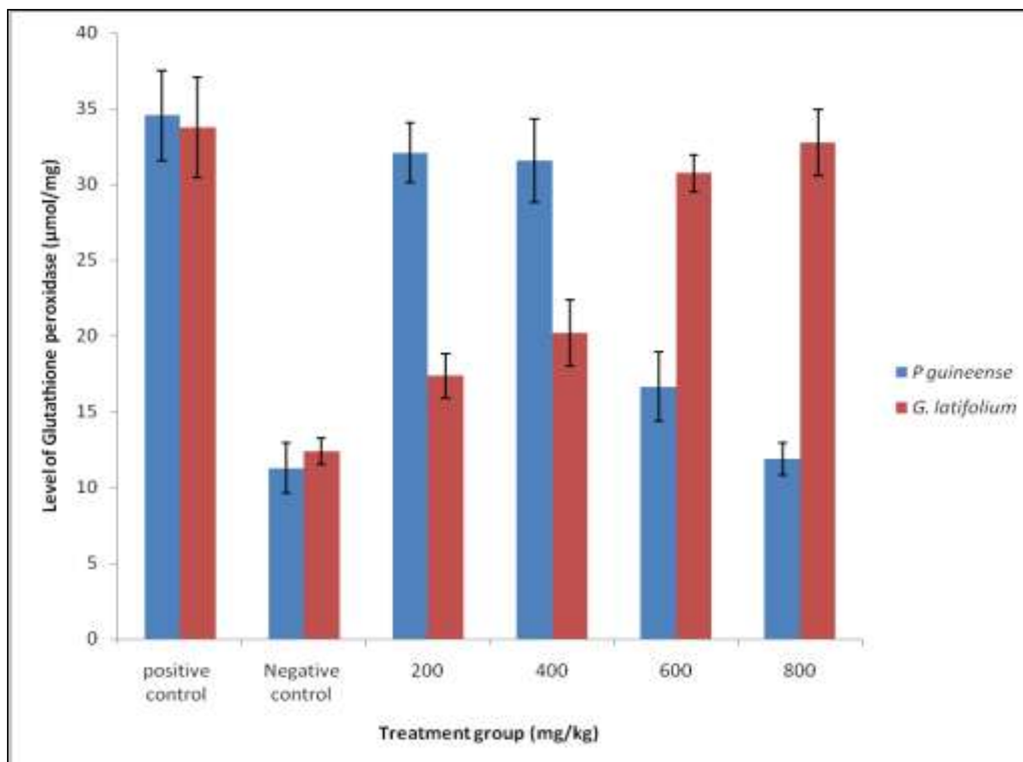


Figure 4: Effect of P. guineense and G. latifolium extracts on the level of Glutathione peroxidase in ethanol- exposed albino rats. The Glutathione peroxidise level in ethanol exposed groups decreased significantly (P<0.05) when compared with normal control and treated groups. The results are mean± SD of eight rats in each group

