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Phytochemical and pharmacological investigation of some medicinal plants for the treatment of hepatoprotective & antioxidant activity

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Abstract---The plant *Luffa acutangula* var. *Amara* is used by native practitioners. Presently, it is planned to make petroleum ether, alcohol, and aqueous extracts of this plant's fruits before undertaking blind screening trials to examine the antidiabetic, hepatoprotective, and antibacterial effects of the fruits and validate native practitioners' claims. The powdered whole fruits were extracted with petroleum ether, alcohol and chloroform water. The LD₅₀ values for all these extracts were found to be 70 mg/kg, 500 mg/kg, and 15g/kg respectively. Alloxan-induced diabetes in 150-200g albino rats was tested for antidiabetic effects and compared to glibenclamide. Albino rats treated with CCl₄, a liver toxicant, had increased SGOT, SGPT, SALP, direct and total bilirubin, serum cholesterol, and serum triglyceride levels. Compared to CCl₄-treated group, findings were significant. In a CCl₄ hepatotoxic model, alcoholic and aqueous extracts significantly decreased wet liver weight and volume, indicating hepatoprotective action. Histopathological abnormalities (fatty infiltration, necrosis, etc.) were normalised in aqueous and alcoholic extract groups. Thus, the current study reveals that *Luffa amara* fruit extracts have antihyperglycemic activity, hepatoprotective activity (as evidenced by both in vitro and histopathological studies), and satisfactory antimicrobial activity, particularly against *Helicobacter pylori*. of the three extracts, alcoholic and aqueous extracts were more effective than petroleum ether extract.

Keywords---*Luffa amara* fruit extracts, Anti-diabetic activity, CCl₄, Hepatoprotective activity, Antimicrobial activity.

Introduction

The liver has two big lobes, right and left, and fills the abdominal cavity slightly below the diaphragm. The hepatic lobule is a column of hexagonal liver cells (hepatocytes). The hepatic artery and portal vein branch between lobules [1,2]. Lobule capillaries are sinusoids, big, permeable channels between liver cells. Sinusoids receive blood from the hepatic artery and portal vein; liver cells use this combination to operate. The portal vein transports blood from the digestive organs and spleen to the hepatic artery. Lobules have central veins. The hepatic veins transport blood from the liver to the inferior vena cava. The liver cells' main digestive function is producing bile [3]. Bile enters tiny bile ducts, called bile canaliculi, on liver cells, which merge to create the hepatic duct, which removes bile from the liver [4]. Viruses cause hepatitis, liver inflammation. Most prevalent hepatitis viruses are A, B, and C. Anorexia, nausea, exhaustion, and jaundice are hepatitis symptoms. Disease severity ranges from minor to deadly. Hundreds of thousands of cases of hepatitis occur in the U.S. each year. While all three viruses cause liver inflammation, they have different mechanisms of transmission and implications for patients [5,6]. Hepatitis A is transmitted fecally orally. Food contaminated by moderate cases is the primary transmission vehicle, while shellfish gathered from sewage-infested water is another source. Most cases of hepatitis A are minor, and recovery confers permanent immunity. People exposed to hepatitis A can have gamma globulin to avoid the illness. Hepatitis B is spread by blood and sperm. 10% of people who recover from hepatitis B become carriers. Carriers may develop chronic hepatitis, cirrhosis, or primary liver cancer. Carriers also infect others, especially their sexual partners. Even infrequent blood contacts should get the hepatitis B immunization [7]. Carriers' sexual partners may also receive the vaccination. This immunisation is currently routine for babies. Hepatitis C can be transferred by blood or mucous membrane contact. Most acquire chronic illness, yet many are asymptomatic for years. Active virus causes liver failure. Liver transplant is the sole treatment. These kinds of hepatitis aren't transferred by blood transfusions. All three viruses are checked in donated blood [8].

Material and Method

Materials

Fruit extracts of *Luffa acutangula* var. *amara* Pet ether extract of fruits of (70mg/kg) Alcoholic extract of fruits of (100mg/kg) Aqueous extract of fruits of (3000mg/kg): Polyethylene glycol-400, gum acacia suspension (5%), Glibenclamide, Glucose estimation kit (Span Diagnostic Ltd.), Elico mini. Sl. 171 spectrophotometers.

