

PHARMACOLOGICAL EVALUATION OF GMELINA ARBOREA FOR ANTIMICROBIAL AND HEPATOPROTECTIVE ACTIVITY

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Abstract: Liver is an important organ and a central one for many of the metabolic functions of the body, decomposition of toxic and waste substances, and disposal of harmful substances from the body. Liver illnesses are still the serious problem of human health. Making active oxygen species is an unavoidable result in aerobic organisms, and their removal is done at a basic level and with regard to the useful physiological functions and their harmful effects. This sensitive balance is for retaining the intracellular redox state which has an important role in optimizing cell functions. Excessive accumulation of free radicals or the body's inability to remove them causes the relocation of redox equilibrium for creating oxidation states in the body. Paracetamol is a common analgesic and antipyretic drug. Several studies have demonstrated the induction of hepatocellular damage or necrosis by acetaminophen higher doses in experimental animals and humans. For screening of hepatoprotective agents, paracetamol-induced hepatotoxicity has been used as a reliable method. Paracetamol is metabolized primarily in the liver and eliminated by conjugation with sulfate and glucuronide, and then excreted by the kidney. The present study aims to evaluate the antimicrobial and hepatoprotective activity of *Gmelina arborea* bark extract. Methanol extract of *Gmelina arborea* afforded protection from such paracetamol induced liver damage, and has shown the most pronounced hepatoprotective effect.

Keywords: *Gmelina arborea*, antimicrobial activity, hepatoprotective activity

Introduction: Many common liver diseases can cause the organ to become inflamed. This inflammation can progress to scarring, or cirrhosis. It is critical that patients with cirrhosis, due to any type of liver disease, seek help because people with cirrhosis are at an increased risk for liver cancer or liver failure [1]. Liver cancer and liver failure can be treated by a multidisciplinary approach including radiation, medication, or surgery, including transplant. In present scenario, liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders [2]. Acute liver failure refers to the rapid development of severe acute liver injury with impaired synthetic function and encephalopathy in a person who previously had a normal liver or had well compensated liver disease. Acute and chronic liver diseases can interfere with the functions of the liver and thereby cause symptoms. The liver has a hefty reserve capacity. In other words, it usually takes substantial damage to the liver before a disease interferes with the functions of the liver and causes symptoms [3]. It is inflammation of the liver, caused mainly by viral infection (viral hepatitis) but also by some liver toxins (e.g. alcoholic hepatitis), autoimmunity (autoimmune hepatitis) or hereditary conditions. Liver involved in multiple functions and it also has great power of regeneration; these makes estimation of the presence or absence of hepatic dysfunction is complicated. The series of biochemical test were used to assess the liver function [4]. The biochemical tests includes the estimation of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP), total bilirubin, protein, triglyceride, total cholesterol, HDL and LDL. Antioxidant assay includes estimation of superoxide dismutase (SOD), catalase, glutathione peroxidase, lipid peroxidation and glutathione transferase in liver tissues. The pathophysiology of almost all the liver diseases is same. The main cause is oxidative stress and other includes ROS induced inflammatory responses. In the treatment of liver diseases, anti-inflammatory plays vital role [5]. Natural products have been the main sources of new drugs. The different strategies have been developed to find the new drugs based on natural products. The traditional and ethic medicines have provided information on the therapeutic effects and resulted in some notable drug discovery of natural products [6]. The special activities of the medicine plants such as the side effects have inspired scientists to develop the novel small molecular. The microorganisms and the endogenous active substances from human or animal also become the important approaches to the drug discovery. The tremendous progress in

technology led to the new strategies in drug discovery from natural products. The bioinformation and artificial intelligence have facilitated the research and development of natural products [7]. Natural products will remain a reliable source for drug discovery. The key to drug discovery from natural products is to find the suitable strategy. Every strategy has its advantages and disadvantages. From herbal medicines to small molecule natural drugs, most of the discoveries were not casual. The indications of the herbal medicine were well known, and scientists purposefully looked for compounds wherein the role played. Although information-based and therapeutic effects based drug discovery from natural products are useful for selection of ethnobotanical plants, these strategies were laborious and relatively low sensitive [8]. Side or toxic effects-based drug discovery from plants help researchers to develop novel drugs. But it needs extensive clinical experience and dialectical thinking. Microorganisms-derived natural products often have some limitations such as high toxicity. Biosynthesis and secondary metabolites will lead to more valuable natural products. Pathogenic factors, targets and animal disease models as the main strategies for disease-based natural drug discovery are more direct than other strategies [9]. An animal model is an important method and means to explore the essence of human disease and the evaluation of the effectiveness of new drugs. The ideal animal model should be similar to the clinical of human disease, especially the animal model of "disease" or "symptom" of traditional medicine should have the characteristics of traditional medicine, similar to clinical syndrome differentiation. Properly making or selecting a reasonable animal model can increase the authenticity and credibility of preclinical pharmacodynamic research and improve the success rate of pharmacodynamic study [10]. Bark is a portion of the vascular plant body consisting of all tissues external to the vascular cambium. Bark has several functions in the living tree. It protects the tree from attack by microorganisms, insects, and mammals, and from chemicals in the atmosphere. Mechanically, the bark contributes to bending strength of the stems. In the wood processing industry and the pulp and paper industry, bark is a major by-product. It is used in energy production in boiler kilns or furnaces. A tannin-based adhesive from bark can be used as a substitute for synthetic phenolic resin, and can be used to produce particleboard. In some countries, bark has been used as a source of remedies for treatment of many diseases. The most popular bark used as a natural medicine is *Cinchona officinalis* bark, which has been used as an antimalarial agent during the past two centuries. The bark also has potential use as an interesting