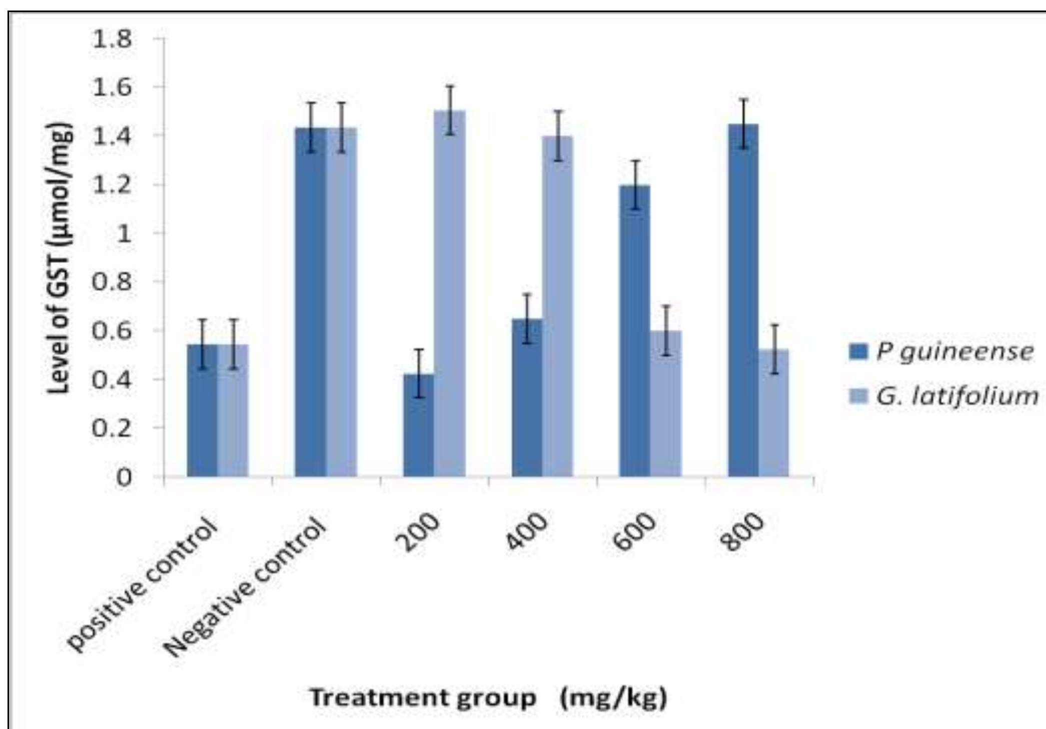


Figure 5: Effect of P. guineense and G. latifolium ethanolic extracts on the level of Glutathione S-transferase (GST) in ethanol-exposed albino rats. The GST level in ethanol exposed groups increased significantly (P<0.05) when compared with normal control and treated groups. The results are mean± SD of eight rats in each group

