Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(1): 12-19
Received: 21-12-2020
Accepted: 30-01-2020

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Evaluation of hepatoprotective effects of arogyavardhini against D-galactosamine-induced hepatotoxicity in rats

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Abstract
Liver disease is among the most serious ailments and can be classified as acute or chronic hepatitis, hepatosis, and cirrhosis. Besides expensive and ineffectual modern therapeutic agents like steroids and chemotherapy, South East Asian countries like India and China have an edge in treating hepatic disorders employing their native botanicals. The present study was designed to evaluate the protective effects of Arogyavardhani on D-galactosamine (d-GalN)-induced fulminant hepatic failure. Rats were given an intraperitoneal injection of d-GalN (270 mg/kg). Arogyavardhani (10 mg/kg and 50 mg/kg) was administered orally for 14 days continuously, and on the last day at 1hour before the d-GalN injection. Rats were sacrificed 24 hours after the d-GalN. Silymarin (100 mg/kg body weight) was given orally as a standard hepatoprotective drug. The liver injury was assessed biochemically, investigating biochemical parameters like ALT, AST, ALP, Bilirubin, Total Protein, and Albumin. The survival rates after the application of Arogyavardhani at 24 h were also observed. D-galactosamine administration induced a significant increase ($P \leq 0.01$) in total bilirubin associated with a marked elevation in the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) as compared to control rats. The pretreatment of Arogyavardhani attenuated these changes in a dose-dependent manner. The survival rate of the OC groups was significantly higher than that of the d-GalN group. Our results suggest that Arogyavardhani has hepatoprotective effects against D-galactosamine induced hepatic damage. Therefore, Arogyavardhani may be used as a hepatoprotective agent against various liver diseases including toxic liver injury.

Keywords: Arogyavardhani, D-galactosamine, hepatoprotective, liver injury, Silymarin

Introduction
The liver is the largest organ of the human body weighing approximately 1500 g, and is located in the upper right corner of the abdomen on top of the stomach, right kidney, and intestines and beneath the diaphragm. The liver performs more than 500 vital metabolic functions [1]. The liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions [2]. Fortunately, the most common, including hepatitis, cirrhosis, liver disorders in children, alcohol retarded disorders, and liver cancer. Treatment options for common liver diseases such as cirrhosis, fatty liver, and chronic hepatitis are problematic. The effectiveness of treatments such as interferon, colchicine, penicillamine, and Corticosteroids are inconsistent at best and the incidence of side effects profound [3]. The World Health Organization (WHO) has estimated that about 71million people infected with hepatitis - C virus, are at the risk of developing liver cirrhosis and/or liver cancer [4]. Liver function tests are used to determine if the liver has been damaged or its function impaired. The following parameters are measured for liver function tests such as Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Total bilirubin and direct bilirubin, Albumin and total protein, and Alkaline phosphatase (ALP) [5-7].
Nature has provided an excellent storehouse of remedies to cure all the ailments of mankind. In ancient days, almost all the medicines used were from natural sources, particularly from plants. Plants continue to be an important source of new drugs even today. Herbal remedies are widely used for the treatment and prevention of various diseases and often contain a highly active multitude of chemical compounds. Modern research is now focusing greater attention on the generation of scientific validation of herbal drugs based on their folklore claim. Ayurvedic medicine is the oldest holistic medicine system and is derived from a Sanskrit world "ayus" (life) and "Veda" (knowledge) which means the "science of life". Ayurveda is the most ancient science of healing which improves longevity.

It has influenced many of the older traditional methods of healing including Tibetan, Chinese and Greek medicine. Hence, Ayurveda is considered by many as the "mother of healing". Ayurveda originated in India more than 3,000 years ago and remains one of the country's traditional health care systems. Its concepts about health and disease promote the use of herbal compounds, special diets, and other unique health practices. Ayurvedic medicines are regarded as a part of complementary and alternative medicine recognized by the World Health Organization (WHO), National Institutes of Health (NIH), and others [8].

