**Mangifera indica (Mango) leaf extract as a recipe for chemical induced acute liver injury**.

**ABSTRACT**

**Background**: Acute liver injury is a precursor of many chronic liver diseases which are of major global health burden. Chemicals including drugs, alcohol and psychoactive substances constitute major causes of acute liver injury.

**Methods**: Varying degrees (mild, moderate and severe) of acute liver injury were induced in equal number of rats. This was followed by daily oral administration of aqueous extract of M. indica leaf at 200 mg/kg for 14 days. Each group had its own control that did not receive the extract.

**Results:** All the extract groups had increase in body weight while the liver injured but non-extract groups lost weight. The relative liver weights of all the extract groups were akin to that of the control. The total protein levels of the moderately and severely injured groups that had the extract were markedly higher than those of their respective control (ie extract not administered). The liver enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) levels of the extract groups were significantly lower than those of respective control. Oxidative stress was less pronounced in the extract groups. Microscopy of the sections prepared from the harvested liver revealed distortion of the liver architecture in all the non-extract but liver injured groups and this was proportionate to the degree of injury. While the liver architecture was preserved in all the extract groups.

Considerable number of non-hepatocyte cells were seen in the non-extract but liver injured groups along with fatty infiltration of the cytoplasm ie hepatocyte steatosis. Karyorhexis was observed in some hepatocytes as evidenced by the fragmentation of the nuclei.

**Conclusion**: Oral administration of the aqueous extract of *Mangifera. Indica* (Mango) leaf is beneficent following chemical induced acute liver injury of varying magnitude in rat.

*Key words*: Acute liver injury, Mangifera indica extract, Carbon tetrachloride.

**1.INTRODUCTION**

According to the Global Cancer Observatory of the International Agency for Research on Cancer; the number of new cancer cases (incidence) world-wide in 2022 was 19,964,811 out of which 9,736,779 died from cancer related causes [1]. Cancers of the liver accounted for 865,269 (4.3%) with 757,948 (7.8%) mortalities. In terms of ranking, cancer of the liver was the 6th as of 2022 and the 3rd commonest cause of cancer related deaths globally. In Sub-Saharan Africa with a population estimates of 1,151,351,784 (17.6% of World Population) as at year 2022; 847,974 of new cancer cases were reported out of which 558,878 died from cancers. In this cohort, the liver accounted for 6.9 % and the 5th in ranking of the new cases and 7.1 % of cancer deaths occupying the 4th position [2]. From the above global and continental statistics, it is obvious that cancers of the liver are of major health concern. Diseases of the liver could be inflammatory (hepatitis), parasitic (amoebic liver abscess) and neoplastic (primary and metastatic). Besides viruses; chemicals such alcohol, drugs and certain food items are major causes of inflammatory liver diseases. Hepatitis is initially acute but may progress to chronicity. This progression may ultimately end up in terminal liver diseases such as cirrhosis and primary liver cancer.

Drugs constitute the largest source of chemicals being consumed by humans worldwide be for prophylaxis or therapeutics. Drug induced liver injury is the commonest cause of acute liver failure in the United States of America and Europe [3-6]. It thus becomes imperative to look for antidotes for chemical initiated acute liver failure.

Carbon tetrachloride has been used in several studies to induce acute liver injury in animal models [7-9]. *Mangifera indica* (mango leaf) is known to contain some phytochemicals of medicinal value.

This study set out to determine the beneficence or maleficence of the aqueous extract of *M.indica* leaf in experimental animals with varying degree of induced acute liver injury.

**2.MATERIALS and METHODS**

**2.1. Plant Materials**

**2.1.1. Plant collection and authentication**

Fresh *Mangifera indica* (mango leaves) were plucked from the mango trees located within the campus of the University of Ibadan, Nigeria. The botanical identification and specie confirmation were achieved with the assistance of the Herbarium Unit of the Department of Botany, University of Ibadan, Nigeria. Samples were deposited for repository and future reference.

**2.1.2. Extract preparation**

After initial washing under potable water with continuous flow, the leaves of *M. indica* were air dried at room temperature and subsequently grounded to fine textured powder. This was then used for the aqueous extract and a yield of 15% was obtained.

**2.2 Animals**

The source of the animals used for this study was the Central Animal House of the College of Medicine situated on the University of Ibadan campus. Their average weight was 240 g and a range of 190-260 g. The period of acclimatization was three weeks in a well ventilated and illuminated environment with optimal ambient temperature (27 ±2o C,12 hours light/day cycle). The animals had liberal feeding with locally sourced but nutritionally balanced pelletized rat feed with unhindered access to water.

**2.2.1Design of the Experiment**

The main thrust of the study was aqueous extract of *M. indica* leaf and acute liver injury of varying magnitude. Thus four main groups with three of them having a subgroup each were created with random allotment of the animals.

