

ROLE OF *PSIDIUM GUAJAVA* LEAF EXTRACT IN ALCOHOL INDUCED HEPATOPATHY OF ALBINO RAT

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ABSTRACT

Healthy Albino Rats (Wister variety) of either sex are reared feeding on pulses, gram and bread. After acclimatization these are divided into one normal-control and three experimental groups. Alcoholic hepatitis is induced in two of the experimental groups by oral administration of 30% ethanol for eight weeks. Oral administration of water- extract of *Psidium guajava* leaves is done to one non-alcoholic and one alcoholic group of Rats throughout the study period. Investigation of AST (Aspartate Transferase), ALT (Alanine Transferase), ALP (Alkaline Phosphatase) activities in blood serum and Liver Lipid Peroxide after the experimental period leads to a conclusion that *P. guajava* leaf- extract may play a good role in stopping hepatocyte destruction due to alcoholic injury. Urea, Creatinine and Uric Acid levels in blood serum and GST (Glutathione S-Transferase) and CYP (Cytochrome p450) activity in liver of the study animals indicate that no major toxicity arise from the administration of *P. guajava* leaf- extract.

Keywords: *Hepatopathy, alcoholism, toxicity, Albino Rats.*

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INTRODUCTION

The apple guava or common guava (*Psidium guajava*; Family- Myrtaceae) is an evergreen shrub or small tree native to Mexico, the Caribbean, and Central and South America. It is commonly known in Assamese Language as “Modhuriaam”. Widely cultivated in tropical and subtropical regions around the world, guava fruits can range in size from as small as an apricot to as large as a grapefruit. Various cultivars have white, pink, or red flesh, and a few also feature red (instead of green) skin. From the very ancient time various ethnic groups of different corners of Assam are using leaf extract of *Psidium guajava* for the treatment of various gastro-intestinal and hepatic disorders. In central and lower Assam water extract of the tender leaves is used orally along with goat-milk to increase appetite of ailing people.

Alcoholic hepatitis is a major social problem amongst lower middle-class people of the third world countries now a day. Though the synthetic antioxidants like “Metadoxine” ($C_8H_{11}NO_3 \cdot C_5H_7NO_3$) to treat alcoholic hepatopathy are available, common people have a tendency to use plant based ethno-medicines. In case of administration of ethno-medicinal components prepared from plant extracts, some problems of

intoxications may occur as a single plant or part of it may comprise hundreds of ingredients. Though some may act as medicines, others may act as toxins and bring irreversible loss to some susceptible organs like liver and kidneys. Hence, toxicological investigation using laboratory animals is a necessary part of promotion of ethno-medicinal components for common men use.

In intoxication, (such as alcoholic intoxication) free radicals (H^0 & OH^0) are formed which in turn brings lysis of the lipid bi-layer of the cell membrane, especially of soft tissues like Liver. The phenomenon is known as “lipid peroxidation”. As a result of this, the cells are destroyed and the contents of the cell are released to the body fluid (serum). The activity of the enzymes AST (Aspartate Transferase), ALT (Alanine Transferase) and ALP (Alkaline phosphatase) are mainly localized in the hepatocytes. Hepatocyte destruction increases their level of activity in blood serum. Present investigation deals with the role of water-extract of *P. guajava* leaves on alcohol-induced liver lipid peroxidation of Albino Rat with supportive investigation of AST, ALT and ALP activities in blood serum both in normal-control and treated

animals. Study on the levels of Urea, Creatinine and Uric acid levels in blood serum are done in normal-control and the ethno-medicine administrated animals to find out probable nephrotoxicity from the use of this ethno-medicine. GST (Glutathione S -Transferase) and CYP (Cytochrome p450) are two main enzymes responsible for xenobiotic metabolism (Jakoby W.B. *et al.*, 1990, and Guengerich F.P. 2001). In case of entrance of any harmful foreign component to the body, the levels of their activities rise several times in various tissues including liver to detoxify the systems. In this study GST and CYP activities in liver samples are investigated both in normal-control and the ethno-medicine administrated animals to find out the probable toxicity on the administration of *P. guajava* leaf extract.

Materials and Methods

To prepare the ethno-medicinal component fresh tender leaves of *Psidium guajava* are homogenized adding deionized water and filtered through whatman's organic-grade filter paper. Filtrate is vacuum-dried at $50 \pm 2^{\circ} \text{C}$ and obtained jelly-like substance is kept in air tight container in deep freeze. This is used within 3 days of preparation. Healthy Albino Rats (Wister variety) of either sex are reared fed on pulses, gram and

bread. After acclimatization they are divided into four groups, each group containing 5 animals. Group-I contains Normal Control Rats, Group-II contains Rats fed with the ethno-medicinal component, Group-III contains Alcohol Administered Rats & Group-IV contains Alcohol Administered Rats feds with the ethno-medicinal component. An experimentally adjusted dose 1000 μl of 40% ethanol is administered orally to each rat of Group-III & IV twice daily for 8 weeks to induce Alcoholic Hepatopathy. 400mg of *P. guajava* leaf extract /kg body weight is administrated orally to Group-II & Group-IV daily for 8 weeks parallely to alcohol administration. A proper hygienic condition is provided to the study animals. No juvenile or pregnant individual is applied for the experimental purposes. The standard guidelines prescribed by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA 2003) are followed during the study.

