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Antihepatotoxic Effects of Aqueous Extract of the Leaves of *Morinda lucida,* Benth on Ethanol-Induced Hepatotoxicity in Rabbit.

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Abstract: The study was designed to evaluate the possible hepatoprotective activity of aqueous extract of the leaves of *Morinda lucida,* against chronic ethanol induced hepatotoxicity in weaner rabbits, since natural products are known to be the best source of remedies for the hepatocellular disease.Thirty (30) healthy all male weaner rabbits were randomly divided into 6 groups of 5 animals each, Group1(Control) was treated with 0.9% Normal Saline,1ml/100g body weight, Group II was treated with 40% ethanol at 1ml/100g body weight, Group III was pretreated with ascorbic acid at 400mg/kg body weight, Group IV was treated with *Morinda lucida* extract only at 300mg/kg of body weight, Group V was pre-treated with *Morinda lucida* extract at 300mg/kg body weight, then 1ml/100g body weight of 40% ethanol was administered one hour later, Group VI was treated with 40% ethanol at 1ml/100g body weight and an hour after, the animals were post treated with *Morinda lucida* extract at 300mg/kg of body weight. Serum enzymes, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, and other biochemical parameters namely, Total protein, Albumin, Total and Direct bilirubin, which are standard indices of hepatocellular damage, were assayed by standard spectrophotometric techniques. The result shows that there was a significant decrease in ALT, AST, GGT, Total and direct bilirubin, between ethanol only treated group and extract pre-treated group with P values of <0.01,<0.001,<0.001,<0.05,<0.01 respectively, These results indicate a probable hepatoprotective activity of the extract in the pre treated and post treated studies. On the contrary, albumin and total protein showed significant increase with the P value of <0.05 and <0.001 respectively, between ethanol only treated group and extract pre treated group. Results of the histopatology study also corroborate the biochemical parameters' results. The result indicates that there was probably a sign of hepatic damage which led to leakages of cellular enzymes into plasma leading to significant increase in some of the hepatic enzymes activities. It also appears that aqueous extract of *Morinda lucida* extract show hepatoprotective effect on ethanol induced toxicity by reversing the changes produced by ethanol in rabbits.

Key word: Hepatotoxicity, Rabbit, Ethanol, *Morinda lucida*, Histopathology

INTRODUCTION

The nutritional importance of ethanol has been extensively studied. The availability of alcoholic beverages is also on the increase and there is no hope that social drinking would ever be out fashioned (Garfield *et al.,* 2003). In human excess alcohol consumption is associated with liver disease and cirrhosis. Alcohol liver disease is a major health problem worldwide (Befrit *et al.,* 1995).

The liver breaks down ethanol so as to eliminate it from the body but its chronic consumption renders the liver incapacitated, the resulting imbalance can therefore injure the liver and consequently interfering with its normal breakdown of protein, fats, and carbohydrates. Animal experimentation has demonstrated that either acute or chronic alcohol administration increases the rate of lipid peroxidation (Diehi *et al*., 1988) which is a classical biochemical feature in hepatotoxic poisoning (Lieber, 1993).

Plant drugs are known to play a vital role in the management of liver diseases particularly in traditional medicine (Dahanukar *et al.,* 2002). Unfortunately, a greater proportion of plants known traditionally to possess medicinal properties have not been subjected to scientific evaluation. This is necessary not only because of the need to discover new drugs for management of liver damages but also to assess the toxicity risks. In the

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West African traditional medicine, *Morinda lucida* has been valued for its antipyretic, antimalaria, and in the treatment of ulcers, leprosy and gonorrhoea. Its leaves and stem extracts has also been recommended for the treatment of hypertension and cerebral complications (Durodola, 1974). This study was therefore undertaken to evaluate the possibility of protecting the liver with aqueous extract of the leaves of *Morinda lucida* from damage due to chronic alcohol consumption.

MATERIALS AND METHODS

Collection and Identification of Plant:

Fresh leaves of *Morinda lucida* were collected in June 2008 from the Teaching and Research Farm, Obafemi Awolowo University Campus, Ile-Ife, Nigeria. The plant was identified and authenticated by Dr F.A Oloyede of Department of Botany, Obafemi Awolowo University, Ile-Ife.