source of strong antioxidants. Pycnogenol, which is extracted from the bark of *Pinus maritima*, has antioxidant activity. Gambhiri consists of dried stem bark of *Gmelina arborea* Linn. (Fam. Verbenaceae), a large deciduous tree, mostly found in southern peninsula and upto Kashmir. Chronic liver dysfunction or injury is a serious health problem worldwide. Chronic liver disease involves a wide range of liver pathologies that include fatty liver, hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. The efficiency of current synthetic agents in treating chronic liver disease is not satisfactory and they have undesirable side effects. Natural remedies from traditional plants and their derivatives are still used all over the world in one from another as they are effective and safe alternate treatments for hepatotoxicity. Natural products will remain a reliable source for drug discovery. The key to drug discovery from natural products is to find the suitable strategy. Every strategy has its advantages and disadvantages [11-15]. The present study aims to evaluate the antimicrobial and hepatoprotective activity of *Gmelina arborea* bark extract.

Material And Methods

Collection and identification of plant material: *Gmelina Arborea* herbs along with inflorescence were collected from tribal region of Bhopal. Transverse sections through midrib from bark of *Gmelina Arborea* obtained as thin sections by Microtome sectioning. Phloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope.

Antimicrobial activity

Test Microorganism: Bacterial pure cultures of *Staphylococcus aureus* (MTCC 3160), *Streptococcus mutans* (MTCC 3160), *Bacillus coagulans* (MTCC 492) and fungal pure culture *Candida albicans* (MTCC 890) were procured from IMTECH, Chandigarh. *Lactobacillus sporogens* (Sporlac powder, Sanzyme (P) Ltd.) purchased from a Pharmacy outlet. The antimicrobial activity was intended to compare the effectiveness of the extracts against oral pathogens and to figure out the most effective extract from the two herbs. The activity was evaluated by agar well diffusion method using nutrient blood agar media against oral pathogenic strains of bacteria (*Staphylococcus aureus*, *Streptococcus mutans*, *Bacillus coagulans* and *Lactobacillus sporogens*) and fungal strain of *Candida albicans*. The sterile borer (8 mm) was used to make wells in Petri plates. All the extracts were diluted in DMSO to get final concentration of 5 mg/ml.

Chlorhexidine (50µg/ml) was used as standard to compare against the test extract. The wells in petri plates were filled with 1ml of extracts and standard chlorhexidine (50µg/ml). The procedure was repeated to obtain triplicates of readings. All the plates were incubated for 24 h at 37°C and zone of inhibition (diameter) was measured in mm. DMSO was used as control. Minimum inhibitory concentration for the extracts, chlorhexidine and control was determined using Micro dilution method with slight modifications.

Hepatoprotective effect of *Gmelina arborea*:

Methanol and aqueous extract of dried bark of *Gmelina Arborea* were found to have higher amount of total phenolic and total flavanoid content than chloroform extract so Methanol and aqueous extract of plant for further in vivo activity like Anti-Inflammatory, Antipyretic activity and hepatoprotective activity. In the present study, hepatoprotective activity of the extracts of *Gmelina arborea* bark were carried out using model of paracetamol induced hepatotoxicity in experimental animals [16-17].

