

Varying influence on different biomarkers related to hepato-recovery events in liver-injured rats when treated with extracts of *Andrographis paniculata*, *Phyllanthus amarus* or their active principles individually and in combination.

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Abstract

Water extracts of *Phyllanthus amarus* and *Andrographis paniculata* were used individually and in combination to investigate their hepato-recovery potential using carbon tetrachloride-treated, liver injured albino wistar rats. Toxicity study performed in female swiss albino mice was found to be satisfactory. HPLC analysis revealed presence of andrographolide in *A. paniculata* and phyllanthin as well as gallic acid in *P. amarus* extracts. Histology analysis indicated extensive injury to liver tissue in experimental rats. Efficacy of polyherbal combination was less compared to individual extracts in reducing bilirubin concentration. SGPT and SGOT reduction by polyherbal combination was found to be similar to that of individual extracts. Co administration of extracts from both plants exhibited maximum reduction in γ GT concentration. However, polyherbal combination was not found to have any significant effect in reducing blood cholesterol when compared to individual extracts. Gallic acid, a component of *P. amarus* when used in pure form, was found to play significant role in reducing most liver injury biomarkers when compared to phyllanthin, the other active component of *P. amarus*. However, the latter was found to be more effective in reducing blood cholesterol compared to gallic acid. We concluded that polyherbal combination of *A. paniculata* and *P. amarus* does not always present a uniform, beneficial effect in cases of liver injury when monitored by several biomarkers. Further, gallic acid was found to be an equally important constituent of *P. amarus* extract along with phyllanthin in affecting hepato-protective activity in albino wistar rats.

Keywords: *Phyllanthus amarus* or their active principles individually and in combination

Introduction

The liver is one of the most important organs of our body. Apart from maintaining and performing vital physiological functions, it also regulates homeostasis of the body. It has role in wide range of biochemical pathways that range from growth, disease resistance and supply of nutrition to energy provision and reproduction [1]. The most important and widely known function of liver however is metabolism of fat, protein and carbohydrates as well as detoxification, bile secretion and storage of vitamins. Thus avoiding liver damage is one of the most crucial internal health care that one should undertake on a routine basis [2].

A large number of drugs are known to damage liver when administered in excess of the recommended dose in humans. Some drugs can cause hepatitis in small doses, even if the liver breakdown system is normal. Large doses of many medications can damage a normal liver.

Acetaminophen, a common constituent of pain killers and fever reducers are recognized as potent chemical agents that cause liver

inflammation. Even when taken at a dose that is marginally higher than that prescribed, the drug is known to induce liver damage. Other drugs such as Nonsteroidal anti-inflammatory drugs (NSAIDs) may also cause drug-induced hepatitis [3, 4].

Yet another disease of the liver is one that is caused by excess consumption of alcohol which results in fatty liver, alcoholic hepatitis, and chronic hepatitis with hepatic fibrosis or cirrhosis [5]. At molecular level, there is enhanced secretion of pro inflammatory cytokins such as TNF alpha, IL6 and IL8, oxidative stress, lipid peroxidation and acetaldehyde toxicity. The pathological symptoms associated with alcohol induced liver injury are wide in spectrum ranging from mild steatosis to hepatocellular carcinoma in the back drop of liver cirrhosis [6]. Although Macrovesicular steatosis is the most common pathological events associated with alcohol abuse [7], increasing evidences of degenerated hepatocytes, infiltrating neutrophils, mallory bodies and fibrosis indicate rapid consolidation of steatohepatitis [6]. However, although liver cells have a high level of recovery rate and even with 75% of the cells being dead, liver has capacity to function as

normal [8], the rate of incidence of liver cirrhosis in people specially with a habit of excess consumption of alcohol is almost 15-20% [9]. Even though data from clinical settings spanning across 35 years place glucocorticoids as a potent drug for liver cell recovery, its corrective effects on liver cell damage are still doubted [10]. Pentoxifylline, a nonselective phosphodiesterase inhibitor that increases intracellular cAMP and cGMP has also been identified as a potent drug with antifibrotic effect which function by blocking profibrogenic cytokine and procollagen I expression [11]. However, this drug is associated with gastrointestinal toxicity, consisting mainly of nausea and vomiting [12].

Complementary and alternative medicine (CAM) has also been under focus to address liver injury. *Silybum marianum* (milk thistle) has been known to be hepatoprotective in nature although a sound conclusion regarding its proven efficacy still remains elusive [13, 14]. In humans, alcoholic hepatitis and cirrhosis has been shown to be linked to reduce hepatic D – adenosylmethionine (SAM), cysteine and glutathionine levels [15]. It has further been demonstrated in primate and other animal models that early stages of liver injury is associated with reduced levels of SAM and that external supplementation with it can reverse effects of liver damage and mitochondrial injuries [16]. However, this medicine also comes under scanner due to conclusions from Cochrane review where adequate supporting evidence for or against SAM was found missing [17]. Betaine (trimethylglycine), found in several food and food supplements [18] has the chemical property of transferring one methyl group from homocysteine to convert it into methionine in the liver. This therefore leads to detoxification of homocysteine and S-adenosylhomocysteine along with restoration of SAM levels, retardation of apoptosis and mounting of negating effects relating to steatosis [19]. Betaine also restores generation of phosphatidylcholine *via* the phosphatidylethanolamine methyltransferase pathway and protects hepatic cells from oxidative stress by retarding breakdown of sulfur-containing amino acids [20] thus leading to over all restoration of effects of alcoholic steatosis. However, betaine has also been shown to enhance LDL cholesterol in blood.

It is therefore found that liver although being one of the most important and sensitive organ of our body is prone to injury by a range of common drugs as well as unhealthy habits such as excess intake of alcoholic drinks. Further, common hepatoprotective agents are either associated with significant side effects or disputed efficacy data.

In this backdrop, we identified two important medicinal plants, *viz.*, *Andrographis paniculata* and *Phyllanthus amarus* and initiated investigation to address two important questions. First, whether co administration of extracts from both the plants in an animal model exceeds the benefits gained from that of individual extracts and two, whether gallic acid plays an equal role in imparting hepatoprotective property by *Phyllanthus amarus* as *Phyllanthin*.

Materials and Methods

Plant collection & Extract preparation

Phyllanthus amarus Schum. & Thonn seedlings and *Andrographis paniculata* Nees were collected from a farmland in Kalyan (Mumbai, India) and Zandu Pharmaceuticals (Vapi, Mumbai, India) respectively, authenticated from Agarkar Research Institute (ARI), Pune (India) and maintained in the greenhouse of the laboratory. Prior to use, the plants were harvested, thoroughly washed in sterile distilled water, dried in oven at 50 °C to complete dryness and powdered. The powder was passed through a sieve (sieve no. 85), stored in airtight containers and used for animal study.

Animal maintenance

The study was carried out as per the guidelines laid down by “OECD guidelines 420”, namely, Fixed Dose Procedure (Evident Toxicity) (OECD, 2000). The animal rooms were maintained on a cycle of twelve hours light and twelve hours darkness, at a relative humidity of 70 ± 5 % and housed in polyurethane rat and mice cages with risk husk bedding, cleaned daily, provided with drinking water *ad libitum* and fed on commercially available mice feed (Amrut Feed, Maharashtra, India).