Experimental Animals:

Thirty (30) healthy weaner rabbits, 5-7weeks old and average weight of 0.50kg were purchased from the Rabbitry Unit of the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife. The animals were acclimatized for 14days in the animal house where they were fed with water and growers marsh obtained from a livestock feed shop in Ile –Ife, Nigeria. The animals were randomly divided into 6 groups containing 5 rabbits each. Group1 (Control) received 0.9% normal saline solution only(1ml/100g body weight). Group II (Ethanol group) received 40%ethanol only (1ml/100g body weight).

Group III(Positive control) received ascorbic acid (400mg/kg body weight) and one hour later 40%ethanol (1ml/100g body weight). Group IV (Extract +Ethanol) were pre-treated with *Morinda lucida* extract (300mg/kg of body weight) and one hour later they were treated with 40%ethanol (1ml/100g body weight). Group V (Extract only) received *Morinda lucida* extract only (300mg/kg body weight). While Group VI (Ethanol +Extract) received 40% ethanol (1ml/100g per body weight), one hour later were post treated with Morinda *lucida* extract (300mg/kg of body weight). All treatment were done orally using polythene cannula, three times a week for 4 weeks. The animals were sacrificed at the end of 4 weeks treatment period. Blood samples were collected from each animal by cardiac puncture using disposable pyrogen free needle and syringe. The blood samples were carefully transferred into well labelled heparinized bottles containing lithium heparin. This was centrifuged at 3000 rpm for 10minutes in a Gallenkhamp Junior Table centrifuged at room temperature. The plasma was decanted into clean bottles and kept in a refrigerator for further analyses. The liver samples were also removed from each experimental animal for further biochemical analyses. One gram of each liver sample was homogenized separately in 10ml of 100mM phosphate buffer, pH 7.4 and centrifuged at 12,000 rpm for 30minutes.The supernatants were decanted into clean sterile bottles, labelled and kept in the deep freezer for further analyses. The remaining liver samples were kept in 10% formal saline for histopathology screening.

Extraction of Morinda Lucida Leaves:

Morinda lucida leaves were air dried under laboratory conditions for 21days, the dried leaves were milled and sieved to obtain uniform powder. One hundred grams of the powdery form of the leaves was suspended in 600ml of distilled water in a conical flask. The mixture was stirred vigorously at room temperature with a mechanical stirrer for 2 consecutive days, 12hours each day.

The preparation was then filtered using muslin cloth, the filtrate was collected into a clean, sterilized conical flask. The extract was then concentrated by evaporation using rotary evaporator, the filtrate was then freeze dried at Obafemi Awolowo University central laboratory (Durodola, 1974)

Biochemical Estimations:

Total protein concentration in the plasma and the liver homogenates were estimated using Biuret reaction method as described by Cannon *et al*., 1974. Plasma albumin concentration was determined using BCG as described by Sigma Chemical Company (Pinnel and Northam 1978). The activity of both plasma and liver homogenate alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed as described by Reitman and Frankel 1957. γ-glutamyl transferase (GGT) activity was determined by the method of Szasz, 1969 while Bilirubin determination was based on colorimetric method as described by Jendrassisk and Grof 1938.

Histological Examination of the Liver:

Samples of liver tissue of the various groups of animals were subjected to histopathological screening.

Statistical Analysis:

The results were presented as Mean ± SD using statistical software package (SPSS for Windows). Comparisons of the (mean ±S.D) were made between different groups of animals, using student't' test and $P<0.05$ is statistically significance.

Results:

Tables 1, 2, 3 showed the comparison of the means of all the parameters measured between the control and ethanol only treated group, ethanol only and extract pretreated group, ethanol only extract post treated group respectively. The results of histological studies are presented in figures 1 to 6.

Table 1: Comparisons of the concentrations (Mean ± S.D) of Total Bilirubin, Direct Bilirubin, Albumin, GGT, Total Protein, AST, ALT between the Control and Ethanol groups.