Hepatoprotective is a class of therapeutic agents that includes many synthetic as well as natural products used for protection against hepatic damage induced by various toxins. Plant-based therapeutic agents like silybin and silymarin from Silybum marianum (milk thistle) are promising hepatoprotectives [9]. However; further research to explore more plant-based hepatoprotectives is always encouraged. It is seen that hepatoprotective botanicals stumble on an important place in traditional Systems of Medicine, where tradition has proposed many plants like *Andrographis paniculata* Nees, *Berberis aristata* DC, *Cassia tora* L., *Phyllanthus amarus Schumach*. & Thonn. etc. for hepatoprotection in humans. Most of these botanicals are available as compound drug formulations. Thus, in our market, several herbal formulations are available for liver disorders but in the real sense, only a few medicinal herbs are tested methodically for hepatoprotective activity. Some herbal formulations claiming to be hepatoprotective may contain chemical constituents with hepatotonic potential. Ayurveda has already formulated many preparations for curing hepatic disorders e.g., Bhungaraajaadi churna, Shadabindu tail, Bhungraaja tail, Bhungraaja ghrita, Ptnarnavaastake kwatha, Purarnavaadi kwatha, Punapnavaadi mandur, Katukyadya lauha, Arogyavardhini, Amritaastak kwatha, Amritarishta and Yograj gugglu [10]. Despite this, ayurvedic treatment options for common liver diseases such as cirrhosis, fatty liver, and chronic hepatitis are not accepted. The effectiveness of treatments such as interferon, colchicine, penicillamine, and corticosteroids are inconsistent at best with the incidence of side-effects. Plants potentially constitute such a group where the side effects are less and treatment is always permanent. In recent years, many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support liver function and treat liver diseases.

Arogyavardhini is a multi-herb supplement that has been used to treat liver and skin conditions in Ayurvedic medicine for centuries [11]. Arogyavardhini is referred to by a few different names, including:

- Arogyavardhini Vati
- Arogyavardhini Gutika
- Arogyavardhini Rasa
- Sarvroghar Vati

The ingredients of Arogyavardhini are Amalaki or Indian Gooseberry, Vibhitaki or Terminalia bellerica, Haritaki, Shilajita, Guggulu or Guggul, Chitraka moola, Kutaki or Picrorhiza kurroa and Neem [12, 13]. These ingredients, in the form of either powder or liquid extracts, are mixed into a paste that's held together with a binding agent like starch. The result is dark black in color and bitter. A 2016 study analyzing the pharmaceutical properties of Arogyavardhini found that it contained nutrients like carbohydrates, proteins, amino acids, starches, and steroids, in addition to plant compounds like flavonoids, alkaloids, tannins, and phenols [13]. Despite this supplement's long history of use in Ayurvedic medicine. In general, the studies that exist were poorly designed and show only weak evidence. The primary belief of traditional Ayurvedic medicine is that optimal health is achieved by balancing three vital energies in the body - otherwise known as doshas. The three dohas are Vata, pitta, and Kapha. Arogyavardhini is believed to bring balance among the three doshas. Thus, in Ayurveda, it has been used to treat numerous conditions, including fever, obesity, acne, eczema, dermatitis, edema, jaundice, liver disorders, etc. [13-15].

Therefore, in the present investigation attempts have been made to find out the effect of Arogyavardhini on the liver of albino rats during hepatitis induced by D-Galactosamine. Galactosamine is a hexosamine derived from galactose with the molecular formula $C_6H_{13}NO_5$. Galactosamine is a hepatotoxic, or liver-damaging, agent that is sometimes used in animal models of liver failure. The incidence of different kinds of liver disease like hepatitis, liver cirrhosis, liver cancer, and other related diseases are very common in Bangladesh. The most common liver diseases in Bangladesh are different types of viral hepatitis. Therefore, this study was carried out to investigate the hepatoprotective effect of Arogyavardhani, a polyherbal formulation commercially available in Bangladesh against galactosamine induced hepatotoxicity in rats.

Materials and Methods

Collection of arogyavardhini (AV)

Arogyavardhini was received as a gift from Professor Madhaw Singh Baghel, Director, Institute for Post Graduate Teaching & Research in Ayurveda, Jamnagar, Gujarat, India. The composition is given in the Table 1.

Table 1: Ingredient of arogyavardhini [12, 13]

Ayurvedic name	Western name	Type of ingredient	Proportion
Haritaki	*Terminalia chebula*	Dried fruit rind	1 part
Bibhitaka	*Terminalia Bellerica*	Dried fruit rind	1 part
Amalaki	*Emblica Officinalis*	Dried fruit rind	1 part
Shuddha Shilajatu	Asphaltum	Processed a black bitumen	3 parts
Shuddha Guggulu	*Commiphora wightii*	Gum resin with guggul shrub	4 parts
Chitika Moola	*Plumbago zeylanica Linn*	Dried root of leadwort plant	4 parts
Katuka (kutaki)	*Picrorrhiza kurroa*	Dried rhizome herbal plant	22 parts
Shuddha Rasa	Parada	Purified mercury	1 part
Shuddha Gandhaka	Gandhaka	Purified sulfur	1 part
Lauha Bhasma	Iron	Ash compound	1 part
Abharaka Bhasma	Mica	Ash compound	1 part
Tamra Bhasma	Copper	Ash compound	1 part
Nimba	*Azadirachta indica*	Leaf juice extract	As needed

Experimental animals
Adult female rats (*Rattus novergicus*: Sprague-Dawley strain) were collected from the central animal house of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, and were used for the present study. The animals were randomized and separated into normal and experimental groups of body weight ranging from 60 to 140 gm.