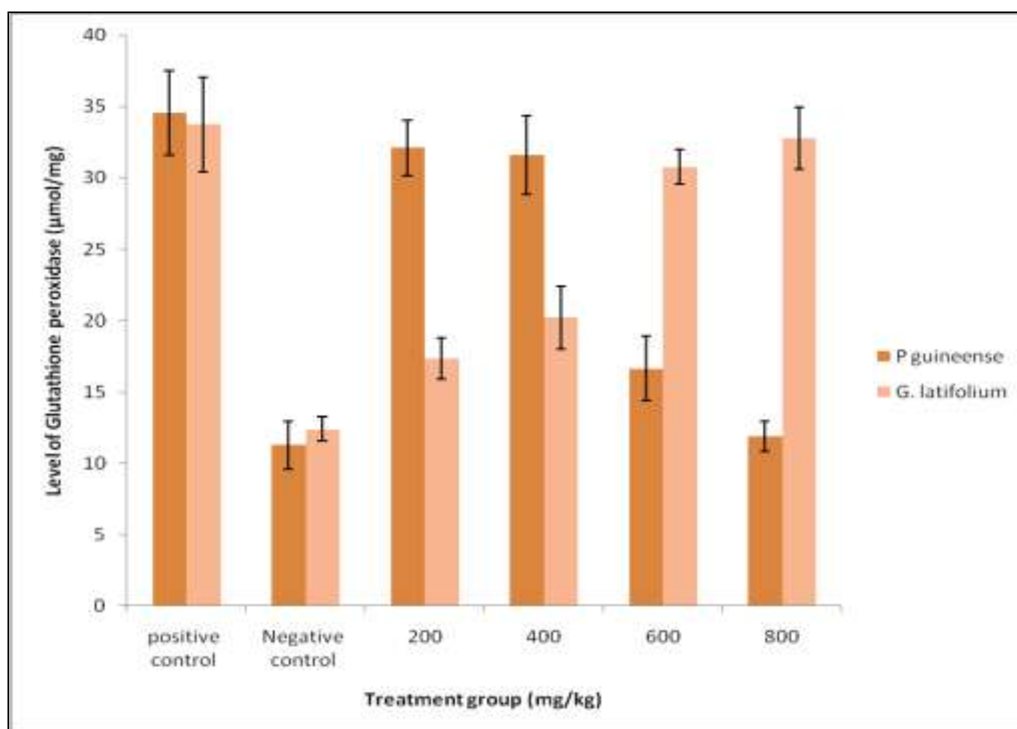


Figure 6: Effect of P. guineense and G. latifolium ethanolic extracts on the level of reduced glutathione (GSH) in ethanol- exposed albino rats. The reduced glutathione level in ethanol exposed groups decreased significantly (P<0.05) when compared with normal control and treated groups. The results are mean± SD of eight rats in each group

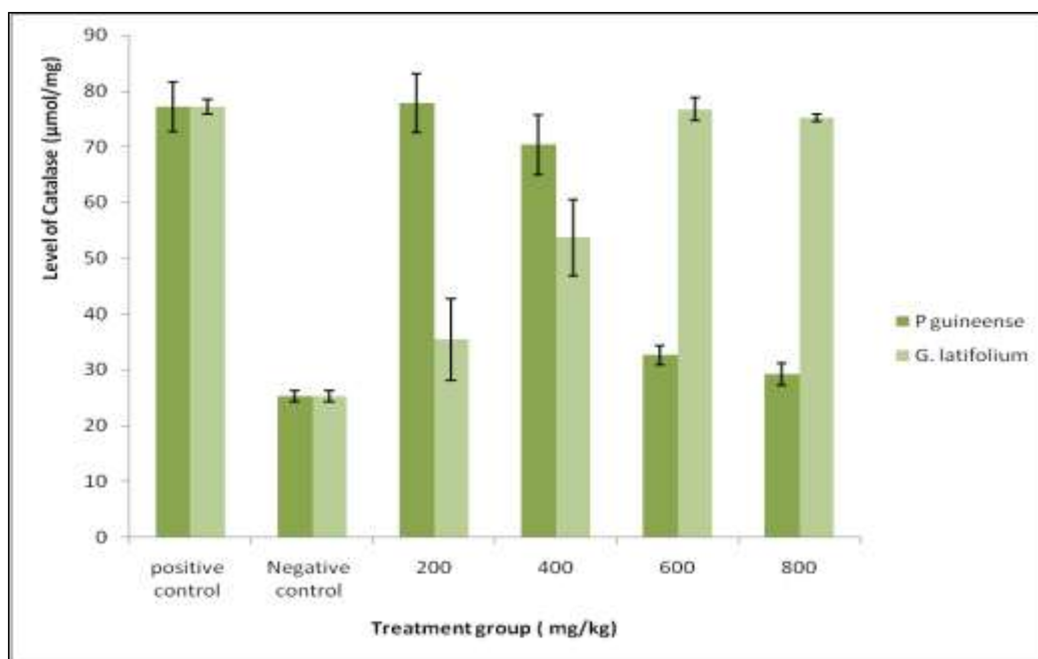


Figure 7: Effect of P. guineense and G. latifolium extracts on the level of Catalase in ethanol- exposed albino rats. The catalase level in negative control groups decreased significantly (P<0.05) when compared with normal control and treated groups. The results are mean± SD of eight rats in each group