Table 1: Experimental design for hepatoprotective effect of *Gmelina arborea* bark extracts

S No.	Group	Treatment
1	Normal control	Normal Saline
2	Disease control	(Paracetamol 1000 mg/kg p.o. for 7 days)
3	Standard	Standard (silymarin) + Paracetamol (1000 mg/kg p.o.)
4	MGA 150	Methanol extract of <i>Gmelina arborea</i> bark (150 mg/kg)
5	MGA 300	Methanol extract of <i>Gmelina arborea</i> bark (300 mg/kg)
6	AGA 150	Aqueous extract of <i>Gmelina arborea</i> bark (150 mg/kg)
7	AGA 300	Aqueous extract of <i>Gmelina arborea</i> bark (300 mg/kg)

IP = intraperitoneally

The preparation of test extract was done freshly prior to dosing. The animals were dosed by intraperitoneally approximately at the same time each day. The dosage volume administered to individual rat was adjusted according to its recently recorded body weight. All the animals were treated with test drugs (extracts) and standard drug, (Silymarin) for 7 days. After this, the food supply was withdrawn and drinking water

was provided ad libitum. Next day, the animals were sacrificed. All the animals were observed twice daily (morning and evening) for morbidity and mortality, throughout the acclimatization and period of study.

Clinical Signs: After test item administration, individual animals were observed for abnormal clinical signs, due to treatment, throughout the study period. The clinical observations included changes in skin and fur, in the eyes and mucosal membrane in the respiratory, circulatory, central nervous and autonomous system and behaviour.

Body weight: Body weight was recorded on day 0 (prior to dosing) and day 8. Change in body weight (%) was calculated on day 8 based on previous body weight

Biochemical Analysis: Using rat capillaries, blood was drawn from retro-orbital plexus and was transferred to the in heparinised eppendorf tubes. It was then centrifuged at 3000 RPM for 10 minutes to separate plasma, which was collected using clean pipette. After collection, plasma samples were subjected to biochemical analysis. Biochemical analysis was performed on blood of animals fasted overnight. The major reason for this is the increased variability that would inevitably result from feeding and would mask more subtle effects and make interpretation difficult.

Histopathology Evaluations: Histopathology of liver tissues of all animals was performed to evaluate the alteration incurred due to treatment of paracetamol and reversal effect of this by various extracts of *Gmelina arborea* bark as well as by a known hepatoprotective compound silymarin, at microscopic level. To evaluate the parameters of oxidative stress and histopathology of liver tissue, portions of liver tissue were taken from each animal separately. After weighing, portions of liver tissue were trimmed and washed. The tissues were then fixed in 10% neutral buffered formalin for 24 hours. Tissue processing was done using automated tissue processor (Leica ASP) to dehydrate in ascending grades of alcohol, clearing in xylene, infiltration in paraffin wax for 5 hours and embedded in paraffin blocks. All the tissue blocks were sectioned at 5 μ m thickness with the Leica Motorised Rotary Microtome. These sections were subjected to staining with haematoxylin and eosin (H & E) with the help of Leica autostainer.

Result And Discussion

Macroscopic evaluation: The bark of *Gmelina Arborea* is typically smooth and whitish-grey to pale white when young, becoming corky and lenticellate as the tree

matures. It exfoliates in thin flakes. The bark also features vertical cracks, ridges, and fissures, giving it a rugged appearance. The taste of *Gmelina Arborea* bark is described as astringent. It can also be characterized as bitter and sweet. The root bark is specifically noted to have a pale brown internal colour and dark brown external color, with a faint, characteristic odour and an astringent taste.



Figure 1: *Gmelina Arborea* bark

Extraction of plant material: The extraction of drug signifies a solid from solid separation, as solid components must be extracted from a solid substance. This type of extraction is generally performed before any separation processing and should be differentiated from solid liquid extraction where the solid drug is extracted with a liquid medium. The liquid liquid extraction is one in which any of the two liquids that are not miscible includes the substance to be extracted.

Antimicrobial activity: The results of antimicrobial activity and statistical analysis of the same suggested significant effectiveness of the extracts of *gmnelia arborea*. Methanol extracts were found to be having higher antimicrobial potential than aqueous extracts of both the herbs. The antimicrobial activity of all the extracts was statistically significant (P value < 0.05) in comparison to standard. Among the extracts, Methanol extracts of *Gmelina Arborea bark* was found to be most effective at all test microbes.