Methods

Method of blood sample collection

Capillary tubes were used to collect blood from a retro-orbital puncture while under the influence of mild ether anaesthesia. Blood was collected in tubes and centrifuged at 3000 rpm at room temperature. Glucose reagent was added to the

measured serum, incubated at 37°C for 10 minutes and analyzed for glucose estimation [9].

Estimation of blood glucose

Trinder's approach involves the use of two enzymes, glucose oxidase (GOD) and peroxidase (POD), as well as the chromogen L-amino antipyrine and the phenol. This technique measures glucose in serum/plasma or cerebrospinal fluid. There was no interference due to the substances like creatinine, fructose, galactose, reduced glutathione, ascorbic acid and xylose. Haemoglobin or bilirubin upto 10mg % does not affect the test [10].

Anti-bacterial activity

Anti-bacterial activity was studied using cup plates. In this method, test solution was added to inoculated agar. Inoculation caused inhibition zones. The test fluid can be put in a tiny cup sealed to the agar surface or on an impregnated filter paper disc [11,12].

Anti-fungal activity

In a beaker, accurately measured portions of peeled potato are combined with two-thirds of a beaker's volume of distilled water, followed by 45 minutes of steaming. The filtrate was collected using muslin cloth and distilled water to make up to the desired volume [13]. Dextrose and agar-agar were added to this mixture and maintained in a beaker in a hot water bath until the agar melted. Then 30ml of molten agar media was poured into each of the boiling tubes. The boiling tubes were plugged tightly with non-absorbent cotton and they were sterilized in an autoclave at 121°C and 15lbs/sq inch pressure for 20min. They were cooled to 46°C and inoculated (1ml/100ml of medium) with the suspension of microorganism and poured into petri dish to give a depth of 3-4mm under aseptic conditions. Allow the medium in the petri dish to settle. A sterile cork borer was used to make 5 cups (8mm diameter) each plate. The entire process was conducted under laminar airflow. 50µl of two concentrations of test solutions of 50µg/ml and 100µg/ml were pipette into first and second cups. Standard ketoconazole solutions of concentration 25µg/ml and 50µg/ml were pipetted into third and fourth cups. Blank was put in the fifth cup. In the case of petroleum ether extract, another blank PEG-400 was placed in the sixth cup, while in the case of alcoholic and aqueous extracts, 5 percent gum acacia suspension was utilised. The plates were then incubated for 48 hours at room temperature to test for antifungal activity. Inhibition zones were measured in millimetres [14-16].

Results and Discussion

Phytochemical investigation

A preliminary examination into the phytochemistry of the plant was carried out. Compounds such as sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, phenolic compounds, tannins, amino acids, proteins, fixed oils, gums, mucilage, and triterpenes were all analysed. Triterpenes and fixed oils were

discovered in petroleum ether extracts; carbohydrates, glycosides, saponins, flavonoids, sterols, amino acids, and proteins were discovered in alcoholic extracts; and carbohydrates, glycosides, saponins, flavonoids, sterols, amino acids, and proteins were discovered in aqueous extract.

Toxicity study

The toxicity study was carried out on the petroleum ether, alcoholic and aqueous extracts of whole fruits of *Luffa acutangula* var. *amara* on albino mice following OECD guidelines (No. 420). The LD₅₀ values for petroleum ether, alcoholic and aqueous extracts were found to be 350 mg/kg, and 500 mg/kg respectively. However, aqueous extract, even at 15g has not produced any mortality, thus proving its practically non-toxic nature. 1/5th dose of LD₅₀ values for the petroleum ether and aqueous extracts were calculated and selected for the present study. Similarly for aqueous extract, 1/5th dose of 15g was selected.

Blind screening studies

When compared to aqueous extract, petroleum ether and alcoholic extracts revealed significant "dose-dependent CNS depression," according to the score sheet. The tests were again carried out for further 7 days, and no delayed toxicity was seen in any of the groups. Tables 1-2 include the results of the readings and observations.

Pentobarbitone induced sleeping time

The effects of fruit extracts of *Luffa acutangula* var. *amara* on pentobarbitone sodium induced sleeping time showed that petroleum ether, alcoholic, and aqueous extracts potentiated pentobarbitone sleeping time. Percent potentiation is 115.56, 119.6, and 113.8 respectively with the extracts mentioned above. Results are compiled in table 3.