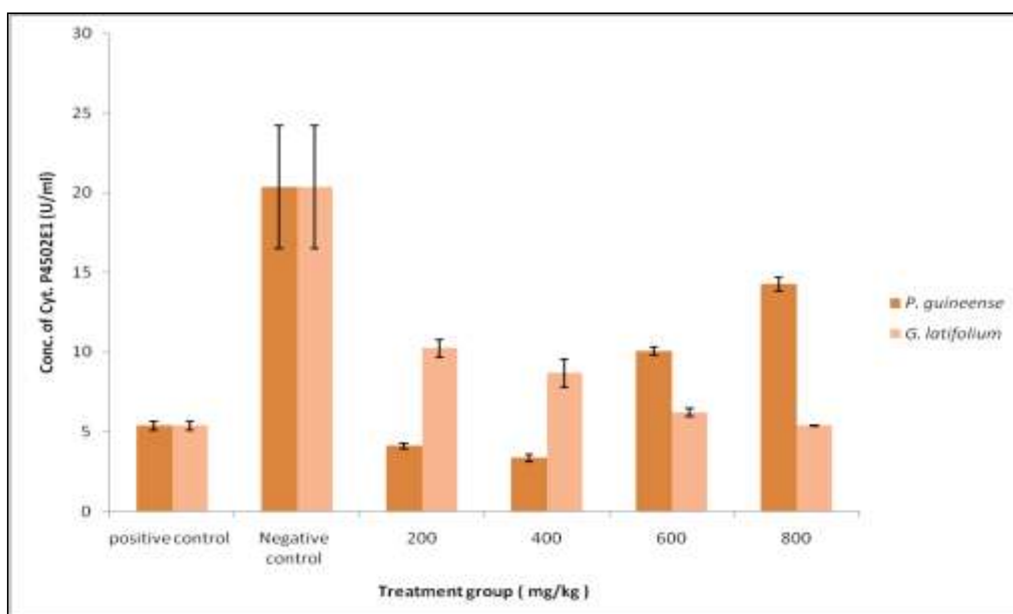


Figure 8: Effect of P. guineense and G. latifolium on Cytochrome P4502E1 in ethanol-exposed albino rats. The level of cytochrome p4502e1 in negative control groups increased significantly (P<0.05) when compared with normal control and treated groups. The results are mean± SD of eight rats in each group.

4. Discussion

4.1. Acute toxicity test of Piper guineense and Gongronema latifolium.

The acute toxicity study in rats showed that at 3000 mg/kg dose *G. latifolium* leaves are safe for consumption and for medicinal uses. At doses above this level however, the rats may exhibits some toxic changes. *G. latifolium* did not produce any major signs of clinical toxicity over the period of 48 hours observation period. This is in line the reports of [24] who stated that *G. latifoolium* is safe at these concentrations.

The acute toxicity study of *Piper guineense* showed the LD_{50} to be 1500 mg/kg dose. It could be assumed that the reactive metabolites of *P. guineense* could have contributed to toxicity of *Piper guineense*, as noticed in behavioural effects within the 48 hours observation (Table 1-2). Nevertheless, certain lethargy,noisy breathing etc were noted in treated rats with *Piper guineense*. Plants with LD_{50} above 1000 mg/kg by oral route are safe or of low toxicity [10]. These results confirm previous work done on the same substances [24]. Lethargy observed in rats could be due to stress caused by handling.

4.2. Malondialdehyde

The present study revealed that administration of 70% ethanol to rats induced oxidative liver damaged which is proved by an increase in malondialdehyde (MDA) level (index of membrane lipid peroxidation defense system) in liver of ethanol – treated rats when compared with normal rats, indicating that the liver is one of the target organs affected by ethanol toxicity (Figure 2). These results are in line with the reports demonstrated that chronic ethanol can cause oxidative damage and generation of reactive oxygen species (ROS) in different tissues including liver [25].

The decrease in such enzymes may be attributed to the ability of ethanol to induce lipid peroxidation which has the main cause of many deleterious effects on the cell membrane and coupled with inactivation of membrane bound enzymes [26]. It has been reported that lipid peroxidation is one of the major causes of ethanol induced toxicity, mediated by the production of free radical derivatives of ethanol [27]. The hepatotoxicity and oxidative damage induced by ethanol administration are also manifested by a significant increase in MDA in the untreated group (negative control). MDA a major reactive aldehyde that appears during the peroxidation of biological membrane polyunsaturated fatty acid [28]. The elevated MDA, found in this study, strongly proves the oxidative damage caused by ethanol [ ]. Treatment with *P. guineense* and *G. latifolium* ethanol extract in ethanol exposed rat recovered the increased MDA level with significant lowering of MDA content. The significant decrease in the MDA concecntration in the treated groups (200mg/kg and 400mg/kg) *P.guineense* and (600mg/kg and 800mg/kg) of *G.latifolium* confirms that treatment with *P. guineense* and *G. latifolium* could effectively protect against the hepatic lipid peroxidation induced by ethanol (Fig 2).This in line with the work of [28] who reported that free radicals involved in various human diseases can be possibly be prevented by antioxidants. Hence, the decrease in the level of MDA in treated groups is in line with previous work of [29-30].