Toxicity study

Female Swiss Albino mice, weighing 20 to 25 g were used to assess the acute toxicity of the plant extracts. Water extracts of the plant materials were administered orally *via* a gavage in single doses to 3 animals in each group which were starved for 18 hours. For assessing toxicity, animals received 2 g and 5 g / Kg body weight of the plant powder slurry made in water. Clinical observations post administration of plant extract included monitoring of condition of the fur, damaged areas of skin, subcutaneous swellings or lumps, areas of tenderness, abdominal distension, eyes (for dullness, dryness, discharges, opacities, pupil diameter, ptosis), color and consistency of the faeces, condition of teeth, breathing abnormalities and gait every 24 hours for a period of 14 days.

Efficacy study

For efficacy study, Albino Wistar rats (Female) of body weight ranging between 100 – 200 grams were procured from Bharat Serum and Vaccines, Mumbai and maintained as described before. The animals were randomly divided into 10 groups of five animals each as described in Table 1. The dosage regimen for each animal of a group is described in Table 2. Rats with liver injury were artificially created by treating with Carbon tetrachloride (CCl₄) [21].

Parameters Observed

Cage side observations included general behavioral changes, daily food and water consumption and daily body weight changes. Biochemical tests on blood and tissue (liver) and histopathological evaluations of the liver were done as described by Preedy [22].

Sacrifice of animals

Animals were sacrificed on the 4th day after administration of the plant extracts. Food was discontinued 18 hours prior to sacrifice of

Table 1: Description of groups of animals used to conduct efficacy study.

Serial No.	Group No.	Description
1	1	Untreated animals
2	2	Rats administered with Carbon Tetrachloride (Hosono-Fukao et al., 2009)
3	3	Rats with induced liver dysfunction and treated with 7 mg/Kg body weight silymarin orally;
4	4	Rats with induced liver dysfunction and treated with 300 mg/Kg body weight <i>A. paniculata</i> extract orally.
5	5	Rats with induced liver dysfunction treated with 660 mg/Kg body weight <i>P. amarus</i> extract orally
6	6	Rats with induced liver dysfunction treated with 500 mg/Kg body weight gallic acid orally.
7	7	Normal rats treated with Methanol + Phosphate Buffer Saline orally (Control)
8	8	Rats with induced liver dysfunction treated with 3.8 mg/Kg body weight phyllanthin dissolved in methanol + PBS orally
9	9	Rats with induced liver dysfunction treated with phyllanthin and gallic acid mixture
10	10	Rats with induced liver dysfunction treated with extracts of <i>P. amarus</i> and <i>A. paniculata</i> (1:1) [Polyherbal combination]

animals but water was provided *ad libitum*. The animals were weighed and then sacrificed by overdose of ether. Blood was withdrawn by cardiac puncture under ether anesthesia and samples were collected in clean, labeled, pre-heparinised vials, immediately stored at 4 C and later analyzed (within 8 hour after collection) for parameters as follows: cholesterol, GOT, GPT, Gamma-GT and total proteins.

Liver was excised during autopsy, rinsed in 0.9% saline, blotted dry of saline & excess of blood and weighed. A small piece (5mm X 5mm) of central portion of the largest lobe of liver was cut and fixed in Bouin's fixative (Picric acid:Formalin:Acetic acid in the proportion 75:25:5) for histopathological examination.

Blood Biochemistry

Evaluation of marker enzymes in the blood included measuring of Bilirubin, SGPT, SGOT, γ -GT and cholesterol. The levels of all these marker enzymes were determined using a commercially available diagnostic reagent kit (Vital Diagnostics Pvt. Ltd, INDIA).

HPTLC estimation

For HPTLC quantification of active principles, the whole plant of *Phyllanthus amarus* was dried, powdered and used for the extraction of active constituent 'Phyllanthin'. Petroleum ether extract reconstituted in chloroform was found to be optimal for the extraction of phyllanthin. Each powdered sample (0.5 g) was mixed with 10 ml petroleum ether and kept overnight. Extract was then filtered through Whatmann's filter paper No. 1 and the filtrate was evaporated to dryness at room temperature. Dried residue was reconstituted in 5 ml chloroform and used for HPTLC analysis (CAMAG, Switzerland). A high purity standard (Sigma Aldrich, USA) at a concentration of 0.5 mg in 1 ml methanol (0.5 mg / ml) was used as reference. For estimation of Gallic acid and andrographolide, the powdered samples (1.0 gm) were mixed with 10 ml methanol, kept overnight and resulting extract filtered through Whatman's filter paper No. 1. The filtrate was evaporated to dryness at room temperature and the dried residue was dissolved in 5 ml methanol and used for HPTLC analysis. A high purity standard (sigma Aldrich, USA) at a concentration of 0.1 mg in 1 ml methanol (0.1mg / ml) was used as reference standard.

Results and Discussion

Phyllanthus amarus belongs to the family Phyllanthaceae and is a member of one of the largest genus within this group [23]. *Andrographis paniculata* is a herbaceous plant belonging to the family Acanthaceae and is a native to India and Sri Lanka. Since a long period of time, both the plants are known for their high medical value [24, 25]. *A. paniculata* has been widely studied for its hepatoprotective property [26, 27]. Extensive studies indicate that andrographolide, the active component in extracts of *A. paniculata* has high degree of antioxidant effect because of its property to activate antioxidant enzymes which in turn catalyze the reaction of oxidants and affect curing in severe liver damage [28]. *P. amarus* too has hepatoprotective properties [29] and is known to have gallic acid and phyllanthin as two important phytochemical components [30]. Toxicity study undertaken to evaluate extracts from both the plants indicated that there was no change in body weight, food and water consumption by the animals from all dose groups (2 g / Kg body weight and 5 g / Kg body weight). Further, there was no mortality recorded even at the highest dose level, i.e., 5 g / Kg body weight, indicating that extracts of *P. amarus* and *A. paniculata* had no significant toxic effect in mice. Medicinal plants in several instances had been found to be toxic to experimental animals. *Fagara zanthoxyloides* for example, is a medicinal plant used extensively in treating malaria and other infections. In a study using extracts of this plant fed orally to mice, Ogwal-Okeng [31] demonstrated distinct signs of cerebral irritation in the animals before death. Histopathological investigations of the viscera indicated congestion and focal necrosis of the liver and renal tubules. Therefore, a favorable toxicity study of *P. amarus* and *A. paniculata* was encouraging and in line with earlier studies.

Estimation of phyllanthin, gallic acid and andrographolide
Phyllanthin bands were resolved in Hexane: Ethyl acetate (2 : 1) solution with 10 minutes tank saturation. The plate was scanned at 254 nm before and 580 nm after derivatization. The maximum absorption was achieved at 254 nm. The active principle phyllanthin appeared as a blue colored band on visual observation after derivatization with 10 % methanolic sulphuric acid and heating the plate at 110 C for 5 minutes. The active constituent resolved at Rf value of 0.45. The peak areas at Rf 0.45 confirm the presence

of phyllanthin in all the tracks. Quantification was achieved using standard phyllanthin with 99% purity. The amount of phyllanthin present quantified in mother plant was 0.57% on dry weight basis (Figure 1a & 1d). For estimating gallic acid and andrographolide, the selected solvent systems were chloroform: ethyl acetate: formic

acid in the ratio 5: 4: 1 and chloroform: methanol (9 : 1) respectively. Twenty minutes of tank saturation gave good resolution of bands. The maximum absorption was achieved at 254 nm.