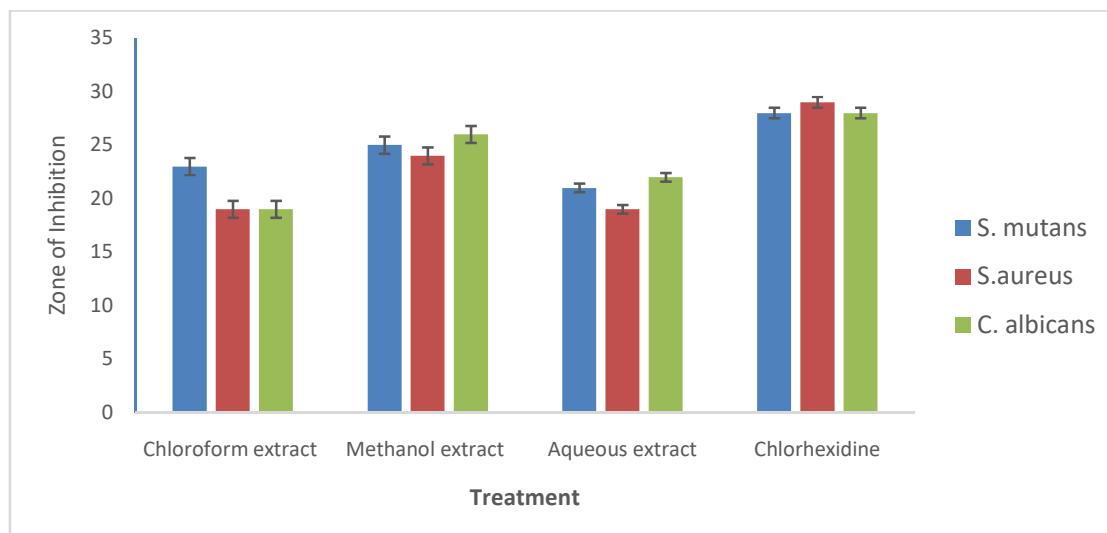


Figure 2: Antimicrobial Activity and zones of inhibition *Gmelina Arborea bark*

Hepatoprotective effect of extracts of the *Gmelina Arborea*: The results of body weight, biochemical analysis, oxidative stress analysis and organ weight, were presented as the mean $\pm$ SD of six rats per group. Graph Pad Prism 6.01 software was used for Statistical Analysis. Descriptive statistics and comparisons between groups were analyzed using one way analysis of variance (one way ANOVA).

Clinical signs and mortality: Administration of paracetamol and different extracts of *Gmelina Arborea* showed no mortality or morbidity in the animals during the period of study. Cage side observations did not show any observable clinical signs related to the compound toxicity. No tremors, convulsions, salivation, diarrhoea, lethargy, or unusual behaviors were observed in extract treated animals throughout the study period. Administration of paracetamol and different extracts of *Gmelina Arborea bark* extracts showed no mortality or morbidity in the animals during the period of study.

Effect on body weight: The recorded body weights of the animals at day 0 and day 8 of the experiment. The change in the body weights of the experimental animals from day 0 to day 8. Treatment with paracetamol induce hepatotoxicity caused significant reduction ($P < 0.01$) in body weight

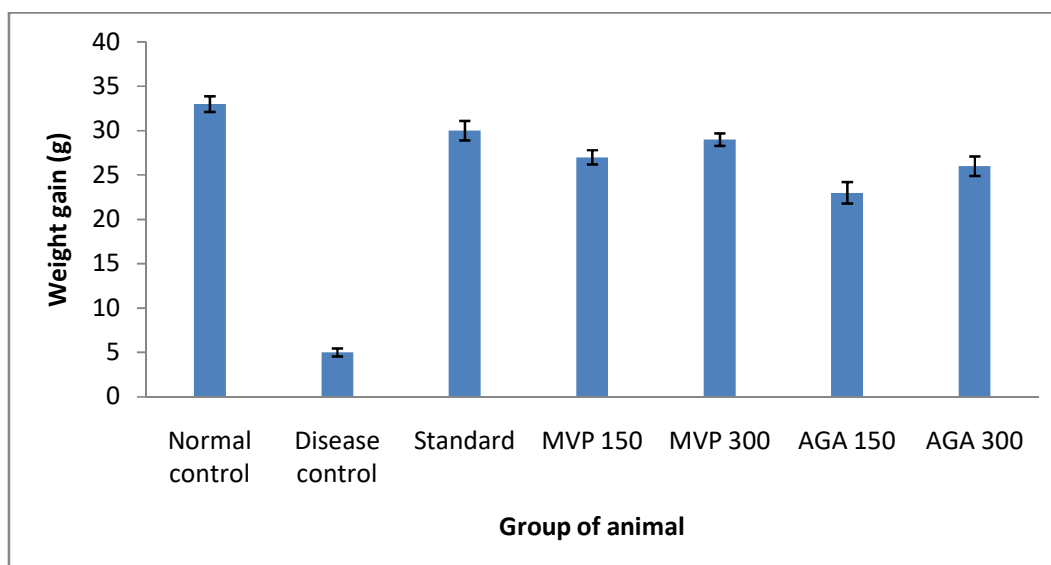


Figure 3: Effect of *Gmelina Arboreabark* extracts on body weight

The initial (day 0) to day 8 body weight of animals from various experimental groups of animals were recorded and gain in body weight has been calculated. In the disease control group the weight gain was lesser than all group. Treatment with *Gmelina Arborea* extract apparently took the animals towards normalcy since the weight gain in animals that of control animals. In the animals that received silymarin a hepatoprotective compound, resulted in a weight gain that was greater than disease control.