Hepatoprotective activity

The hepatic enzymes viz; SGPT, SGOT, SALP, TG, CHO, BID, BIT, Total proteins, ALB and HDL levels were assessed in groups of rats treated with CCl₄, standard drug silymarin, CCl₄ + Petroleum ether, CCl₄ + alcoholic and CCl₄ + aqueous extracts. The findings from the in vitro tests revealed that treatment with aqueous extract significantly decreased the elevated levels of hepatic parameters caused by CCl₄ exposure, with the exception of serum HDL, albumin, and total proteins, which remained elevated despite treatment with silymarin and the other three extracts mentioned above. The alcoholic extract had less hepatoprotective efficacy than the aqueous extract, while the petroleum extract had the least. ANOVA shows that there is a significant difference between the groups, but the student 't' test shows that aqueous and alcoholic extracts have substantial activity and petroleum ether extracts have the least. The alcoholic and aqueous extracts reduced the elevated levels of SGPT, SALP, SGOT, TG, CHO, BID, BIT, and elevated the reduced levels of total proteins, HDL, and albumin. Results are given in table 4 and 5.

Wet liver weight and wet liver volume

CCl₄ treatment in rats resulted in their liver enlargement - evident by increased wet liver weight and wet liver volume. Significant restoration of wet liver weight and wet liver volume, similar to that in normal rats, was observed in groups treated with silymarin and the three fruit extracts of *Luffa acutangula* var. *amara*. Results are compiled in table 6.

Serum profile studies

Results of serum profile studies are shown in table 4.

Histopathological studies of liver:

Normal control group: Shows normal lobular architecture of liver with hepatocytes arranged in single cords are shown in figure 1.

Carbon tetrachloride treated group: Figure 2 demonstrates perivenular necrosis and steatosis in the liver, with varying degrees of steatosis ranging from ballooning degeneration to necrosis.

Standard drug treated group: This group, which received silymarin 30 minutes before CCl₄ treatment, has normal lobular architecture, indicating that the medication has hepatoprotective properties, and no alterations detected with CCl₄ shown in figure 3.

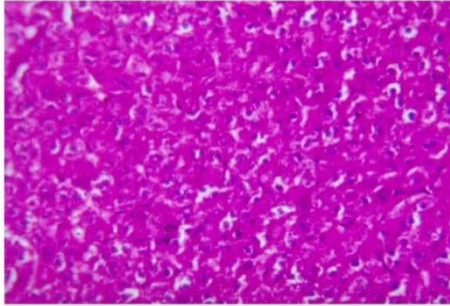
Petroleum ether extract treated group: A section from the liver showing normal architecture, with congestion in perivenular areas (prominent fatty change) hepatitis and early necrosis, suggesting least hepatoprotective action of petroleum ether extract against CCl₄-induced hepatotoxicity shown in figure 4.

Alcoholic extract treated group: Hepatic cells in the portal and central veins appear normal, with very slight fatty alteration in the perivenular cells (vacuoles are clearly visible in the cytoplasm of the hepatocytes) confirming the hepatoprotective activity of *Luffa acutangula* fruits, which contain an alcoholic extract shown in figure 5.

Aqueous extract treated group: A section of liver showing the portal tract and minimal fatty changes with near normal lobule architecture. There are small vacuoles in few hepatic cells around the central vein thus indicating the protective action of aqueous extract shown in figure 5 and 6.

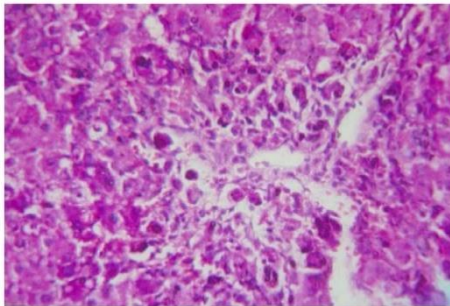
**Histopathological Photomicrographs
of Liver**

Fig - 1



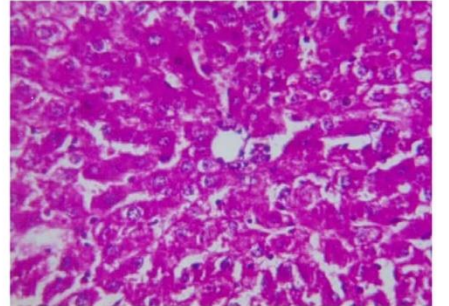
**Group - A Normal Control
H & E - 100X**