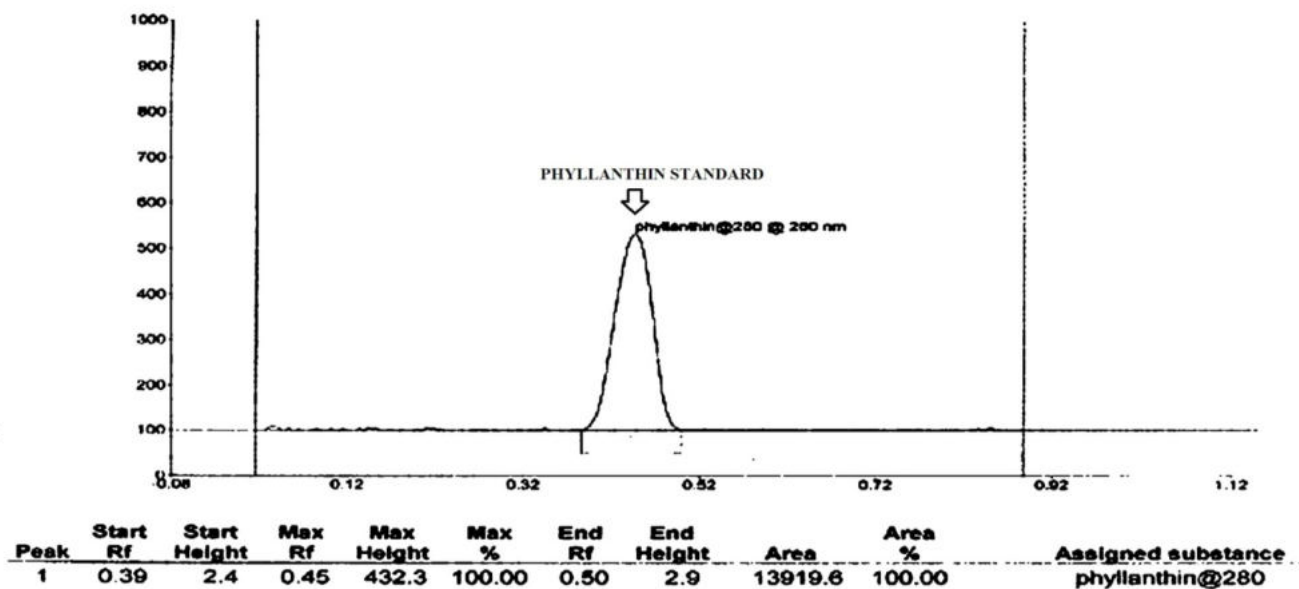


Figure 1a

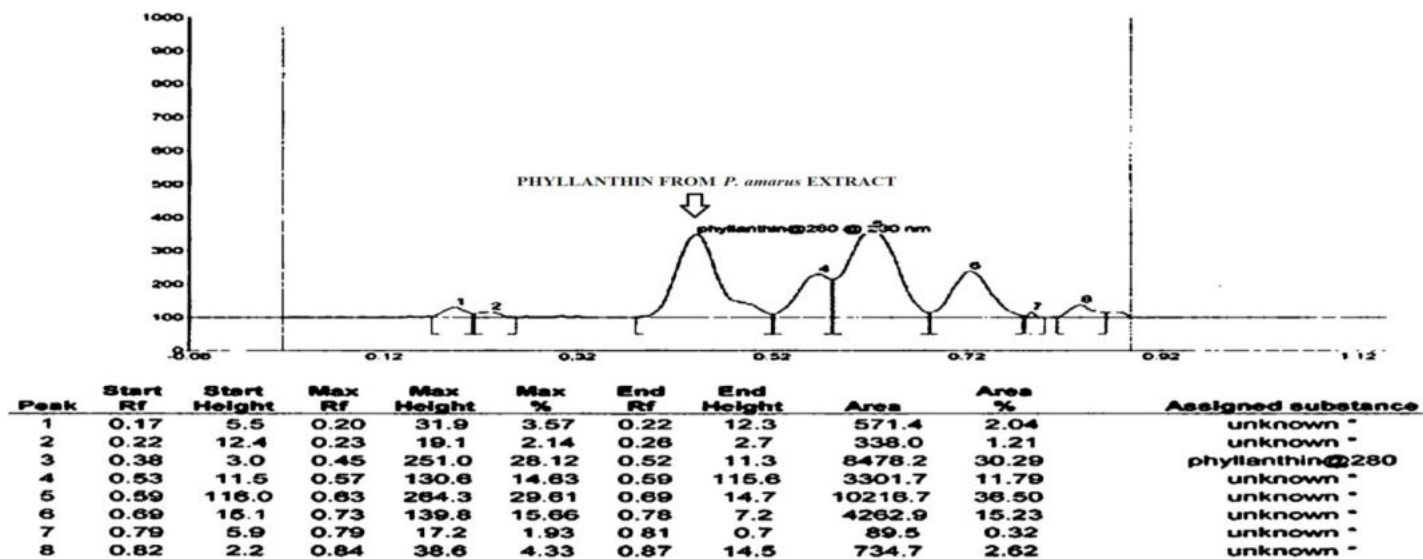


Figure 1b

Gallic acid and andrographolide appeared as a blue and brown colored band on visual observation after derivatization with 10 % methanolic sulphuric acid and heating the plate at 110 C for 5 minutes. Gallic acid resolved at Rf value of 0.41 (Figure 1c and 1d)

while andrographolide resolved at Rf value of 0.43 (Figure 1e and 1f) in all tracks. Quantification was achieved using respective standards (Sigma Aldrich, USA). The amount of gallic acid and andrographolide present in *P. amarus* and *A. paniculata* were

found to be 0.29 % and 5.77% respectively on dry weight basis. This was in line with results reported earlier on estimation of these

compounds[30,32].