Biochemical Analysis:

Effect on the levels of AST in blood plasma: AST (Aspartate Aminotransferase), also known as SGOT (Serum Glutamic-Oxaloacetic Transaminase), is an enzyme found in the liver, heart, muscles, and other tissues. An AST blood test measures the amount of this enzyme in the blood. Elevated AST levels in the blood can indicate damage to these tissues, with liver damage being a primary concern. The levels of AST activity in the blood plasma of animals in different experimental groups were performed. In the animals from disease control AST level were increased in comparison to normal control group. Methanol extract of *Gmelina Arborea* bark extract showed dose dependent activity on AST levels in blood plasma of animals. Methanol extract were found more active than aqueous extract. All extract of *Gmelina Arborea* control AST level in dose dependent manner. Methanol extract showed maximum control than aqueous extract.

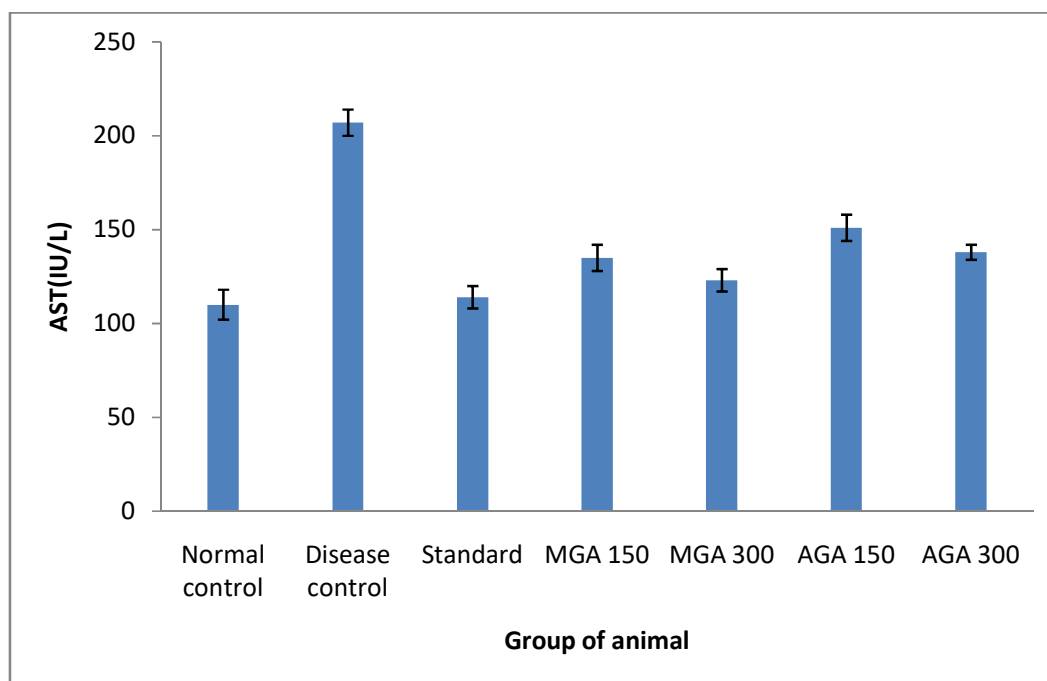


Figure 4: Effect of *Gmelina Arboreabark* extract on AST levels in blood plasma of animals

Effect on ALT levels in blood plasma of animals: ALT (Alanine Aminotransferase) is an enzyme primarily found in the liver, and its levels in blood plasma can indicate liver health in animals. Elevated ALT levels can suggest liver damage or disease, while normal levels generally indicate healthy liver function. The levels of ALT activity in the blood plasma of animals in different experimental groups were performed. In the animals from disease control ALT level were increased in comparison to normal control group. Methanol extract of *Gmelina Arborea* bark extract showed dose dependent activity on ALT levels in blood plasma of animals. Methanol extract were found more active than aqueous extract. All extract of *Gmelina Arborea* control ALT level in dose dependent manner. Methanol extract showed maximum control than aqueous extract.