Animal care
All of the rats were kept in plastic cages with dimensions of 30 x 20 x 13 cm and softwood shavings were employed as bedding in the cages. Feeding of animals was done ad libitum, along with drinking water, and maintained in natural day and night cycles. The animals were housed in a well-ventilated hygienic experimental animal house.

Acute toxicity study
The acute oral toxicity test was performed following the guidelines of the Organization for Economic Co-operation and Development (OECD) for testing of chemicals with minor modifications (OECD Guideline 425). Sixteen female mice (non-pregnant, 35-40 g body weight) were divided into four groups of four animals each group. Arogyavardhini (AV) was administered orally to the test animals at different concentrations (10, 20, 50, 100, 200, 300, and 400 mg/kg body weight). After administration of the extracted solutions, mortality or signs of any toxicity was observed for 1 hour. Then the test animals were observed every 1 hour for the next 5-6 hours. The animals were kept under observation for 1 week. Mice that received doses above 100 mg/kg exhibited ptosis (drooping of upper eyelids) and were lethargic.

Dose of administration
Arogyavordini (AV) solution (10 mg/kg body weight) and (50 mg/kg body weight) for groups were prepared in distilled water and given orally. Galactosamine (270 mg/kg body weight) solution was prepared in distilled water. Dose of D-GalN in D.W. 270 mg/kg b.w i.p. on the 14th day.

Study design
Rats of bodyweight 60-140 gm were selected of 30 rats was divided into 6 groups of 5 animals each.

Route to administration
For the toxicological studies, the liquid preparation was administered orally [Per oral (p.o.) route]. D-GalN (270 mg/kg) & Ketamine (500 mg/kg) b.w. (body weight) were administered intra-peritoneally (i.p.).

- Group I: Control: The animals received distilled water (D.W.) 10 ml/kg b.w. p.o. for 13 days.
- Group II: Positive Control (PC): Also received distilled water 10 ml/kg b.w. p.o. for 13 days. A single dose of D-GalN in D.W. 270 mg/kg b.w was given i.p. on the 14th day.
- Group III: Standard Silymarin: The animals received silymarin100 mg/kg b.w. p.o. was given for 13 days. The animals received a single dose of D-GalN in D.W. 270 mg/kg b.w. i.p on the 14th day.
- Group IV: Toxicant + AV 10mg/kg: AV 10 mg/kg b.w. was given p.o. for 13 days. The animals received a single
- Group V: Toxicant + AV 50mg/kg: AV 50 mg/kg b.w. was given p.o. for 13 days. The animals received a single dose of D-GalN in D.W. 270 mg/kg b.w i.p. after 1 hour of vehicle on the 14th day.
- Group VI: AV 50mg/kg: AV 50 mg/kg b.w. was given p.o. for 13 days.

Chemicals and Reagents
The chemicals and reagents that were used during the experiment are listed below (Table 2).

Table 2: Chemicals and Reagents

Chemicals and Reagents	Sources
Galactosamine HCl	Atomax Chemicals Co. Ltd., China.
Heparin	Rotex Medica, Germany
G-Ketamine IM/IV Injection (Ketamine HCl, USP, 50 mg/ml)	Gonoshasthaya Pharmaceuticals Ltd. Bangladesh
Bilirubin, Total Protein, Albumin Protein, ALP, AST, ALT Liquicolor-Human	HUMAN GmbH, Germany

Chemicals and Reagents sources

- Galactosamine HCl Atomax Chemicals Co. Ltd., China.
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- G-Ketamine IM/IV Injection (Ketamine HCl, USP, 50 mg/ml) Gonoshasthaya Pharmaceuticals Ltd. Bangladesh
- Bilirubin, Total Protein, Albumin Protein, ALP, AST, ALT Liquicolor-Human HUMAN GmbH, Germany

All other reagents and chemicals that were used in this work were of analytical grade and were prepared in all glass-distilled water.

Preparation of samples for biochemical studies
From the post vena cava of the animal, blood samples were collected, and immediately blood was transferred to the tubes having heparin. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate serum for biochemical analysis. The liver was dissected and part of it was taken for lipid peroxidation test.

Assessment of liver functions
Biochemical parameters like Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Bilirubin and alkaline phosphatase (ALP) [16-18], and total protein (TP) and albumin [19, 20] were determined by Dimension RXL (Max)/ vittros-250 auto analyzer using kits in Medinova Diagnostic Center.