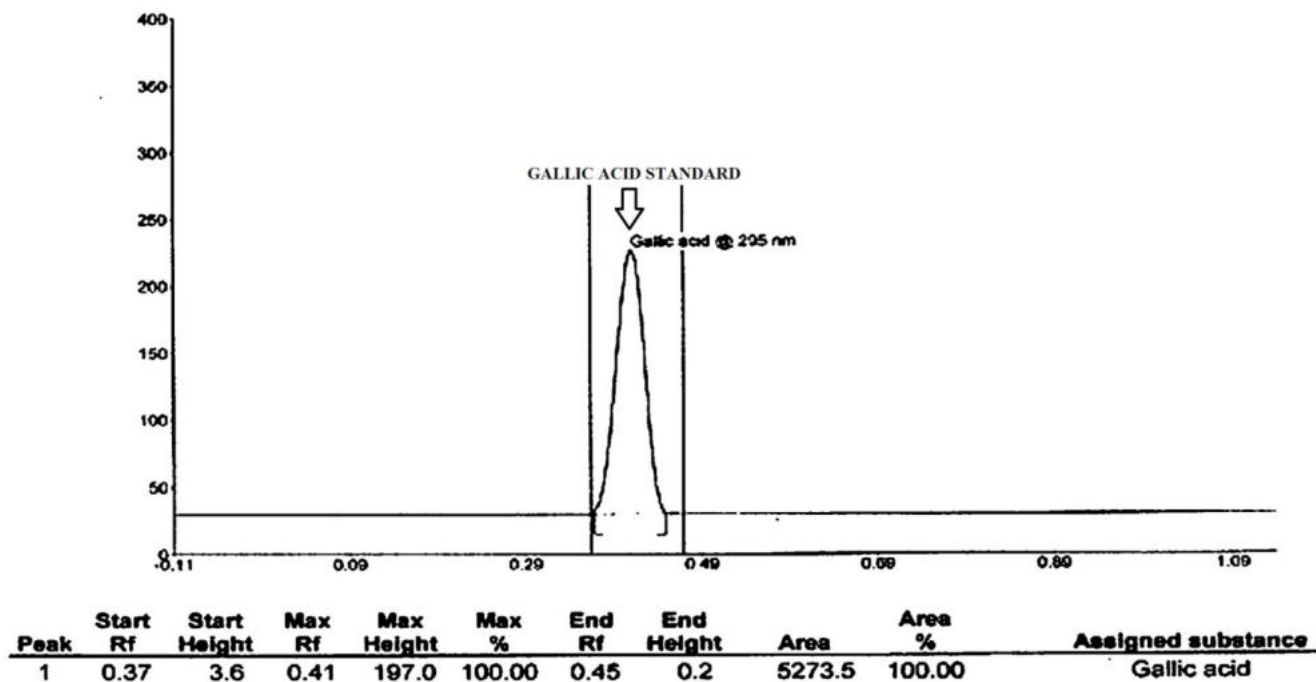


Figure 1c

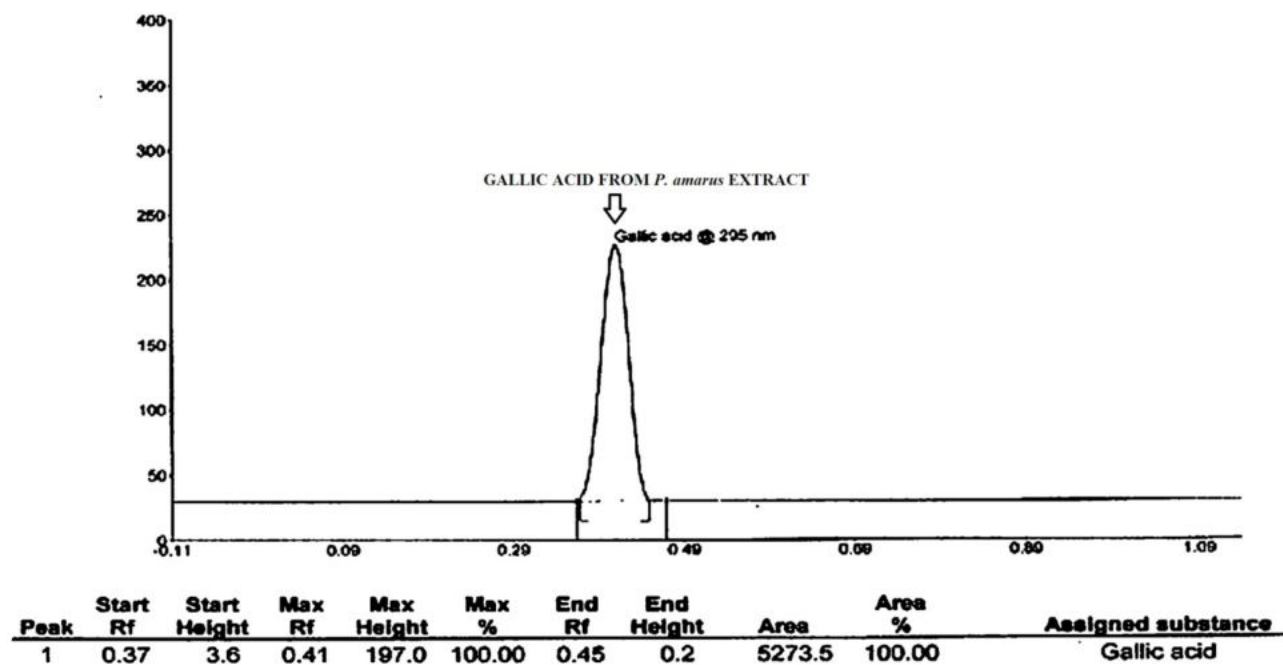


Figure 1d

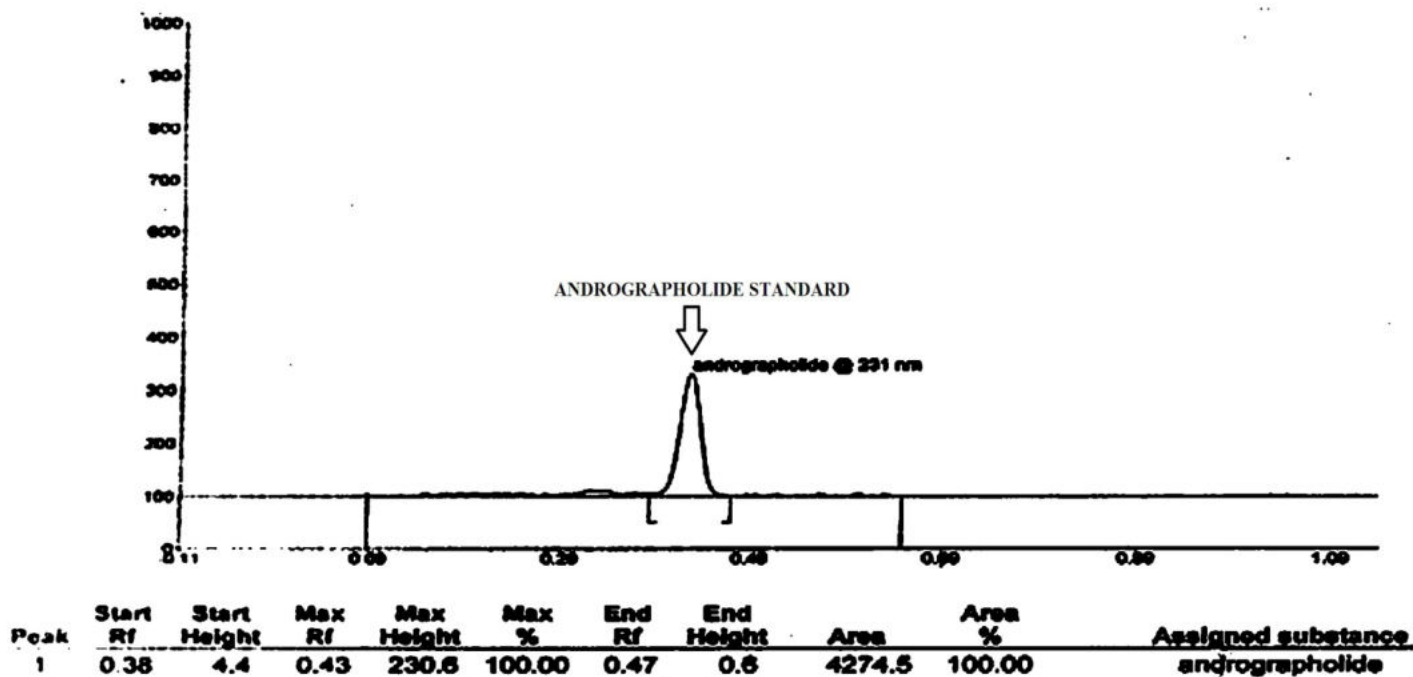


Figure 1e

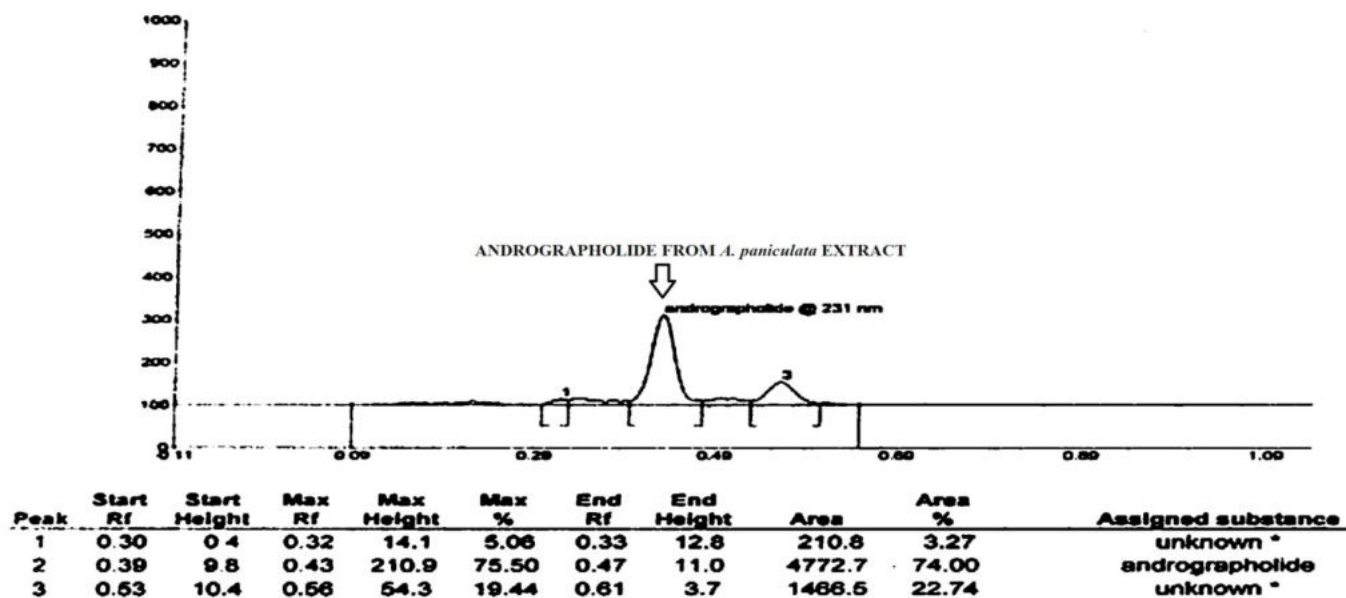


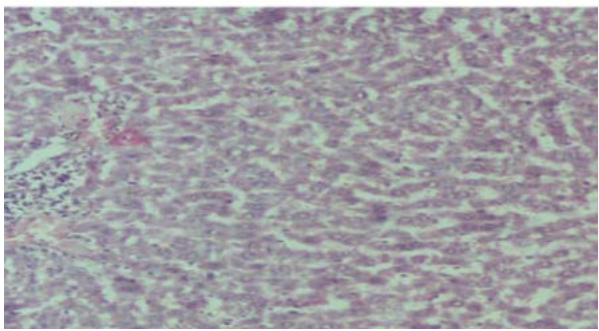
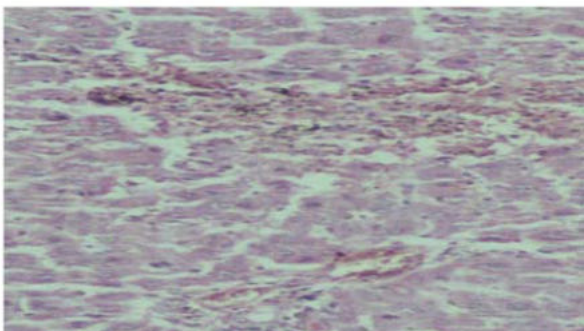
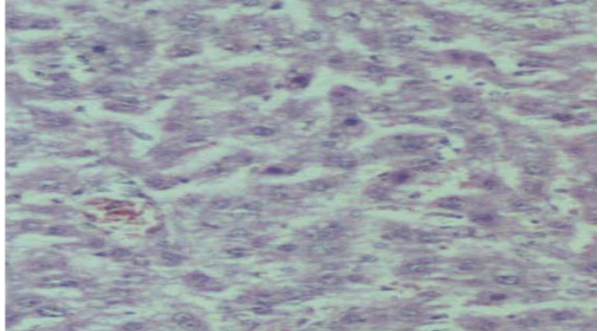
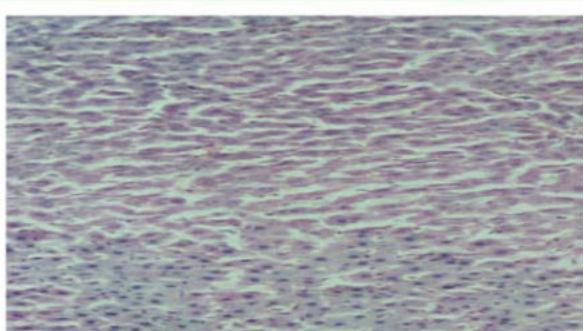
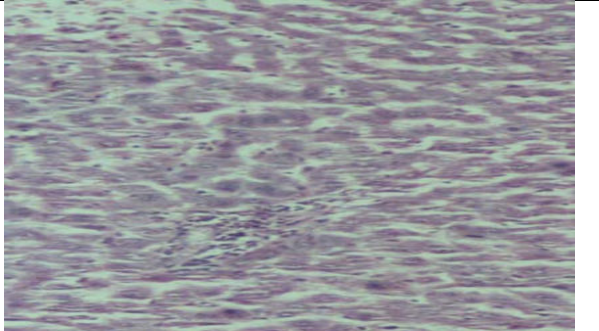
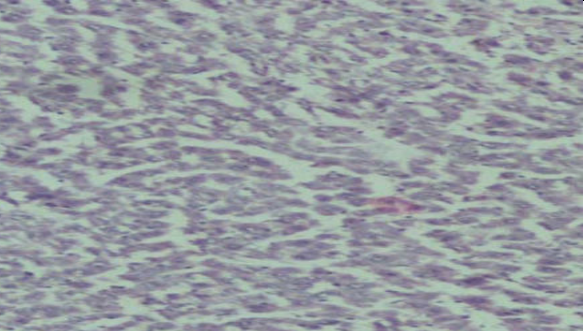
Figure 1f

Figure 1: Chromatograms showing presence of active components of *A. paniculata* and *P. amarus*. (a) Gallic acid standard (b) Gallic acid detected in *P. amarus* extract (c) Phyllanthin standard (d) Phyllanthin detected in *P. amarus* extract (e) Andrographolide standard and (f) Andrographolide detected in *A. paniculata* extract.

Treatment with CCL4 indicated extensive damage to liver tissue as was observed by histological examination (Figure 2b). Histological

sections of rats from all 10 groups included in this study is shown in figure 2a – Figure 2j.