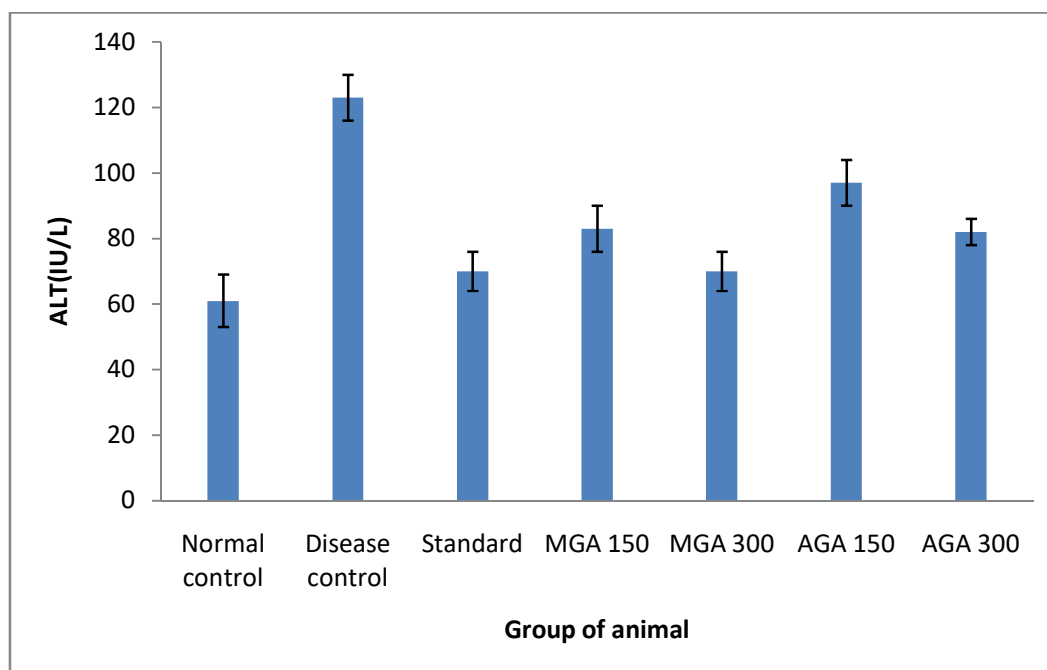


Figure 5: Effect of *Gmelina Arboreabark* extract on ALT levels in blood plasma of animals

Effect on ALP levels in blood plasma: Alkaline phosphatase (ALP) is an enzyme found in various tissues, including the liver, bones, and intestines. Elevated ALP levels can indicate liver or bone disorders, while low levels can suggest other conditions like hypothyroidism or deficiencies. Disease control animal level of ALP was much higher, than normal control. The difference in normal control and disease group was quite significant. The methanol extracts showed reduced levels of ALP activity, at both dose level. Administration of standard (Silymarin), the level of ALP activity was found to be significant reduction and close normal control animals. Methanol extract of *Gmelina Arborea* bark extract showed dose dependent activity on ALP levels in blood plasma of animals. Methanol extract were found more active than aqueous extract. All extract of *Gmelina Arborea* control ALP level in dose dependent manner. Methanol extract showed maximum control than aqueous extract.

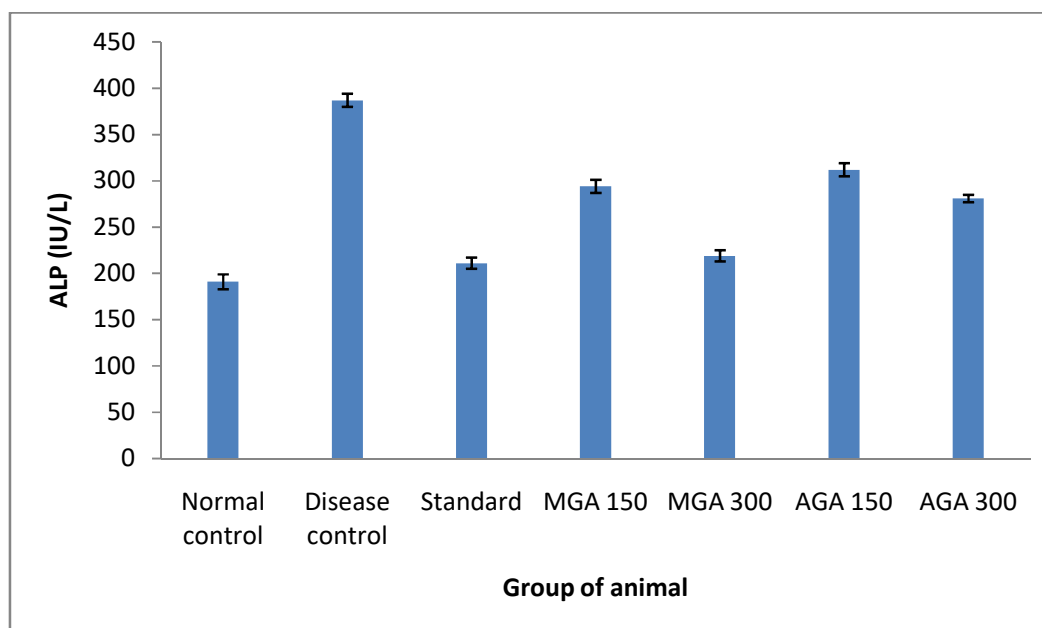


Figure 6: Summary of the *Gmelina Arboreabark* extract on ALP levels in blood plasma