Statistical analysis
All the grouped data were statistically evaluated with SPSS (Chicago, IL) version 16 software. All the results were expressed as mean ±SEM (Standard error of mean) values for five animals in each group. The data obtained from the hepato-protective study were subjected to one-way ANOVA followed by Dunnett's test for statistical significance.

Results and Discussion
Liver regeneration is an example of tissue recovery after injury. This proliferation process can be induced in experimental conditions by partial hepatectomy or by various hepatotoxic chemical agents (tetrachloromethane, D-galactosamine, thioacetamide). D-galactosamine (GalN) is known for inducing the features of acute hepatitis in rats. The toxic effect of GalN is connected with an insufficiency of UDP-glucose and UDP-galactose and the loss of intracellular calcium homeostasis. These changes affect cell membranes and organelles, and the synthesis of proteins and nucleic acids

[21]. After GalN application, the location of proteoglycans is changed in the rat liver [22]. GalN also inhibits the energy metabolism of hepatocytes [23]. This and a further study (Sire O. *et al*., 1983) [23] show that GalN injures the enzymes involved in the transport of substrates to the mitochondria and modifies the phospholipid composition of membranes.

Damage to liver structure and function induces the regenerating process. An important role in this regeneration process is played by the cytokine IL-6. It prevents the progression of liver necrosis and thus enhances the survival of intoxicated animals, and is also involved in initiating liver regeneration [24, 25]. After GalN injury, the liver responds by activation of progenitor cells that proliferate and then differentiate into mature hepatocytes. Adult hepatocytes can also proliferate after GalN injury but these hepatocytes do not undergo dedifferentiation [26]. Other findings have demonstrated that hepatocytes can divide to restore the liver mass after GalN liver injury [27]. Rats have been used successfully to investigate D-GalN induced hepatotoxicity models [28, 29]. Therefore, we selected rats to study the hepatotoxic effect of D- GalN and hepatoprotective action of Ayurvedic preparation. There are several pathological mechanisms on which these tests are based. Damaged hepatocytes and biliary epithelium may release cell constituents (e.g., enzymes) into the blood resulting in increased levels of these analytes. The most commonly measured 'liver' enzymes are alanine aminotransferase (ALT, formerly sGPT), aspirate aminotransferase (AST, formerly known sGOT), Alkaline phosphatase (ALP), sorbitol dehydrogenase (SDH), and gamma-glutamyl transferase (GGT). There are some other tests used less frequently. e.g., ornithine carbamyl transferase, isocitrate dehydrogenase, and arginase. Increased levels of ALT, AST, and SDH are usually associated with damage to hepatocytes [30-33]. Some measurements like ALT and GGT are indicators of hepatic injury rather than liver-function tests (LFTs). The use of the following plasma enzymes: ALT, AST, ALP, GGT, and GLDH-with OCT or SDH as optional additions, remembering that plasma ALP has a high intestinal component and plasma GGT is low in the rat. However, AST is the least liver-specific as the skeletal and cardiac muscles contain a significant concentration of the enzyme, and damage to these cell types will also increase serum levels of AST [34]. Whereas, elevated levels of ALP [35] and GGT [36] are mainly associated with biliary diseases. For assessing hepatotoxicity, the core tests should include tests for hepatocellular and cholestatic injuries because both or either may occur. Given that it is not always possible to predict whether a xenobiotic will cause either cellular or biliary injury, the choice of enzymes and bilirubin/bile salts needs to include markers for both of these types of toxicity and to take into consideration the intracellular locations of these enzymes. These common tests are far more effective when used in groups, and reliance should not be placed on any single test for the diagnosis of hepatotoxicity. The recognition of an increase in several of these test values and a pattern of changes offer more evidence of an adverse effect due to a xenobiotic.

Effects of D-galactosamine

The activities of serum AST, ALT, ALP (hepatic marker enzymes for liver damage), and Bilirubin were increased markedly in D-GalN treated animals as compared to normal control mice; this indicated liver damage in the toxicant treated group. The results are given below in Table 3 and fig 1.