	
Figure 2a: Untreated animals	Figure 2b: Carbon tetrachloride
	
Figure 2c: Silymarin	Figure 2d: <i>A paniculata</i> extract
	
Figure 2e: <i>P. amarus</i> extract	Figure 2f: Gallic acid



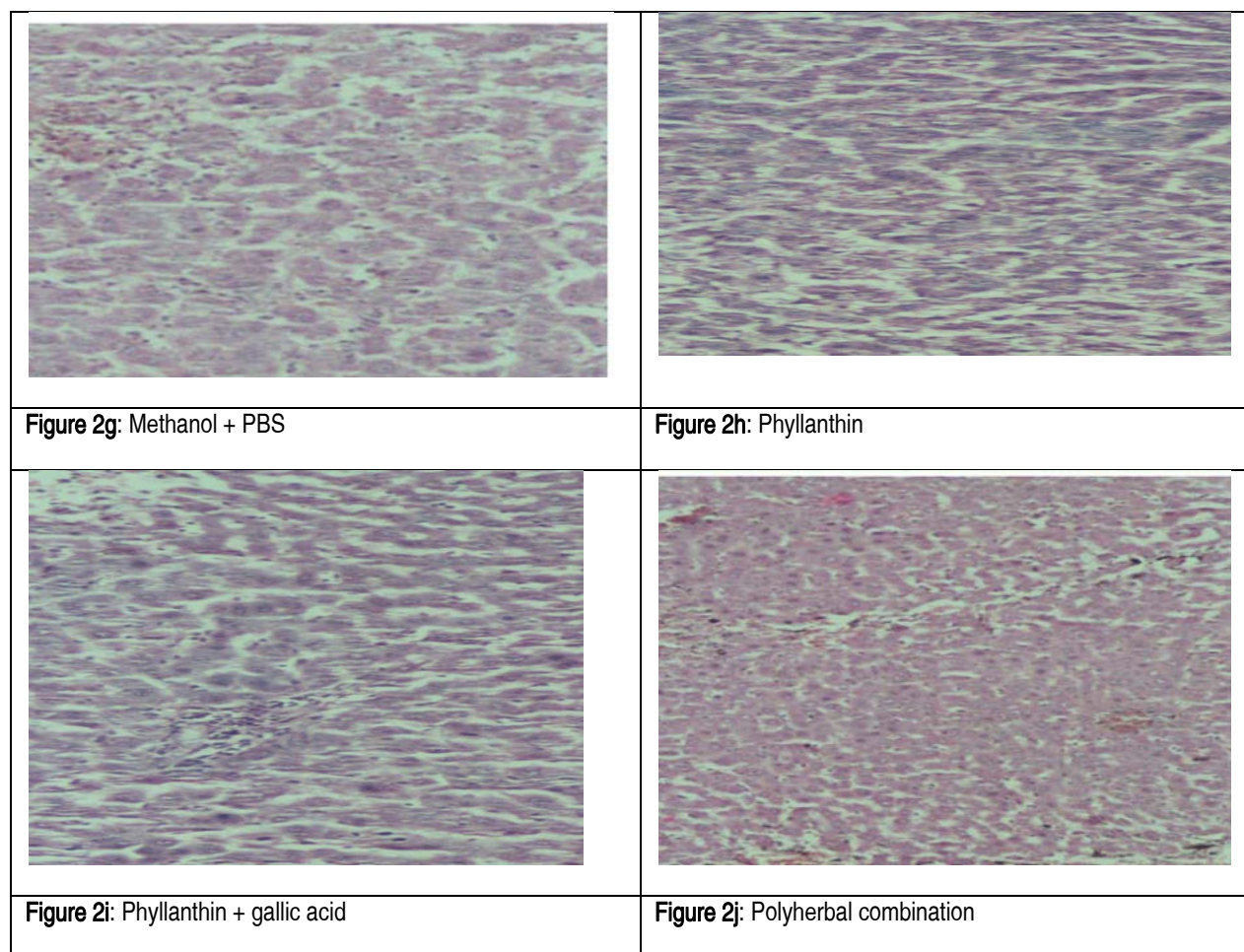


Figure 2 a-j: Histology slides showing section of liver from rats treated with different combinations of plant extracts and phytochemicals along with controls.

Bilirubin is a break down product of heme present in the blood cells and is secreted into the bile by liver. The total bilirubin estimation takes into account both conjugated as well as unconjugated ones and its presence in the blood is a factor of recovery of damaged liver. Extracts from both *A. paniculata* and *P. amarus* reduced bilirubin concentration in the blood. The recovery efficiency was however less compared to the standard drug Silymarin [33]. The efficiency of polyherbal mixture comprising of extracts from both plants was less compared to individual ones. Gallic acid was found to exhibit the highest efficiency in reducing total bilirubin in the blood followed by phyllanthin. Although gallic acid and phyllanthin were administered in pure form, it appears that the hepatoprotective property of *P. amarus* can be related to both Gallic acid as well as Phyllanthin (Figure 3a).

Sinosoidal membrane transporters assist hepatocytes in uptaking bilirubin from the blood which is then excreted into bile by canalicular membrane as bilirubin glucuronides. Out of a vast range of organic anion transporting polypeptides, Oatp1 and HLST transport bilirubin monoglucuronide. On the other hand, MRP3, a

protein which is located in the lateral membrane, transports bilirubin glucuronides back into the blood when there is a defective biliary bilirubin excretion [34]. It is possible that active component within the plants extracts or their components either enhance binding of bilirubin glucuronides with respective anion transporting polypeptides or inhibit MRP3 preventing bilirubin from re-entering the blood stream. Since the latter case is usually associated with a defective biliary bilirubin excretion which may not be the case in our animal model, interaction with the anion transporting peptides appears more likely.

Elevated Serum Glutamic Pyruvic Transaminase (SGPT) enzyme is associated with various liver problems such as viral hepatitis and liver damage [35]. Checking this enzyme in blood is therefore an accepted method for evaluating liver health. In our study, *P. amarus* extract was found to be more effective compared to *A. paniculata* and the later did not have any significant effect in reducing SGPT in the CCL4-treated rats. The reduction in SGPT by the polyherbal combination was similar in profile compared to *P. amarus* and therefore it might be the action of *P. amarus* alone



which was reflected in polyherbal combination (Figure 3b). This was further substantiated by the observation that gallic acid and phyllanthin both had SGPT-decreasing effect individually as well as

in combination. This value was lower than phyllanthin extract or the polyherbal combination possibly because it was used in pure chemical form.