The details of the groups are as below-;

1 Control (CN)- Extract only

2 A- Mild Acute Liver Injury (ALIM)

B- Mild Acute Liver Injury with Extract (ALIME)

3 A- Moderate Acute Liver Injury (ALID)

B- Moderate Acute Liver Injury with Extract (ALIDE)

4 A- Severe Acute Liver Injury (ALIS)

B- Severe Acute Liver Injury with Extract (ALISE)

**2.2.2 Induction of Lead Toxicity**

Based on documented protocol [7,8], liver injury was induced by single oral administration of carbon tetrachloride (CCl4) in olive oil (as a vehicle) in equal (50:50) proportion via a 16 G orogastric tube. For induction of mild acute liver injury, 1ml(1.6g)/kg body weight of CCl4 was administered. Induction of moderate and severe acute liver injury was achieved at the respective dosage of 2.5 ml(4g)/kg and 5ml(8g)/kg of CCl4.

**2.3 Conduct of the Experiments**

Animals in the Control (CN), Mild acute liver injury extract (ALIME), Moderate acute liver injury extract (ALIDE) and Severe acute liver injury extract (ALISE) groups had once daily oral dose of the M. *indica* leaf extract at 200 mg / kg body weight for 14 consecutive days. All the non-extract groups ie ALIM, ALID and ALIS had normal rat feed and water for same duration.

On day 15, blood samples were collected from the animals for biochemical analyses (liver function test consisting of total protein, alanine aminotransferase(ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). The activities of Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Catalase; and the degree of lipid peroxidation as measured by Malondialdehyde (MDA) levels were used to assess the extent of oxidative stress occasioned by the chemical induced liver injury.

Thereafter, the animals were euthanized and the livers were harvested for histopathological evaluation.

Also, the animals were weighed at both the commencement and conclusion of the experiments.

**2.4** **Ethical Conduct**

The animals used in this study, were handled in accordance to the guidelines as prescribed by the ethical conduct of animal research of the University of Ibadan. Also, the principles of laboratory animal care as contained in the 8th edition (2011) of the Guide for the Care and Use of Laboratory Animals by the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals were observed [10].

**2.5Data Analysis and Processing**

The numerical aspects of the results were analyzed with Statistical Package for the Social Sciences (SPSS) version 24 and expressed as percentages, means plus standard deviation of means (SD). The student t- test was used for intra and inter group comparison and level of significance was set at p<0.05.

**3.RESULTS**

All the non-extract groups with induced liver injury had weight loss while the extract groups and control had marginal weight gain. The mean weight of the liver was similar across the groups. While the relative weight of the liver of two of the extract groups ie the mild and moderate liver injury groups were significantly lower than their counter parts (ALIM vs ALIME; ALID vs ALIDE).

The serum protein levels of all the liver injured groups with the exception of the ALIM were significantly lower than that of the control. However, the protein levels of the ALIDE and ALISE groups were significantly higher than that of the respective control.

The alanine aminotransferase (ALT) levels of all the experimental groups were markedly elevated with reference to that of the control group. Intra group comparison of the ALT level revealed significant difference between the values for the ALIM and ALIME groups. For the aspartate aminotransferase (AST), the values were similar across the groups. The alkaline phosphatase (ALP) levels of all the experimental groups were significantly elevated with reference to the control group. However, only the ALP level of group ALISE was significantly lower than its corresponding non-extract group ie ALIS as regards intragroup comparison.

The activities of GPx in all the groups were markedly depressed when compared with that of the control. Intragroup comparison of the GPx activity revealed a significant elevation in the moderate and severe liver injury groups i.e. ALID vs ALIDE; ALIS vs ALISE. The sodium dismutase (SOD) activity was similar across the groups. No appreciable difference was observed in the degree of lipid peroxidation as deduced from the values of malondialdehyde (MDH) across the groups. (Table 1)

Microscopy of the sections prepared from the harvested liver revealed distortion of the liver architecture in all the liver injured groups that did not receive the extract of *M.indica* leaf. This distortion appeared most pronounced in the group in which severe liver injury was inflicted with 8mg (5ml) /kg of CCl4 i.e ALIS. While all the liver injured groups that received the plant extract showed normal liver architecture.

Considerable number of non-hepatocyte cells were seen in the non-extract but liver injured groups. Another feature of these groups was hepatocytes with fatty infiltration of the cytoplasm ie hepatocyte steatosis. Karyorhexis was seen in some hepatocytes as evidenced by the fragmentation of the nuclei. (Plate 1).