Table 3: Effects of D-GalN on liver-function specific plasma parameters on rats

Group	ALT	AST	Bilirubin	ALP	TP	ALB
Control	33.2±6.62	57.4±6.28	0.22±.02	112±9.2358	47.6±1.77	26.6±.93
PC	1498± 815.8 [b]	1744.8±1040.3[c]	1.8240±0.20[a]	443±141.69[a]	46±1.73	27.2±1.28

*Results are represented as mean±SEM, a = P<0.001, b = P<0.01 and c = P<0.05 compared to the control group

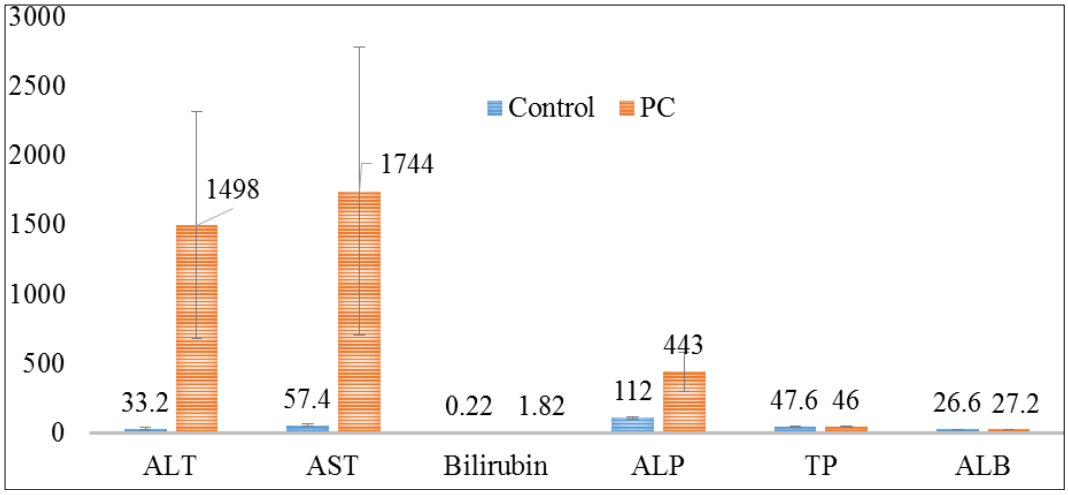

Fig 1: Effects of D-GalN on liver-function specific plasma parameters on rats

Liver damage induced by D-GalN usually shows disturbances of liver cell metabolism which leads to characteristic changes in the liver serum enzymes [37]. The increased levels of AST, ALT, and ALP, which are hepatic serum marker enzymes in the toxicant D-GalN group, may be interpreted as a result of the liver cell destruction or changes in the cell membrane permeability indicating severity of hepatocellular damage induced by D-GalN hepatotoxicant [38].

Effects of arogyavordini (AV) on serum transaminase levels

Plasma ALT is the most useful enzyme for detecting hepatocellular injury in most laboratory animal species. Plasma ALT can increase or decrease following microsomal enzyme induction effects in the rat and the dog [39, 40] and when there is a heavy fatty infiltration of hepatic cells, where plasma enzymes may reflect the displacement of cytoplasm as

the fat load increases. In the present study, plasma ALT and ALP are used to assess liver function. These enzymes are important in assessing and monitoring the degree of liver cell inflammation and necrosis. Elevation of ALT activity is found in cirrhosis of the liver, obstructive jaundice, hepatic congestion, and myocardial infarction. Organs rich in AST include the heart, liver, and skeletal muscles. Hence, plasma AST rises in myocardial infarction, muscle necrosis, and/or hepatic disorders. These study reveals that Control & AV 50 mg/kg pretreated animals had normal values of transaminase. Pretreating rat with the Arogyavordini (AV 10 mg/kg) significantly suppressed the plasma AST (P<0.05) 90.81% and ALT (P<0.05) 92.14%, activity when compared with the positive control. Arogyavordini (AV 50 mg/kg) also significantly suppressed the plasma AST (P<0.05) 91.43% and ALT (P<0.05) 92.48%, activity when compared with the positive control (Table 4 & Fig 2).

Table 4: Effects of AVR on liver-function specific plasma parameters (ALT and AST) on rats

Group	ALT	AST
Control	33.2±6.628	57.4±6.28
PC	1498±815.82	1744±1040
STD	91.2±25.15[f]	139.4±35.98[f]
AV 10mg/10ml + D-Galn	117.6±15.81[f]	160.4±22.30[f]
AV 50mg/10ml + D-Galn	112.6±23.75[f]	149.6±31.90[f]
AV 50mg/10ml	50.2±7.19	68.2±7.35[f]

*Results are represented as mean±SEM, f = P<0.05, compared to the positive control group