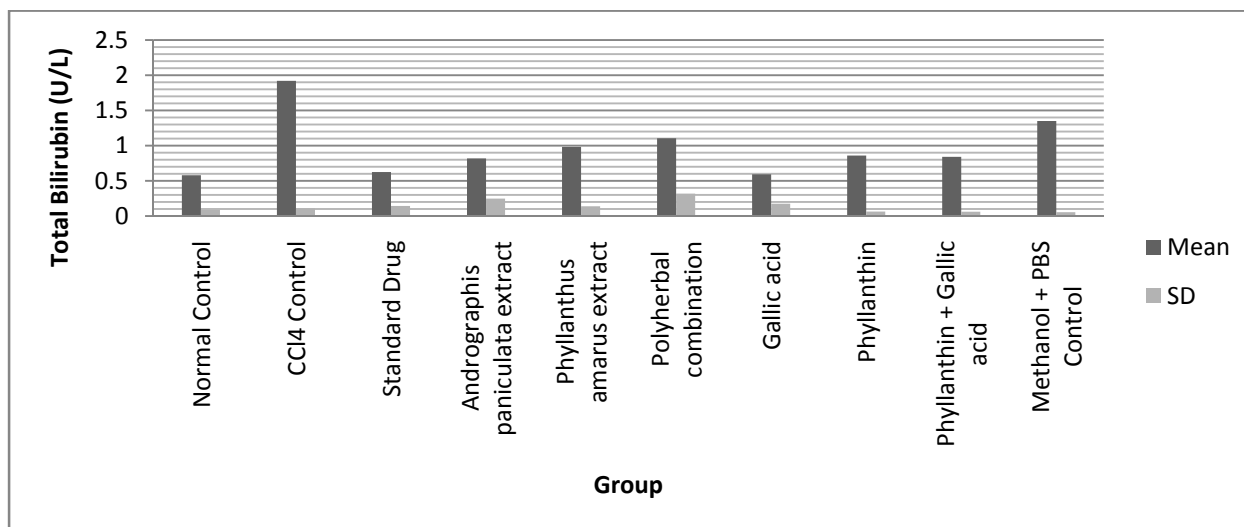


Figure 3a

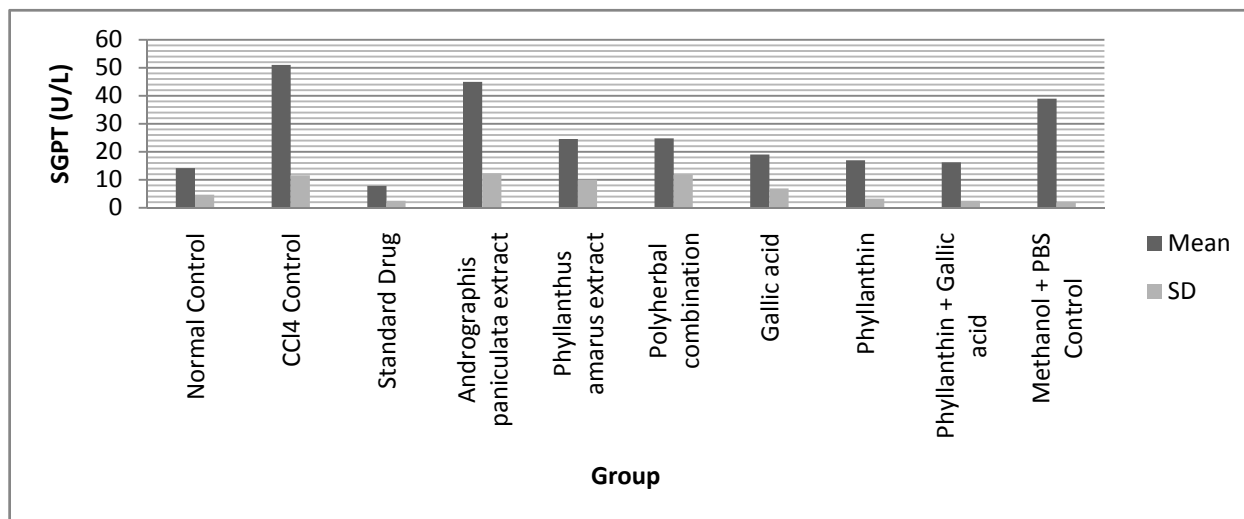


Figure 3b

Serum glutamic-oxaloacetic transaminase (SGOT) profile was found to be similar to that of SGPT. The enzyme was found to be high the control group with artificially damaged liver as anticipated. However, like SGPT, *A. paniculata* did not have a significant lowering effect on SGOT as compared to *P. amarus*. Interestingly, gallic acid exhibited the maximum lowering of SGOT and the value was almost similar to the standard drug Silymarin (Figure 3c). It may be concluded therefore that gallic acid qualified to be one of the primary active component candidate in the *P. amarus* extract

along with the well known phyllanthin as far as SGOT as a liver health parameter is concerned.

An elevated SGOT in the blood can be from sources other than the liver also and hence this assay is not as direct an indicator as SGPT in human setting [36]. However, since elevated SGOT in our animals were detected after liver damage, we included this test also to measure hepatoprotective properties of the plant extracts.

γ -glutamyltranspeptidase (GGT) was found to be elevated in mice administered in prior with the liver damaging CCL4. This enzyme is secreted by the liver and an enhanced value in the blood indicates