4.3. Scavenging Enzymes

Antioxidant enzymes superoxide dismutase (SOD), glutathione (GSH), and catalase, ethanol significantly ($P < 0.05$) decreased superoxide dismutase (SOD), reduced ghtathione (GSH), catalase and increased glutathione s-transferase antioxidant enzymes. The

decrease in SOD activity along with GSH, catalase explains the increase in lipid peroxidation which is an indicator of oxidative stress that persists in the cell as shown in Figure 3. *Gongronema latitolium* and *Piper guineense* leaf extracts significantly increased (P<0.05) serum superoxide dismutase (SOD), reduce glutathione (GSH), catalase and decreased glutathione S-transferase (GST). Consequently, GPx is a selenium dependent enzyme has high potency in scavenging reactive free radicals [31]. In the present experiments, the levels of glutathione peroxidise activity in liver was elevated during alcohol intoxication to compensate the free radical scavenging effect utilized by the GSH as the substrate [32].When GPx activity in liver increased, the glutathione level is decreased in ethanol fed rats. Treatment with the herbal extract *G. latifolium* and *Piper guineense* significantly decreased the level GPx to normal level (Figures 3-7).Under oxidative stress conditions, the concentration of the glutathione, diminished through conjugation to xenobiotics, and by secretion of both the glutathione conjugates and glutathione disulfide from the affected cells [33]. Superoxides dismutase had been reported as one of the most important enzymes in the enzymatic antioxidant defence system [34]. It removes superoxide anion by converting it to hydrogen peroxide, diminishing the toxic effect caused by this radical. SOD work in conjunction with H_2O_2 removing enzymes, such as catalase and glutathione peroxidase. The defensive antioxidant enzyme next to SOD is catalase.CAT traps the harmful hydrogen peroxide and converts into water and oxygen. The activity of catalase was found to be decreased in ethanol intoxicated rats .The inhibition of catalase activity during ethanol induced toxicity may be due to the increased generation of reactive free radicals, which can create oxidative stress in the cells .The administration of herbal drugs *Gongronema latifolium* and *Piper guineense* inversed the catalase level in the liver and protected from the free radical induced oxidative stress [35].This results supports that, the antioxidant properties of the herbal drug was excellent as compared with the untreated and normal control.

The increase in the levels of antioxidant profiles that is SOD, GSH and catalase by *Gongronema latifolium* and *Piper guineense* leaf in a dose dependent fashion may be attributed to have biological significance in eliminating reactive free redicals that may affect the normal functioning of cells. This radical scavenging activity of extracts could be related to the antioxidant nature of polyphenols or flavonoids, thus contributing to their electron/hydrogen donating ability. Thus, the herbal drugs exert a beneficial effect in regenerating the endogenous scavenging enzymes as reported by [36].

4.4. Cytochrome P45O2E1

In the present study, the effects of ethanol extract *G. latifoliu*m and *Piper guineense* on CYP activities in ethanol exposed wistar rats were investigated to explore the possibility of therapeutic efficacy with herbal medicines.Cyp2e1 is induced by chronic alcohol consumption and assumes an important role in metabolizing ethanol to acetaldehyde at elevated ethanol concentrations km 8 to 10 mM, compared to 0.2 to 2.0mM for hepatic ADH [37]. The administration of ethanol extract of *Gongronema latifolium* and *Piper guineense* decreased the level of cyp2e1 significantly [P < 0.05] toward normal [Figure 8] . The results of this study implicated that *G. latifoliu*m and *Piper guineense* reduced the activities of Cyp2e1 significantly [P<0.05] towards normal. This is in line with the work of [38 -42].This may be attributed to the presence of phytochemicals in the extracts. This may be achieved by the extracts ability to stabilize various radicals involved in oxidative process [43-45].Our results were in agreement with the previous reports [46-52].