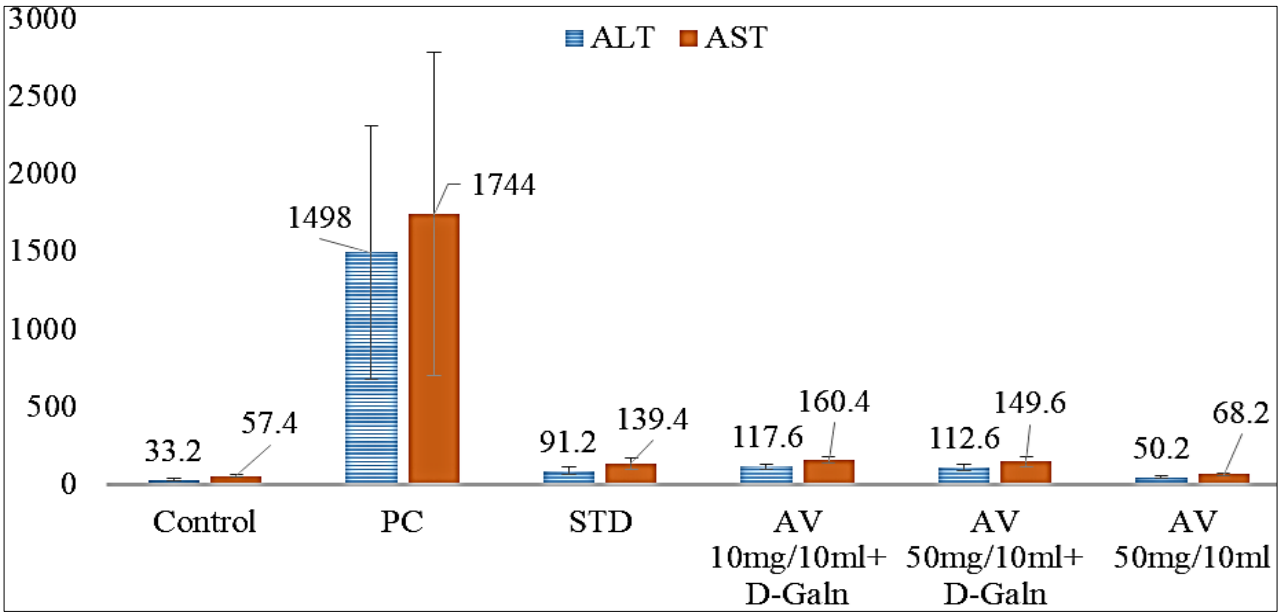

Fig 2: Effects of AV on rat serum ALT and AST level after D-GalN administration

Reduction in the levels of AST and ALT towards their normal values is an indication of stabilization of plasma membranes as well as repair of hepatic tissue damage caused by D-GalN. This effect is in agreement with the view that serum levels of the transaminases return to normal with healing of the hepatic parenchyma and regeneration of hepatocytes [28].

Effects SRT and AV on serum bilirubin level

Bilirubin is formed from the breakdown of hemoglobin; it is then conjugated before secretion in the bile. Erythrocytes are destroyed primarily in the spleen to release hemoglobin, which is then broken down to heme and separated from the globin parts of the hemoglobin molecule. The heme is converted first to biliverdin by the reversible action of biliverdin reductase and then to unconjugated bilirubin. The unconjugated bilirubin reacts more slowly in analytical methods and the plasma total bilirubin reflects the balance between production and excretion. In most laboratory animals, the plasma total bilirubin is lower than in humans due to the low renal threshold for circulating conjugated bilirubin. In some genetic rat strains (e.g., the Gunn rat), bilirubin cannot be converted to glucuronide due to an enzyme deficiency, and therefore plasma bilirubin is higher in these strains. Plasma bilirubin may be increased due to drug-related inhibition of uridine diphosphate glucuronosyltransferase [41] or inhibitors of hepatic bilirubin transporters. Metabolites such as bilirubin are normally removed by the liver. Liver dysfunction often results in elevated bilirubin levels due to inadequate clearance or conjugation with proteins. Pretreating rat with the Arogyavordini (AV 10 mg/kg) significantly reduced the serum bilirubin (P<0.001) 90.13% when compared with the positive control. Pretreating rat with the Arogyavordini (AV 50 mg/kg) markedly reduced the serum bilirubin (P<0.001) 89.04% when compared with the positive control (Table 5 & Fig 3).