Parameters	CONTROL	ETHANOL	P
Total bilirubin (U/L) plasma	1.74 ± 0.16	14.59 ± 0.44	< 0.001
Direct bilirubin (U/L) plasma	2.95 ± 0.11	5.84 ± 0.00	<0.001
Albumin(mg/ml) plasma	4.90 ± 0.26	4.50 ± 0.32	NS
Albumin(mg/g) liver	2.89 ± 0.16	1.9 ± 0.10	<0.01
GGT(U/L) plasma	4.16 ± 0.17	67.164 ±1.86	<0.001
Total protein (mg/L) plasma	6.81 ± 0.17	3.125 ± 0.08	< 0.001
Total protein (mg/g) liver	6.22 ± 0.51	4.34 ± 0.09	<0.001
AST(U/L) in the plasma	12.5 ± 0.39	52 ± 1.05	<0.001
AST(U/g) in the liver	12 ± 0.66	78 ± 1.61	<0.01
ALT(U/L) in the plasma	15 ± 0.64	26 ± 0.62	<0.01
ALT(U/g) in the liver	20 ± 0.70	45 ± 1.36	<0.001

Table 2: Comparisons of the concentrations (Mean ± S.D) of Total Bilirubin, Direct Bilirubin, Albumin, GGT, Total Protein, AST, ALT between the Ethanol and Extract + Ethanol Groups.

Parameters	ETHANOL	EXTRACT+ETHANOL	P
Total bilirubin (U/L) plasma	14.59 ± 0.44	12.77 ± 0.18	<0.05
Direct bilirubin (U/L) plasma	5.84 ± 0.00	2.71 ± 0.16	<0.01
Albumin(mg/ml) plasma	4.50 ± 0.32	5.25 ± 0.32	<0.05
Albumin(mg/g) liver	1.9 ± 0.10	4.17 ± 0.08	<0.001
GGT(U/L) plasma	67.16 ± 1.86	8.11 ± 0.28	<0.001
Total protein (mg/ml) plasma	3.15 ± 0.08	8.18 ± 0.20	<0.001
Total protein (mg/g) liver	4.34 ± 0.09	21.25 ± 0.61	<0.001
AST(U/L) plasma	52 ± 1.05	12 ± 0.32	<0.001
AST(U/L) liver	78 ± 1.6	22.5 ± 0.68	<0.001
ALT(U/L) plasma	26 ± 0.62	18 ± 0.39	<0.01
ALT(U/L) liver	45 ± 1.36	35 ± 0.77	<0.01

Table 3: Comparisons of the concentrations (Mean ± S.D) of Total Bilirubin, Direct Bilirubin, Albumin, GGT, Total Protein, and AST, ALT between the Ethanol and Ethanol + Extract Groups.

Parameters	ETHANOL	ETHANOL+EXTRACT	P
Total bilirubin (U/L) plasma	14.59 ± 0.44	9.43 ± 2.67	<0.05
Direct bilirubin (U/L) plasma	5.84 ± 0.00	4.42 ± 0.20	<0.001
Albumin(mg/ml) plasma	4.50 ± 0.32	5.35 ± 0.05	<0.05
Albumin(mg/g) liver	1.9 ± 0.10	4.17 ± 0.08	<0.05
GGT(U/L) plasma	67.16 ± 1.86	17.38 ± 2.40	<0.001
Total protein (mg/ml) plasma	3.15 ± 0.08	6.40 ± 0.83	<0.01
Total protein (mg/g) liver	4.34 ± 0.09	21.25 ± 0.61	<0.05
AST(U/L) plasma	52 ± 1.05	21.29 ± 11.66	<0.001
AST (U/L) liver	78 ± 1.61	22.5 ± 0.68	<0.001
ALT(U/L) plasma	26 ± 0.62	21.32 ± 1.63	<0.05
ALT(U/L) liver	45 ± 1.36	35 ± 0.77	<0.001

Discussion:

Alcohol is mainly metabolized in the liver and its excessive consumption can lead to acute and chronic liver diseases including hepatitis, liver cirrhosis and fatty liver. Although diverse mechanisms are involved in the ethanol-induced hepatotoxicity, accumulating evidence has supported the importance of oxidative stress mediated by reactive oxygen species (ROS) or reactive nitrogen species (RNS) (Albano, 2006, Cederbaum, 2006).