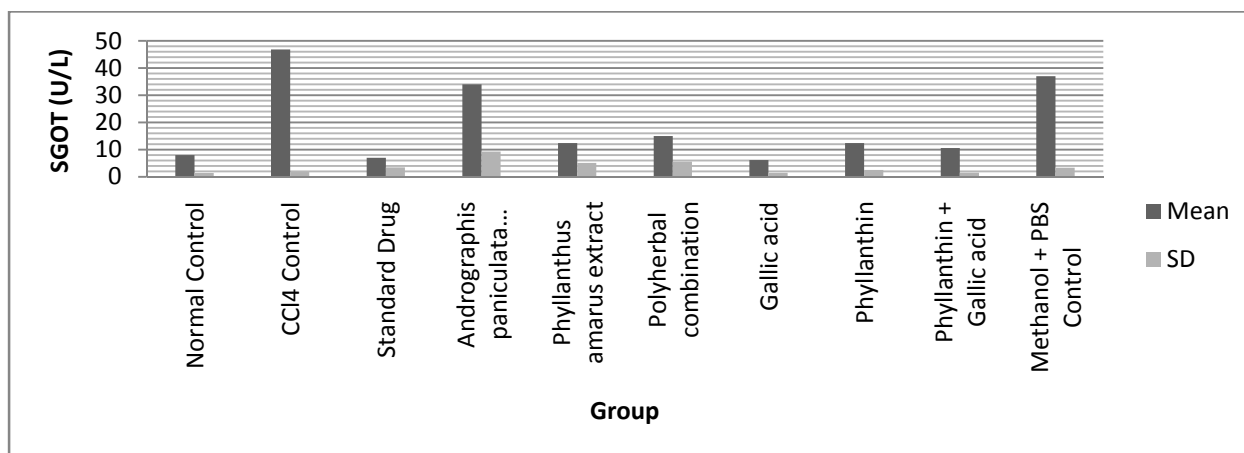


Figure 3c

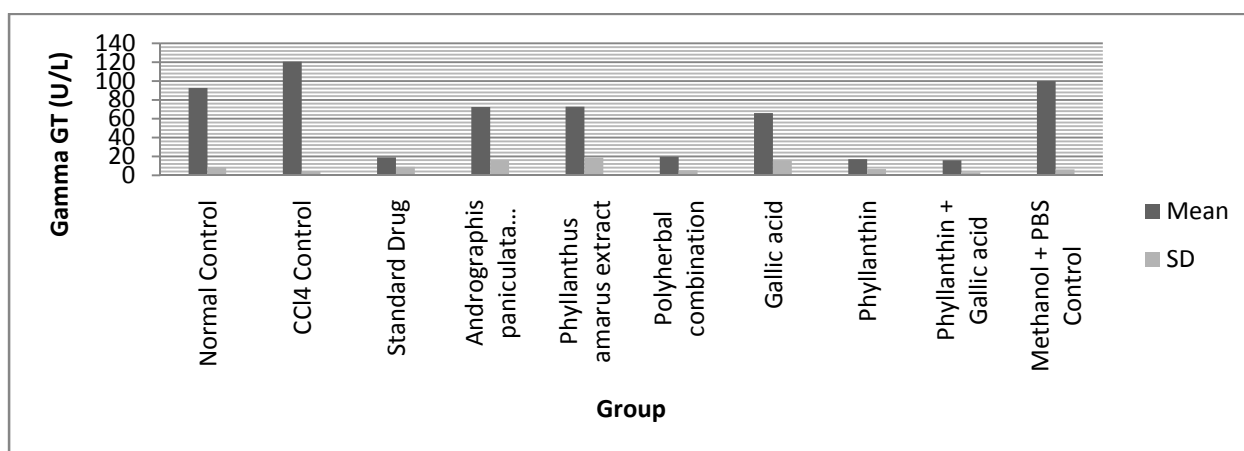


Figure 3d

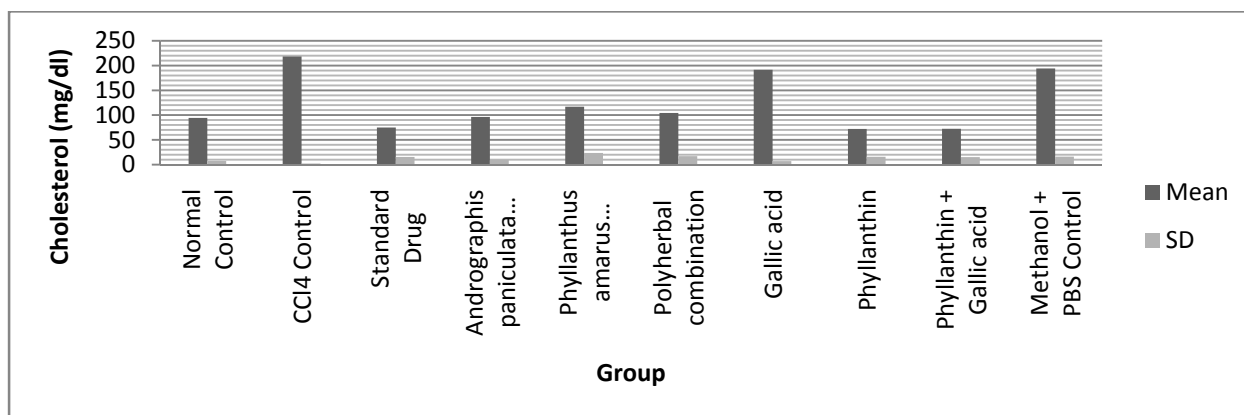


Figure 3e

Figure 3: Estimation of various biomarkers in blood of mice with artificial liver injury done by administering Carbon tetrachloride. a, b,c,d & e : Total bilirubin, SGPT, SGOT, γ -GT and Cholesterol respectively. CCl4 control: Mice with liver injury done by administering Carbon tetrachloride; Normal control: Untreated mice; Standard drug: Silymarin (Lamarin, Serum Institute of India, Pune, INDIA).



either an induced hepatic microsomal production caused by agents such as alcohol or it may also indicate leakage of GGT from the hepatocytes [37]. While individual extracts of *A. paniculata* and *P. amarus* exhibited moderate reduction in GGT, the combination of both demonstrated marked reduction in GGT concentration in blood. This is encouraging since it points towards curing of hepatocytes inside the damaged liver thus reducing leakage of the enzyme into the blood stream. Data indicate that phyllanthin rather than gallic acid was more effective in reducing γ GT levels and similar results in gallic acid and phyllanthin combination might be because of the contribution of the latter. *A. paniculata* and *P. amarus*, both individually as well as in combination, was similar in terms of their capacity to reduce total cholesterol from the blood of liver-injured mice. However, phyllanthin and not gallic acid was found to have the maximum reducing effect of cholesterol and its contribution was observed even when phyllanthin and gallic acid were used in combination. Ethanol is considered to be one of the most common causes of liver damage in humans. Ethanol metabolism reduces AMP-activated protein kinase thus enhancing the sterol regulatory element-binding protein (SREBP). This results in activation of lipogenic genes in the liver thus explaining enhanced cholesterol in hepatocyte damaged livers [38]. The reduction in cholesterol observed after administering the plant extracts may be due to reduced transcription of SREBP either by increasing the AMP-activated protein kinase or by some other physiological pathway. CCL4 damages liver cells by facilitating release of CCl₃ radical in free form followed by destruction of lipid and proteins within the liver [39]. Recently, using *A. paniculata* and *P. niruri* plant extracts recovery of artificially damaged liver in rats was demonstrated by Anil et al., [40] by way of reduction in levels of major liver biomarkers. However, a similar study by our group indicates that the extract of *A. paniculata* and *P. amarus* does not have equivalent effect in normalizing liver biomarkers. Further, dramatic synergistic effect was only observed in reduction of GGT levels by the polyherbal extract compared to individual ones. We conclude that a polyherbal combination does not always present a uniform, enhanced synergistic effect when assessed by several biomarkers known to reflect liver health.

P. amarus [41, 42] and *A. paniculata* [43] has been widely reported for their anti oxidant properties which is believed to be the primary reason for their hepatoprotective attribute. However, it appears that curing of liver from injury by enhanced anti oxidation using these two plant extracts is perhaps a holistic process of recovery and hence, not all biomarkers respond equally to these two plant extracts either individually or in combination. This is best exemplified in case of SGOT where the reduction of this enzyme was markedly varied in case of *A. paniculata* and *P. amarus*. Further, synergistic effect too was noted, for example, in case of γ -GT where the polyherbal combination demonstrated superior

efficacy in reducing serum concentration of this enzyme in comparison to the individual extracts. We further noted that out of the two major components of *P. amarus* extract, *viz.*, phyllanthin and gallic acid [30], the latter was a dominant contributor to hepatoprotective property of *P. amarus* compared to the former.

Polyphenolic metabolites are a large group of phytochemicals to which gallic acid (GA, 3,4,5-trihydroxybenzoic acid) and its derivatives belong and therefore are natural antioxidants [44]. The "no-observed-adverse effect-level" (NOAEL) of gallic acid is as high as 120 mg/Kg/day in rat [45] which explains our encouraging toxicity results. However it is noteworthy to mention that while the antioxidant property of gallic acid is widely studied in solutions, this data does not reflect its effect in a multi cellular system such as the liver where it has to be metabolized and transported to the point of action (reaction sites). In fact, a special animal model has been created exclusively for such a purpose where the phenomenon of free radical scavenging in the real cell membrane systems has been artificially reproduced by incorporating a stable free radical, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), into the model bilayer membrane prepared from phospholipids [46]. This therefore may explain the reason for varying effect of different natural antioxidants originating from plants in influencing liver biomarkers. Phyllanthin, the other active constituent of *P. amarus* is primarily a lignin [47, 48]. This compound is linked through C8-C8' of phenyl propanoid units while hypophyllanthin, a similar active principle found in this plant, is linked in addition through C2-C4' to make a tetrahydronaphthalene ring system [48]. The hepatoprotective property of this compound is primarily due to its ability to scavenge free radicals. It can remove superoxides and hydroxyl radicals thereby retarding lipid peroxidation [49]. Interestingly, this compound was also reported to have antigenotoxic properties [50] which further expand the spectrum of its beneficial effects.

Andrographolide, the active principle in *A. paniculata* has been demonstrated to possess cardio-protective properties also. Treatment with this compound was found to protect cardiomyocytes against hypoxia-related injury and increased the cellular reduced glutathione (GSH) level and similar other antioxidant enzyme activities [51]. It is possibly a similar effect that is mounted in hepatocytes within the liver which in turn activates rapid regeneration of the tissue. While the general method of hepatoprotective property if the *P. amarus* and *A. paniculata* is a strong anti oxidant property, it is important to note that the nature and structure of the active compounds from both the sources vary. It is therefore natural to undergo varying transportation and activation within a multi-cellular system as is explained above in case of gallic acid. This is possibly the reason for varying influence of these plant extracts and their active principles on various biomarkers related to hepato-recovery events as observed in our study.

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