Table 5: Effects of AV on plasma bilirubin levels

Group	Bilirubin
Control	0.22±.02
PC	1.82±0.20
STD	0.26±.04[d]
AV 10mg/10ml+ D-Galn	0.18±.02[d]
AV 50mg/10ml+ D-Galn	0.2±.00[d]
AV 50mg/10ml	0.2±.03

Results are represented as mean±SEM, d = P<0.001, compared to the positive control group

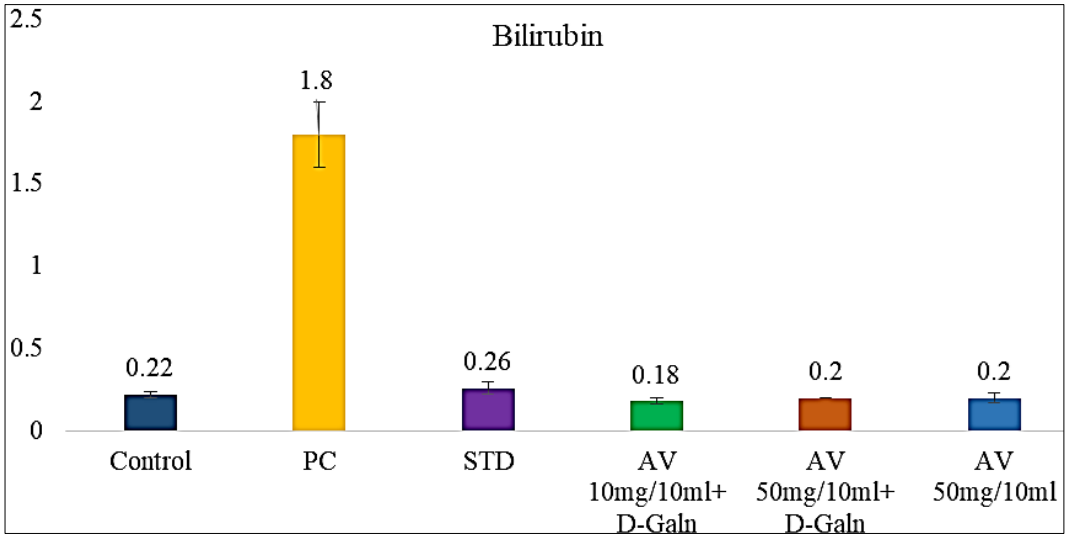

Fig 3: Effects of AV on rat serum bilirubin level after D-GalN administration

Effects of AV on alkaline phosphatase (ALP) levels

Alkaline phosphatase (ALP) isoenzyme measurements are useful in elucidating the nature of increased ALP and associated with a hepatopathy with progressive changes of the plasma membrane and other subcellular organelles [42, 43]. ALP activity when considered is related to the hepatocyte function. That means an increase in its activity is due to elevated synthesis in the presence of increased biliary pressure [44]. In Arogyavordini (AV 10 mg/kg) treated rat was a significant lowering of ALP level (P<0.01) 75.67% when compared with the positive control. Administration of rats with increased dose of Arogyavordini (AV 50 mg/kg) caused significantly reduced ALP level (P< 0.001) 71.69% when compared with the positive control (Table 6 & Fig 4).

Table 6: Effects of AV on ALP levels

Group	ALP
Control	112±9.23
PC	443±141.70
STD	136.6±8.14^{e}
AV 10mg/10ml+ D-Galn	107.8±9.88^{d}
AV 50mg/10ml+ D-Galn	125.4±17.11^{e}
AV 50mg/10ml	92.8±2.33

Results are represented as mean±SEM, d = P<0.001, compared to the positive control group, e = P<0.01, compared to the positive control group