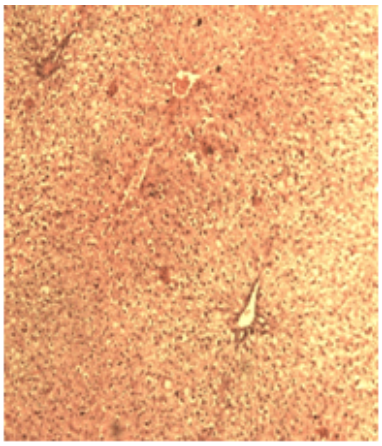

Fig. 1: Control group - Essentially normal.

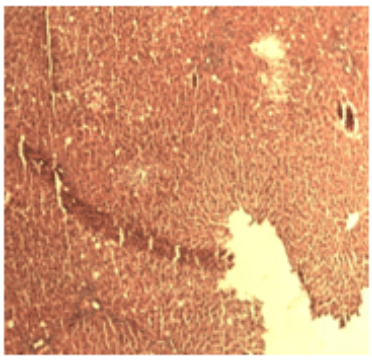

Fig. 2: Ethanol Only -.Severe perinuclear vacuolation of the hepatocytes indicative of some fatty change.

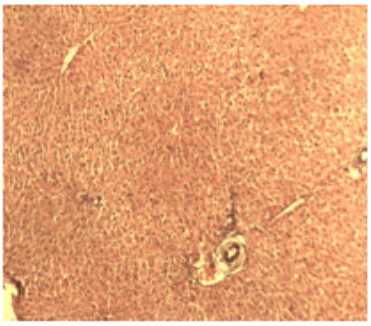

Fig. 3: Positive Control-Mild perinuclear vacuolation of the hepatocytes indicative of some fatty change.

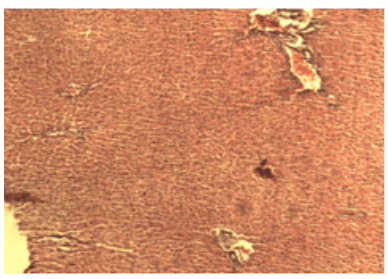

Fig. 4: Extract pre treated group-Mild perinuclear vacuolation of the hepatocytes indicative of some fatty change.

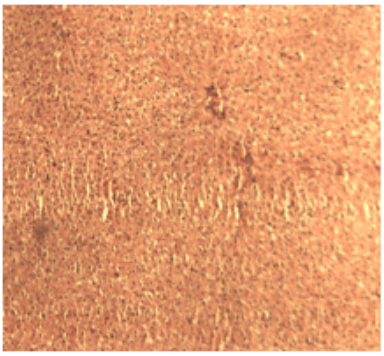

Fig. 5: Extract Only-Essentially Normal.

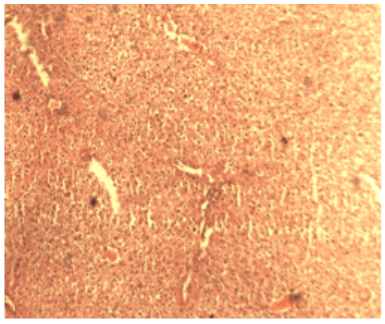

Fig. 6: Extract post treated- Moderate perinuclear vacuolation of the hepatocytes indicative of some early fatty change.

Damage to the liver after chronic ethanol ingestion is a well-known phenomenon, and the obvious sign of hepatic injury is the leakage of cellular enzymes into plasma [Cederbaum, 2006]. This study was designed to investigate the possible hepatoprotective effect of aqueous *Morinda lucida* extract on the alcohol induced toxicity in rabbits. Hepatic marker enzymes such as; Alanine aminotransferase (ALT), Aspartate aminotransferase (AST)), Gamma glutamyl transferase (GGT), and other biochemical parameter (Total protein, Albumin, Direct and Total bilirubin) activities were determined by standard techniques.