5. Conclusions

The results showed that the plant under study have bioactive components of medicinal value.It showed that *G latifolium* is not toxic in the doses investigated while *P.guineense* where found to have adverse effect at high dose. This work suggest that ethanol is a potential toxicant, which induced liver damage through lipid peroxidation process as indicated in the levels of oxidative marker index and scavenging enzymes investigated. It also suggested that the ethanol leaf extracts of *G.latifolium* and *Piper guineense* have the potential efficacy in protecting tissues and organs from peroxidative damage in ethanol-exposed rats' ress index and increase in level of scavenging enzymes. Thus medical properties of these plants should be explored and assessed.

6. References

1. Nagappa, A.N, P. A. Thakurdesai, N. V. Rao, and J. Singh, (2003). Antidiabetic activity of Terminalia catappa Linn fruits. Journal of Ethnopharmacology, vol. 88: 45–50,
2. Holman, R and R. C. Turner, (1999). "Oral agents and insulin in the treatment of NIDDM," in Text Book of Diabetes, J. Pickup and G. Williams, Eds., pp. 467–469.
3. Kameswara, B, M. M. Kesavulu, and C. Apparao, (2001). "Antihyperglycemic activity of Momordica cymbalaria in alloxan diabetic rats," Journal of Ethnopharmacology, vol. 78: 67–71
4. Kameswrarao,B, R. Giri, M. M. Kesavalu, and C. Apparao (1997). "Herbal medicine," in The Management by Indigenous Resources. Journal of Science Education in Developing Countries 90: 375–377.
5. Seitz, H.K., and Stickel, F., (2006). Risk Factors and Mechanisms of Hepatocarcinogenesis with Special Emphasis on Alcohol and Oxidative Stress. Biological Chemistry, 387:349–360.
6. Seifert, w.f., bosoma, A. and Brouwer, A. (1994). Liver Fibrosis in rats. Journal of Hepatology. 19:193-301.
7. Sharma, S., Hullati, K. K., Prasanna, S. M., Kuppast, I. J. and Sharma, P. (2009). Comparative study of Cuscuta reflexa and Cassytha filiformis in diuretic activity. Pharmacognosis. Resource. 1: 327-330.
8. Shah JH. (1988). Alcohol decreases insulin sensitivity in healthy subjects. Alcohol Alcoholism. 23(2):103-9.
9. Shakhashiri, B.Z., (2009). Chemical of the Week; Ethanol. General Chemistry, 1:1-2.