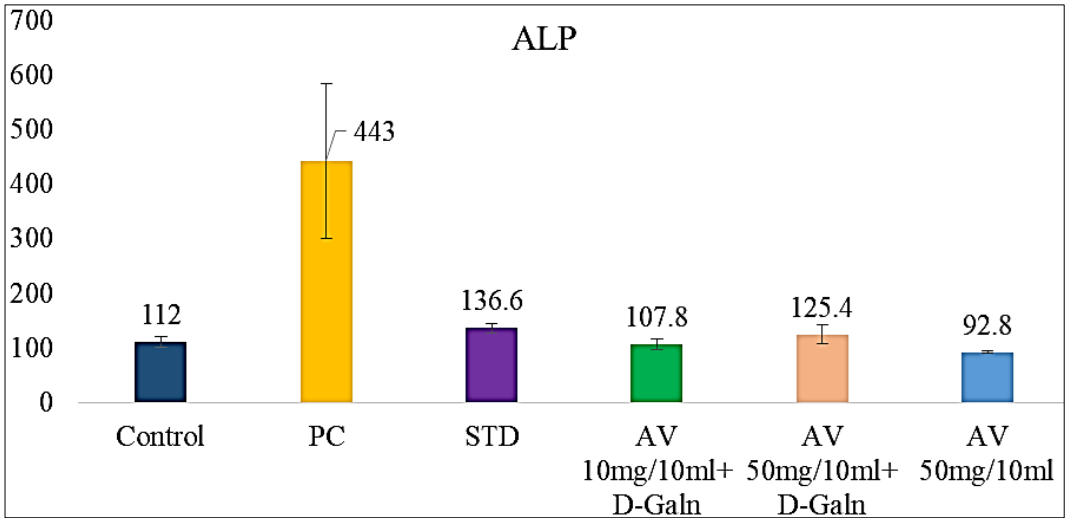

Fig 4: Effects of Avon rat ALP level after D-GalN administration

Effects of AV on plasma protein level

Hepatocytes are the most active sites for protein synthesis. Most proteins found in plasma are produced by the liver. The levels of plasma proteins reflect the balance between the rates of synthesis, utilization, and degradation. Severe liver damage has been associated with decreased production of various proteins resulting in reduced serum levels of total protein, albumin, and/ or globulin [45, 46]. Following hepatocellular injury, protein synthesis may be reduced; however, this may not be evident immediately because the protein half-lives are relatively long compared to the half-lives of enzymes. The protein pattern seen following significant chronic hepatocellular damage is hypoproteinemia with a reduction in albumin, accompanied by a relative increase of globulins.

In our study, reduction of Total protein occurs for the positive control group compared to the control group. Pretreating rats with the Arogyavordini (AV 10 mg/kg) & (AV 50 mg/kg) did not increase the total protein (TP) level when compared with the positive control group. There was no reduction in the serum albumin (Alb) level occurring for the positive control group. Though any impact is not evident by now, it may show some significant impact if it is kept under treatment for a longer period (Table 7).

Table 7: Effects of AV on plasma protein level

Group	TP	Alb
Control	47.6±1.77	26.6±.93
PC	46±1.73	27.2±1.28
STD	41.4±4.74	27±.71
AV 10mg/10ml+ D-Galn	46.6±1.21	27.2±1.16
AV 50mg/10ml+ D-Galn	44.4±1.36	25.8±.58
AV 50mg/10ml	47.4±1.44	26.4±.68

The results are represented as mean±SEM

Effects of AV on liver weight

Normally, if rats are exposed to toxic substances, these substances will cause a certain degree of damage to the target organ(s). Hence, the weight of the damaged organ(s) will either increase (swell) or decrease. D-GalN increased the weight of the liver when compared with the Control group but not significantly. Pretreating rat with Silymarin (STD) did not significantly reduced the weight of the liver when compared with the positive control. Pretreating rat with the Arogyavordini (AV 10 mg/kg & AV 50 mg/kg) not significantly reduced the weight of the liver when compared with the positive control. Rat treating with only Arogyavordini (AV 50 mg/kg) have liver weight lower than the control group but this change was not significant (Table 8).

Table 8: Effects of AV on liver weight

Group	Weight of liver
Control	4.2590±0.31
PC	4.6860±0.23
STD	4.2352±0.26
AV 10mg/10mg+ D-GalN	4.2298±0.24
AV 50mg/10mg+ D-GalN	4.3964±0.21
AV 50mg/10ml	3.6760±0.23

The results are represented as mean±SEM

As demonstrated in our study, the administration of D-GalN significantly elevated serum levels of hepatic enzymes, indicating considerable hepatocellular damage. Our study confirmed the protective effects of Arogyavordini against D-GalN in rats. In rats, Arogyavordini hepatoprotective activity is quite similar to silymarin, a reference hepatoprotective agent.

Conclusions

Liver injuries induced by D-GalN are the best-characterized system of xenobiotic-induced hepatotoxicity and commonly used models for the screening of anti-hepatotoxic and/or hepatoprotective activities of drugs. D-GalN hepatotoxicity induces liver injury closely resembling human viral hepatitis with necrosis, inflammation, and regeneration. The present study was undertaken to evaluate the hepatoprotective effect of Arogyavardhini, a classical Ayurvedic preparation, having therapeutic activity against hepatic disorders, against D-GalN-induced liver injury in Sprague Dawley rats. Evidenced by biochemical studies, it is concluded that Arogyavardhini offered protection due to the phytochemicals present which provide hepatoprotective activity induced by D-GalN hepatotoxicity. These observations provide biochemical data supporting the traditional uses of Arogyavardhini for the treatment of some hepatic disorders and suggest the possible utilization of this formulation as a new drug for hepatotoxicity. However, the marked or unknown mechanism of Arogyavardhini is still to be proved. Hence, more research is needed on Arogyavardhini to make it a novel medicine.

Acknowledgments

The authors are grateful to the Department of Pharmacy Gono Bishwbidyalay, Savar, Dhaka-1344, and Department of Pharmacy, Jahngirnagar University, Savar, Dhaka for the laboratory facility to conduct the research work.

Conflict of interest

All the authors declared that they do not have any conflicts of interest in publishing this research article.

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