Blood samples are collected retro-orbitally from the inner canthus of the eye using micro-haemorit capillaries under light ether anesthesia and kept in separate labeled micro-centrifuge tubes (Punitha *et al.*, 2005). These are allowed to clot at room temperature for 20 minutes. The sera of

respective blood samples are extracted by centrifugation. and kept in separate labeled micro-centrifuge tubes in 0° to 4°C. Then animals are sacrificed by high dose of ether anesthetization and livers are dissected out and kept in deep freeze in proper labeled vials. Measurement of Liver Lipid Peroxidation is done by the photometric evaluation of molar extinction co-efficient of thiobarbituric acid (Ohkawa *et al.*, 1979). Serum-AST activity is measured by using AST (GOT) reagent kit (IFCC/Kinetic) (Bergmeyer, H.U. *et al.*, 1978). Serum-ALT activity is measured by using ALT (GPT) reagent kit (IFCC/Kinetic) (Bergmeyer, H.U. *et al.*, 1980). Serum-ALP activity is measured by ALP reagent kit (GSCC/Kinetic) (Bretandiere J. P. *et al.*, 1977). Serum Urea level is measured by using Urea reagent kit (Mod. Berthelot) (Fawcett J. K. *et al.*, 1960). Serum Creatinine level is measured by using Creatinine reagent kit (Alkaline Picrate) (Kammcrat C., 1978). Serum Uric Acid level is measured by using Uric Acid reagent kit (Uricase/PAP) (Fossati P. *et al.*, 1980). GST activity in liver is measured by using GST Assay kit (Kinetic) (Habig, W. H. *et al.*,

1974). CYP activity in liver is measured by using Cytochrome p450 assay kit (Kinetic) (Schenkman J.B., 1993). Total protein estimation in liver samples is also done (Lowry, O. H., 1951) and GST and CYP activities are expressed as activity per mg of liver protein. AST, ALT, ALP and Creatinine assay kits are procured from Ranbaxy-RFCL (India) LTD. Urea, and Uric acid assay kits are procured from Crest Biosystems (India) LTD. GST and CYP assay kits are procured from Sigma-Aldrich Inc. (USA). Thiobarbituric acid is procured from Research Fine Chem (India) LTD. Folin-Ciocalteu's phenol reagent (used in protein estimation) is procured from Sigma-Aldrich Inc. (USA). The other reagents and chemicals are procured from Ranbaxy-Ranchem, (India) LTD. All the biochemical investigations and evaluations are done in a semi-automated biochemistry analyzer ("Lab Life Chem-Master" manufactured by Ranbaxy- Diagnova LTD) with proper programming. All the data obtained during the period of investigation are analyzed following the methods of Croxton, F.E. (1959) with the help of MS Excel.

Table 1: Showing mean values of different study parameters of hepatic function obtained during the experiment.

Study parameters	Group-I Normal-Control Rats	Group-II Rats fed with <i>P. guajava</i> leaf extract	Group-III Alcohol administered Rats	Group-IV Alcohol administered Rats fed with <i>P. guajava</i> leaf extract
Serum AST (IU/L)	273.56±0.916	213.26±0.719 -22.043%*	485.046±0.599 +77.309%*	316.152±1.806 -34.820%*
Serum ALT (IU/L)	71.622±0.377	64.324±0.399 -10.190%*	216.412±0.596 +202.159%*	74.988±0.822 -63.730%*
Serum ALP (IU/L)	815.214±0.845	744.53±0.766 -8.671%*	2038.652±15.272 +150.076%*	864.876±1.0396 -65.349%*
Liver Lipid Peroxide (n mol/mg)	233.61±0.5496	224.592±1.1976 -3.860%*	474.508±1.1096 +103.120%*	276.08±1.1076 -41.818%*
GST activity in Liver (µmol/min/mg)	0.385±0.001	0.315±0.002 -18.181%*	0.395±0.001 +2.597%*	0.371±0.001 -6.075%*
CYP activity in Liver (µmol/min/mg)	382.698±0.881	363.746±0.649 -4.952%*	416.086±0.849 +8.724%*	393.38±0.719 -5.457%*
+...% percent increase, -...% percent decrease * Significant at p<0.001				

Table 2: Showing mean values of different study parameters of renal function obtained during the experiment.