10. Sheille R., Mondello L., Marriott P., Dugo G. (2002). Characterisation of lavender essential oils by using gas chromatography–mass spectrometry with correlation of linear retention indices and comparison with compre- hensive two-dimensional gas chromatography. Journal of Chromatography A, 970: 225–234.
11. Sofowora, A. (1982). Medicinal plant and traditional medicine in Africa. John Wiley and sons, New York. p. 44.
12. Sofowara,A., (1984). Medicinal Plants and Traditional Medicines in Africa. Idadan: Spectrum Book Ltd.p. 23
13. Sofowora, A. (2006). Medicinal plant and traditional medicine in Africa. Reprint edition, spectrum Books Ltd.Ibadan,p. 150-160.
14. Sovljanski R (1990). Heavy metal contents in medicinal and spice plants cultivated in Ygoslavia. Herbal Hung. 29(3):59-63.
15. Spala,O.O., Isaiah,O.N., Sammy,S.K., John,C.B., and Charles,M.M., (2007). The Larvicidal Activity of P.Guineense and Spilanthes Mauritiana Crude-Powder Against Anopheles Gambiae and Culex quinquefasciatus in Kili District, Kenya. Journal of Biological Sciences, 7:1215-1220.
16. Suresh, V., Sruthi, V., Padmaja, B. and Asha, V.V. (2011). I vitro anti inflammatory and anti cancer activities of Cuscuta reflexa Roxb. Journal Ethnopharmacology. 134: 872-877.
17. Szuki, K., Tanka, M., Watanable, N. amnd Saito, S. (2008). Neurotrophin receptor is a marker for precursor of fibroblast in mouse fatal liver. Journal of Gastroenterology. 1:270-281.
18. Tapsell, L.C., Hemphill, I., and Cobiac, L., (2006). Health Benefits of Herbs and Spices: the Past, the Present, the Future. Medical Journal of Austria, 185(4):4–24.
19. Tiku, S. C. (2007). Piper guineense leaf extract on reproductive organ of male rats. Journal of Natural Product. 44: 78-81.
20. Ugochukwu, N.H., and Babady, N.E., (2002). Antioxidant Effects of G.latifolium in hepatocytes of rat models of non-insulin dependent diabetes mellitus. Fitoterapia, 73(7-8):612–618.
21. Ujowundu,C.O., Kalu, F.N., Nwaoguikpe,R.N., Okechukwu,R.I., and Ihejirika,C.E., (2012). The Antioxidative Potentials of G.latifolium on Diesel Petroleum Induced Hepatotoxicity. Journal of Applied Pharmaceutical Science, 2(1):90-94.
22. Sivropoulou A., Papanikolaou E., Nikolaou C., Kokkini S., Lanaras T., Arsenakis M. (1996), Antimicrobial and cytotoxic activities of Origanum essential oils. Journal Agricultural Food Chemistry. 44: 1202-1205.
23. Searle AJ and Willson RL (1980). Glutathione peroxidase: effect of superoxide, hydroxyl and bromine free radicals on enzyme activity; International Journal Radiant Biology 37: 213-217.
24. Senthil, K. R., Ponmozhi, M., Viswanthan, P. and Nlini, N. (2003). Enzyme catalyzed reaction. Journal of Nutrition and Biochemistry14: 452-458.
25. And Willson RL.(1980).Glutathione peroxidase effect of superoxide hydroxyl and bromine free radicals on enzyme activity,.International journal radiant biology 37:213- 217.
26. Senthil, K. R., Ponmozhi, M., Viswanthan, P. and Nlini, N. (2003). Enzyme catalyzed reaction. Journal of Nutrition and Biochemistry. 14: 452-458.
27. Tamás, L., Huttová, J., Mistrk, I., and Kogan, G., (2002). Effect of Carboxymethyl Chitin-Glucan on the Activity of Some Hydrolytic Enzymes in Maize Plants. Chemistry Papers, 56(5): 326–329.
28. Taylor, R. and Agius, L. (2008). Enzyme property. Journal of Biochemistry 250: 650-740
29. Wang, CS; Chang, Ting-Tsung; Yao, Wei-Jen; Wang, Shan-Tair; and Chou, Pesus (2012). Impact of increasing alanine aminotransferase levels within normal range on incident diabetes. Journal of Formos Medical Association111 (4): 201–8.
30. Wanner, Barry L.; and Patrick Latterell (August 7, 1980). Mutants Affected in Alkaline Phosphatase Expression: Evidence for Multiple Positive Regulators of the Phosphate Regulon in Escherzchza Coli. Genetics 96 (2): 353–366.
31. Tiku, M.L, Narla, H., Jain, M., Yalamanchili, P., (2007). Glucosamine Prevents in Vitro Collagen Degradation in Chondrocytes by Inhibiting Advanced Lipoxidation Reactions and Protein Oxidation. Arthritis Research Therminology, 9(4):76.
32. Thomasson H. (2000). Alcohol elimination: Faster in women? Alcoholism Clinical Experimental Resources. 24(4):419- 20.
33. Tsukamoto, H., and Xi, X.P., (1989). Incomplete Compensation of Enhanced Hepatic Oxygen Consumption in Rats with Alcoholic Centrilobular Liver Necrosis. Hepatology, 9:302–306.
34. Tuma, D.J., (2002). Role of malondialdehyde-acetaldehyde adducts in liver injury. Free Radical Biology and Medicine, 32:303–308.
35. Tuma, D.J., and Casey, C.A., (2003). Dangerous By products of Alcohol Breakdown: Focus on Adducts. Alcohol Research and Health, 27:285–290.
36. Tuma, D.J., Thiele, G.M., and Xu, D., (1996). Acetaldehyde and Malondialdehyde React Together to Generate Distinct Protein Adducts in the Liver During Long-Term Ethanol Administration. Hepatology, 23:872–880.
37. Udosen,E.O., Egbung,G.E., Onofiok,U.L., Robert,A.E., Ekam,V.S., Iwara,A.I., and Odey,M.O., (2012). Effect of Ethanol Root and Twig Extract of G.latifolium (Utazi) on Kidney Function of Streptozotocin Induced Hyperglycemic and Normal Wistar Rats. Journal of Medicine and Medical Sciences, 3(5):291-296.
38. Ueno, Y., Adachi, J., and Imamichi, H., (1996). Effect of the Cytochrome P-450IIE1 Genotype on Ethanol Elimination Rate in Alcoholics and Control Subjects. Alcoholism: Clinical and Experimental Research, 20:17–21.
39. Ugochukwu, N.H., Babady, N.E., Cobourne, M., and Gasset, S.R., (2003). The Effect of G.latifolium Leaf Extract on Serum Lipid Profile and Oxidative Stress of Hepatocytes of Diabetic Rats. Journal of Biological Sciences, 28:1–5.
40. Vanderlinde, R.E. (1986). Review of pyridoxal phosphate and liver disease. Journal of Clinical Laboratory Science. 16:79-93.