Bilirubin is produced from the breakdown of haemoglobin (the oxygen- carrying protein pigment) in red blood cells. The liver clears bilirubin from the body by excreting it through bile into the intestine. Elevated bilirubin levels may be indicative of liver disorders or blockage of bile ducts; this is in agreement with this study where the mean values of total and direct bilirubin significantly increased in ethanol only treated group compared with control group. In chronic acquired liver diseases, the serum bilirubin concentration is usually normal until a significant amount of liver damage has occurred and cirrhosis is present. In acute liver disease, the bilirubin is usually increased in relation to the severity of the acute process.

Excess alcohol consumption has also been linked with alteration of liver metabolism and liver damage, with leakage of cytoplasm liver enzyme gamma glutamyl transaminase (GGT) into blood. GGT is a liver enzyme found on the cell surface on all cells, with particularly high concentrations in the liver, bile ducts, and kidney.GGT may be raised in liver dysfunction, even where the condition is considered sub clinical. In particular, it is raised with alcohol toxicity (Yokoyama, 2007). GGT index has been reported high in alcoholic liver disease and measurement of GGT has been claimed to be an extremely sensitive test and marker of ethanol-induced hepatic damage (James, 1993. The result shows that mean value of GGT increased significantly in ethanol only treated rabbits, this is consistent with findings in literature (Ruppin, 1982), who reported that serum GGT concentration may be elevated in response to many drugs and in regular drinkers. After treatment with the extract, this enzyme decreased significantly as shown in Table 3 which indicates possible hepatoprotective effect of the extract.

Protein concentration in Table 1 shows significantly reduction in ethanol only treated group. Doyle *et al.*1994 supported the decrease in protein concentration; they attributed the decrease to inhibition of hepatic protein secretion by ethanol, the same lowering effect of ethanol ingestion was proved to be reflected on other liver parameters such as albumin.

In the current research, albumin concentration reduced significantly in ethanol only treated group, which is in support of the previous findings. The mean values of total protein and albumin were significantly decreased by ethanol intake when compared with control group as shown in Table 1, which is in agreement with Ahmed *et al*. 2002, who found significant decrease in serum protein and albumin in ethanol only treated rabbits, significant reduction in the concentration of these metabolites is an indication of severe liver injury because liver is the principal organ responsible for the synthesis of these proteins but albumin synthesis is also sensitive to amino acid supply and thus nutrition state plays important roles in albumin concentration (Harper, 1961)

The comparison of extract pre-treated and post-treated groups showed significant decrease in all the parameters except in albumin and total protein. The observed decreases in the enzymes activity discussed above shows that, the extract preserved the structural integrity of the liver from the toxic effect of ethanol. Decrease in plasma bilirubin of the ethanol administered rabbit after treatment with the extract indicates the effectiveness of *Morinda lucida* in the maintenance of normal functional status of the liver. Whereas, significant increase in total protein and albumin might indicates the ability of the extract to stimulate the regeneration of hepatic tissue which increase protein synthesis in damaged liver and improvement of the functional status of the liver cells in pre treated and extract post treated groups which is in agreement with Hussein and Farrington 2007.

Administration of aqueous extract of *Morinda lucida* showed significant hepatoprotective activity in all the parameters except direct bilirubin and plasma albumin which was not significant, liver total protein was not significant in ascorbic acid group which may be due to their increased utilization for scavenging oxygen derived radicals, this is in line with the previous findings on ascorbic acid which has been reported for its hepatic stimulant activity (Hussein and Farrington 2007).

In this study, the extract pre treated group has a significant increase in all parameters except in plasma total protein and plasma AST. The total bilirubin, direct bilirubin, GGT, total protein and ALT concentration in the rabbit liver was significantly increased.

Pre-treatment with the extract of *Morinda lucida* decreased levels of plasma enzyme markers, thus suggesting that the extract possessed compounds that protected the hepatocytes from alcohol induced liver injury and subsequent leakage of enzymes in to the circulation. Decreased level of the enzyme markers in the extract post treated group compared to ethanol only treated group was an indication that the extract also possessed a curative effect.

In conclusion, the results of this study shows that, the aqueous extract of *Morinda lucida* extract may offer hepatoprotective effect on alcohol-induced toxicity in liver as observed in the histopathology pictures. Further studies are recommended to determine the mechanism of the hepatoprotective action of the plant and the phytochemical(s) responsible.

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