Fig - 2



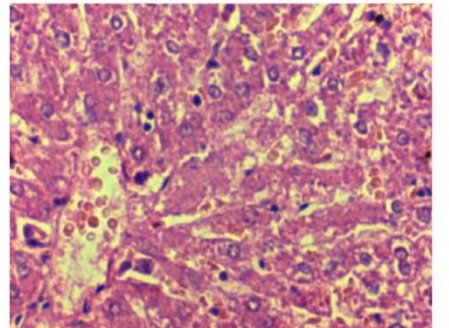
**Group - B Carbon tetrachloride Control
H & E - 100X**

Fig - 3

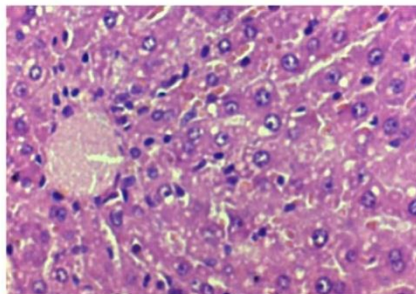
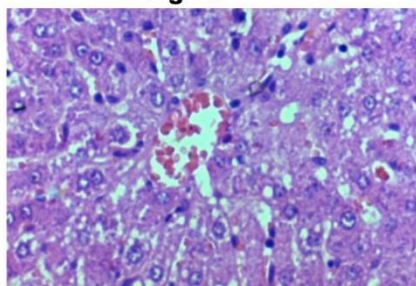


**Group - C Silymarin + CCl₄
H & E - 100X**

Fig - 4



**Group - D Pet Ether Extract + CCl₄
H & E - 40X**

Fig - 5**Group - E Alcoholic Extract + CCl₄
H & E - 40X****Fig - 6****Group - F Aqueous Extract + CCl₄
H & E - 40X**

Pentobarbitone sleeping time

The effects of *Luffa acutangula* var. *amara* fruit extracts on pentobarbitone sodium-induced narcosis revealed that both alcoholic and aqueous extracts shortened the duration of pentobarbitone sodium-induced sleeping time. Percent potentiation is 134.8 and 140.8 respectively. However, petroleum ether extract showed less effect on the duration of pentobarbitone sodium induced sleeping time with percent potentiation is 170. Results are compiled in table 7. Results are analyzed by unpaired students't' test and ANOVA and the same confirms significant effects in all the extracts.

Antidiabetic activity

The petroleum ether extract has reduced the blood glucose level to only 36.53% at the end of 6th hour. Its effect started at the end of 1st hour. Alcoholic extract has reduced the blood glucose levels significantly to the extent of 64.14% at the end of 10th hour. Blood glucose lowering effect started at the end of 1st hour. Aqueous extract has reduced to the extent of 65.17% at the end of 10th hour, with effect starting from the 1st hour. The recovery of blood glucose started around 12th hour and has not been recovered fully by 24 hours. Standard glibenclamide produced blood glucose reduction at the end of 1st hour itself but the peak effect was seen

Table 9 B Antifungal activity of fruit extracts of *Luffa acutangula* var. *amara* (100 μ g/ml)

Name of the extract	Dose μ g/ml	Average zone of inhibition (in millimeter)	
		Candida albicans	Aspergillus niger
Standard			
Ketoconazole	50	19	29
Pet ether extract	100	14	4
Alcoholic Extract	100	18	6
Aqueous Extract	100	12	1

Conclusion

Carbohydrates, flavonoids, sterols, triterpenes, glycosides, saponins, amino acids, proteins and fixed oils are present in the fruit extracts of *Luffa acutangula* var. *amara*. All extracts showed "Dose-dependant CNS-depression". This was evident by increase in pentobarbitone sleeping time. Percentage blood glucose reductions with aqueous and alcoholic extracts were comparable to that of standard glibenclamide. Petroleum ether extract showed less significant activity. Test was done using GOD/POD method. Alcoholic and aqueous extracts showed good hepatoprotective activity in CCl₄- induced hepatotoxic model by in-vitro serum examination and histopathological observation of rat livers. The results were compared with the standard drug silymarin. Petroleum ether, alcoholic, and aqueous extracts showed good antibacterial activity against gram +ve and gram -ve bacteria. Petroleum ether, alcoholic and aqueous extracts also showed good antifungal activities. However, further studies are needed in this regard to isolate and characterize the phytochemical constituents, and also to explore their medicinal uses of the plant.

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