41. Vasudeva, R.Y (2002). Immunomodulatory activity of Achyranthes aspera on the elicitionof antigen-specific murine antibody response. Pharmacology Biology 57:175-178.
42. Vaughn, K.C. (2003). Dodder hyphae invade the host: a structure and immunocytochemical characterization. Protoplasma, 220: 189-200.
43. Veldman, R.G,and Meinders, A.E. (1996). On the mechanism of alcohol-induced pseudo- Cushing's syndrome. Endocrinology Revolution 17:262-5.
44. Vicent, C. L. O'Neil, P. G.and Wang., (2011). Malondialdehyde, Encyclopedia of Reagents for Organic Synthesis. America Journal of Clinical Nutrition 94: 422–430.
45. Vijaya K.S, Sankar P,and Varatharajan R,(2009). Anti-inflammatory activity of roots of Achyranthes aspera. Pharmaceutical Biology 47:973-975l.
46. Voon B.H and Kueh H.S (2009).The nutritional value of indigenousfruits and vegatables in Sarawak.Asia Pacific Jonal of clinical nutrition 8(1):24-31.
47. Seifert, W. F., Bosma, A. and Brouwer, A. (1994). Liver fibrosis in rats. Journal of Hepatology 19: 193-301.
48. Sunderman F.W Jr. marzouk A, Hopfer S M, Zaharia O and Reid M
49. C (1985). Increased lipid peroxidation in tissues of nickel chloride-treated rats; Annual Clinical Laboratory Science. 15 229-236.
50. Sillanaukee P. (1996). Laboratory markers of alcohol abuse. Alcohol Alcoholism. 31(6):613- 16.
51. Singh SP, Pullen GL, Srivenugopal KS, Yuan X-H, Snyder AK. (1992). Decreased glucose transporter I gen expression and glucose uptake in fetal brain exposed to ethanol. Life Science. 51:527-36.
52. WHO (2005). Quality Control Methods for Medicinal Plant Materials, Revised, Geneva.Wong MK, and Tan Paper company, Wee.pp 76.
53. Worrall, S., and Thiele, G.M., (2001). Protein Modification in Ethanol Toxicity. Adverse Drug Reactions and Toxicological Reviews, 20:133–159.