Effect on Bilirubin level of animals: Paracetamol (acetaminophen) can increase bilirubin levels, particularly in cases of overdose. This is because paracetamol can cause liver damage, leading to impaired bilirubin processing and elevated bilirubin levels in the blood. Animals that received methanol extract of *Gmelina Arborea barkat* the dose of 150 mg/kg, the concentration of bilirubin was 0.50 ± 0.016 mg/dL, while at dose 300 mg/kg bilirubin was 0.46 ± 0.019 mg/dL. Standard treated animal showed bilirubin content of 0.42 ± 0.017 mg/dL. This too suggests that the methanol extract of *Gmelina Arborea bark* at the dose of 300 mg/kg are able to normalize the Bilirubin level in animals.

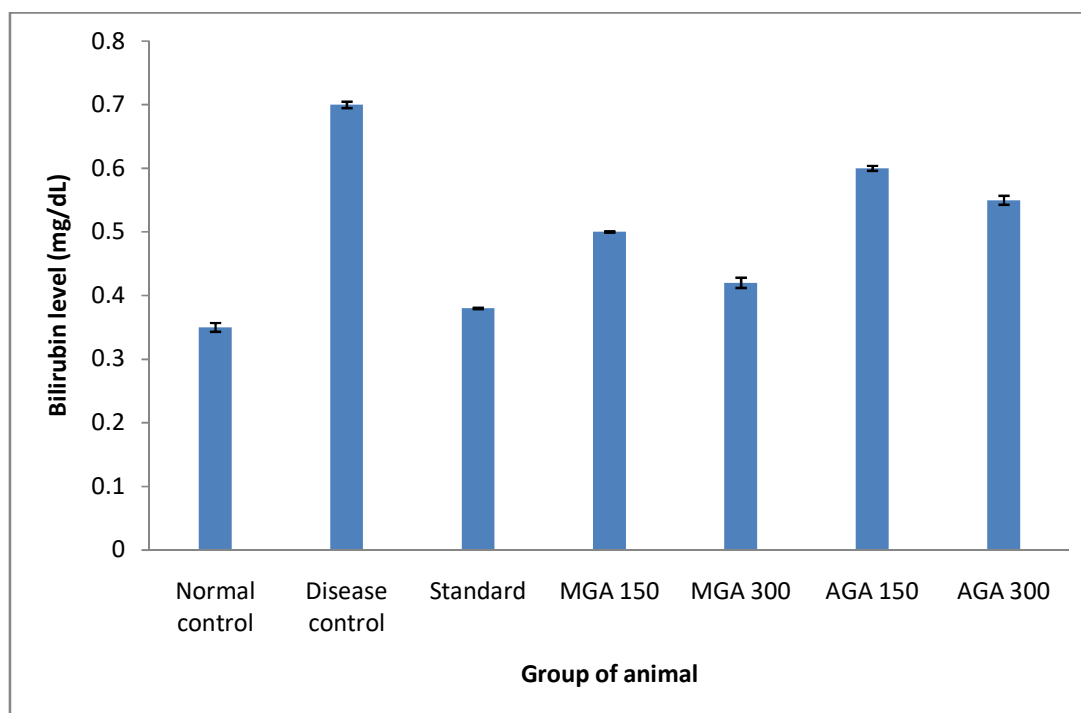


Figure 7: Summary of the *Gmelina Arboreabark* extract on Bilirubin level in blood plasma

Histopathology: Hepatocellular necrosis can be defined as death of hepatic parenchyma which initiates an inflammatory response. Pyknosis is the terminus of cell death, where the nucleus shrinks in size and chromatin condenses to solid, structureless masses. Cytoplasmic vacuolation is a morphological phenomenon where giant vacuoles are formed in animal cells, leading to cell death. Normal control Group, histological sections revealed normal histology. Disease control Group, animals showed minimal focal perivascular leukocytic infiltration, focal mild hepatocellular necrosis, hepatocytes with pyknotic nuclei, condensed cytoplasm and severe cytoplasmic vacuolation over a large area. Standard treated group did not reveal any lesion of pathological significance.