Study parameters	Group-I Normal-Control Rats	Group-II Rats fed with <i>P. guajava</i> leaf extract
Serum Urea (IU/L)	16.428±0.004	16.432±0.007 N.S.
Serum Creatinine (IU/L)	1.232±0.010	1.238±0.006 N.S.
Serum Uric Acid (IU/L)	1.328±0.006	1.334±0.007 N.S.
N.S.= Not significant		

RESULTS AND DISCUSSION

It has been observed that the activities of Serum AST, ALT and ALP are significantly decreased on the administration of *P. guajava* leaf extract ($p < 0.001$) in Group-II animals in comparison to normal control animals of Group-I with -22.043%, -10.190% and -8.671% deviations respectively. Liver lipid peroxide levels are also show the similar trend in this Group with -3.860 deviations (Table-1). This indicates that the water extract of *P. guajava* leaves has role in hepatostimulation and hepatoprotection. Several fold increase of the mentioned biochemical parameters ($p < 0.001$) are observed in alcohol administered rats of Group-III, with 77.309%, 202.159%, 150.076% and 103.120% deviations respectively from their normal control levels of Group- I Rats. This indicates large scale destruction of the hepatocytes. But these could be successfully minimized ($p < 0.001$) with the administration of the ethno-medicinal component in Group-IV animals showing -34.820%, -63.730%, -65.349% and -41.818% deviations respectively from those of Group-III animals (Table-1). It has also been observed that no significant augmentation of Urea, Creatinine and Uric Acid levels in blood serum of group-II rats

from normal control; leading to a conclusion that the ethno medicinal component plays no nephrotoxic role (Table-2).

Studies on GST and CYP in liver samples revealed that, the levels of their activities slightly decreased in group-II rats from normal control on administration of *P. guajava* leaf extract. It is due to antioxidant property of the ethno-medicine. Similar picture is observed in case of alcoholic and ethno-medicine administered rat groups (group-II and IV). From the phytochemical investigations of *P. guajava* leaves by Olajide O.A. *et al*, (1999) it is come to know that these leaves are rich in some flavonoid compounds, namely morin, morin-3-O-lyxoside, morin-3-O-arabinoside, quercetin, and quercetin-3-O-arabinoside . The flavonoids play a good role as potent antioxidants minimizing the free radicals by scavenging on them (Middleton E. Jr., 1996). So they can successfully minimize Lipid Peroxidation of the soft tissue cell membranes including those of liver.

CONCLUSION

From this study it could be concluded that oral administration of *P. guajava* leaf-extract successfully minimizes hepatocyte destruction caused by alcoholic intoxication. No major toxicity arises from the use of this

ethno-medicinal component and this may be a good ingredient for the treatment of alcoholic hepatopathy.



REFERENCES

Begum S., Hassan S.I., Siddiqui B.S., Shaheen F., Ghayur M.N. and Gilani .H., Triterpenoids from the leaves of *Psidium guajava*. *J. Phytochem.*; **61(4)**:399-403., (2002).

Bergmeyer, H. U. and Holder, M., Method for the measurement of catalytic concentrations of enzymes. *J.Clin. Chem. Acta.* **105**:147F-154F., (1980).

Bergmeyer, H. U., Scheibe, P. and Wahlefeld, A. W., Optimization of methods for aspartate aminotransferase and Alanine aminotransferase. *J. Clin.Chem.* **24**:58-73. (1978).

Bretandiere, J. P., Vassault, A., Amsellem, L., Pourci, M. L., Phung, H. T. and Baily, M., Criteria for establishing a standardized method for determining alkaline phosphatase activity in human serum. *J. Clin. Chem.* **23**:2263-2274. (1977).

CPCSEA: CPCSEA Guidelines for Laboratory animal facility. *Ind. J .Pharm.* **35**: 257-274. (2003).

Fawcett, J. K. and Scott, J. E., A rapid and precise method for determination of urea. *J. Clin. Pathol*: **13**:156, (1960).

Fossati, P. and Prencipe, L., Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-

aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *J. Clin. Chem.* **26**:227-231, (1980).

Guengerich F. P., Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. *J. Chem. Res. Toxicol.* **14** (6): 611–50, (2001).

Habig, W.H., Pabst, M.J. and Jakoby, W.B., Glutathione S-transferases. The first step in Mercapturic acid. *J. Biol. Chem.* **249**: 7130-7139 (1974).

Jakoby W.B., and Ziegler, D.M., The enzymes of detoxication. *J. Biol. Chem.* **265** (34): 20715–8 (1990).

Kammcrat, C., Modification of the alkaline picrate assay for creatinine to prevent spuriously elevated values by keto acids. *J. Clin. Chem. Acta.:* **84**:119 (1978).

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., Protein measurement with the Folin-Phenol reagents. *J. Biol. Chem.* **193**: 265-275(1951).

Middleton E. Jr. Biological properties of plant flavonoids: *An overview: Inter. J. Pharm.*; **34(95)**:344-348(1996).

Ohkawa, H., Ohishi, N. and Yagi, K.,
Assay for lipid peroxides in animal tissues
by thiobarbituric acid reaction. *J. Anal.*
Biochem. : **95**:351-358, (1979).

Olajide, O. A.; Awe, S. O. and Makinde,
J. M., Pharmacological studies on the leaf
of *Psidium guajava* . *J. Fitoterapia*, **70**:25-
31(1999).

Schenkman J.B., Cytochrome P450, p. 3-
14. In Schenkman J.B. and Greim H (eds.).
Handbook of Experimental Pharmacology,
Springer-Verlag, Berlin Heidelberg 105, p.
(1993).