Group received a dose of 300 mg/kg of methanol extracts of *Gmelina Arborea* bark revealed normal histology. Group administered with hepatoprotective compound silymarin did not reveal any lesion of pathological significance and displayed normal histology. Treatment of animals with methanol extract of *Gmelina arborea* provide protection against paracetamol induced hepatotoxicity.

Liver is an important organ and a central one for many of the metabolic functions of the body, decomposition of toxic and waste substances, and disposal of harmful substances from the body. Liver illnesses are still the serious problem of human health. Making active oxygen species is an unavoidable result in aerobic organisms, and their removal is done at a basic level and with regard to the useful physiological functions and their harmful effects. This sensitive balance is for retaining the intracellular redox state which has an important role in optimizing cell functions. Excessive accumulation of free radicals or the body's inability to remove them causes the relocation of redox equilibrium for creating oxidation states in the body

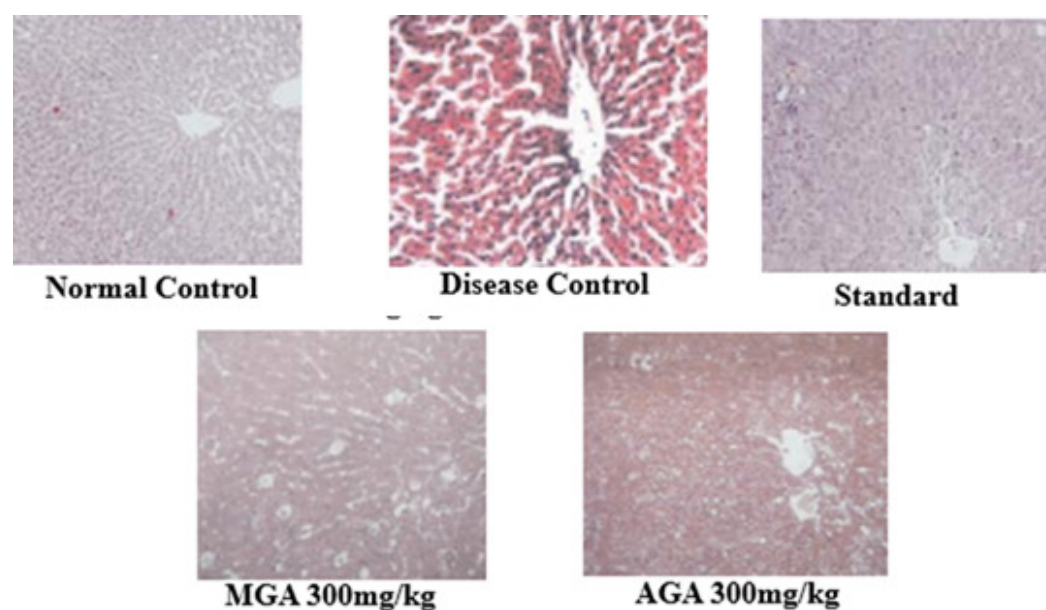


Figure 8: Histopathology of Liver of the animals (MGP methanol extract of *G. arborea*; AGP Aqueous extract of *G. arborea*)

Paracetamol is a common analgesic and antipyretic drug. Several studies have demonstrated the induction of hepatocellular damage or necrosis by acetaminophen higher doses in experimental animals and humans. For screening of hepatoprotective agents, paracetamol-induced hepatotoxicity has been used as a reliable method. Paracetamol is metabolized primarily in the liver and eliminated by conjugation with sulfate and glucuronide, and then excreted by the kidney. Moreover, paracetamol hepatotoxicity has been attributed to the formation of toxic metabolites, when a part of paracetamol is activated by hepatic cytochrome P-450 to a highly reactive metabolite Nacetyl-p-benzoquinoneimine (NAPQI).

Among all extract methanol extracts of *Gmelina Arborea* exhibits the excellent hepatoprotective properties as indicated by maximum prevention of increased serum biochemical parameters on paracetamol induced toxicity.

From our results, it can be concluded that decrease levels of catalase, and increased serum marker enzymes and lipid peroxidation level in paracetamol treated animals was due to hepatocellular damage. Methanol extract of *gmnelia arborea* afforded protection from such paracetamol induced liver damage, and has shown the most pronounced hepatoprotective effect.

Conclusion: Liver is an important organ and a central one for many of the metabolic functions of the body, decomposition of toxic and waste substances, and disposal of harmful substances from the body. Liver illnesses are still the serious problem of human health. Making active oxygen species is an unavoidable result in aerobic organisms, and their removal is done at a basic level and with regard to the useful physiological functions and their harmful effects. This sensitive balance is for retaining the intracellular redox state which has an important role in optimizing cell functions. Excessive accumulation of free radicals or the body's inability to remove them causes the relocation of redox equilibrium for creating oxidation states in the body

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