**Table 1. Morphological, Biochemical and Antioxidants Parameters**

(mean values)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | CN | ALIM | ALIME | ALID | ALIDE | ALIS | ALISE |
| Body weight gain (%) | 7.96 | -3.16 (loss) | 11.08 | -0.50 (loss) | 7.12 | -0.62(loss) | 4.49 |
| Liver weight (g) | 8.58±1.44 | 7.74±1.93 | 7.28±1.77 | 8.02±1.19 | 6.84±1.01 | 7.58±1.06 | 8.56±1.73 |
| Relative liver weight (g) (/100g body weight) | 3.63±0.45 | 4.17±0.51 | 3.13±0.26β | 4.06±0.57 | 2.98±0.70β | 3.43±0.55 | 3.45±0.48 |
| Total protein(g/dl) | 5.80±0.24 | 5.57±0.71 | 1.12±0.03δ | 1.31±0.12 δ | 2.69±0.35 δ | 1.65±0.10 δ | 2.04 ±0.41δ |
| ALT(U/L) | 54.14  ±  48.87 | 263.77  ±  19.99α | 182.74  ±  12.25α β | 234.68  ±  92.39α | 216.03  ±  12.33α | 221.88  ±  30.04α | 230.36  ±  32.81α |
| AST(U/L) | 0.77±0.10 | 0.75±0.09 | 0.75±0.05 | 1.17±0.07 | 0.60±0.13 | 0.81±0.06 | 0.46±0.11 |
| ALP(U/L) | 371.48  ±  98.08 | 1051.49  ±  26.88α | 1051.49  ±  44.69α | 1256.83  ±  34.62α | 1096.92  ±  51.99α | 1418.75  ±  66.43α | 756.67  ±  34.47α β |
| GPx (mmol/L) | 19.07  ±  1.51 | 11.54  ±  1.60 δ | 11.54  ±  1.92 δ | 7.87  ±  0.83 δ | 13.47  ±  0.81δβ | 9.26  ±  0.56 δ | 14.35  ±  0.53δβ |
| SOD(µmg/protein) | 0.96±0.381 | 0.81±0.17 | 1.32±0.33 | 0.81±0.47 | 1.12±0.11 | 0.87±0.09 | 1.06±0.17 |
| MDA(nmol/L) | 0.27±0.07 | 0.13±0.02 | 0.07±0.07 | 0.23±0.10 | 0.05±0.03 | 0.71±0.15 | 0.51±0.06 |

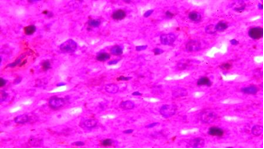
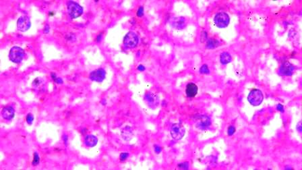
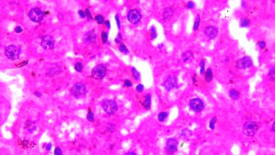
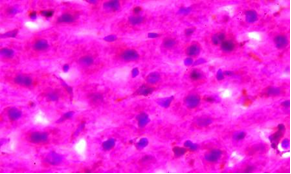
β**-**Significantly different from the corresponding control (ie ALIME vs ALIM; ALIDE vs ALID)

δ- Significantly lower than the Control group

α- Significantly higher than the Control group

ALT-alanine aminotransferase(ALT); AST-aspartate aminotransferase.

ALP-Alkaline phosphatase; GPx- Glutathione peroxidase; SOD-Superoxide dismutase; MDA- Malondialdehyde.

**H**

**S**

**H**

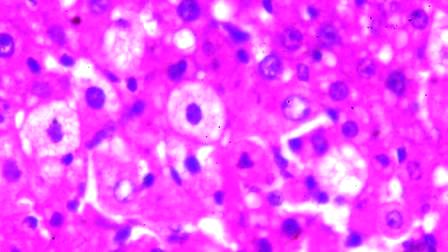
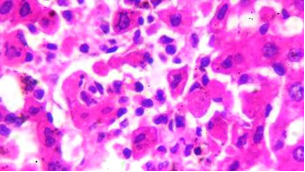
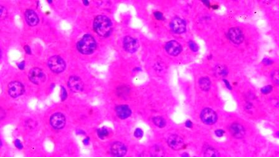
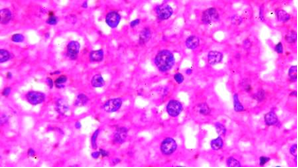
**ALIME**

**SHC**

**ALIM**

**CN**

**CN**

**KHC**

**KHC**

**KHCC**

**NHC**

**SHC**

**ALID**

**ALIDE**

**ALIS**

**ALISE**

**Plate 1. Photomicrographs of the Liver specimens from the groups (H & E x 400)**

Legend; H-Hepatocyte; KHC- Hepatocyte with karyorhexis; NHC- Non hepatocyte cell; S- Sinusoid; SHC- Hepatocyte with steatosis.

Groups; **CN**- Control, **ALIM**-Mild acute liver injury, **ALIME**-Mild acute liver injury with extract, **ALID**-Moderate acute liver injury, **ALIDE**-Moderate acute liver injury with extract, **ALIS**-Severe acute liver injury & **ALISE**-Severe acute liver injury with extract.

**4.DISCUSSION**

The liver being one of the major organs is largely responsible for the metabolism of macronutrients into micronutrients these are then utilized for various purposes such tissue repairs, syntheses and growth. Thus in an acutely injured liver, affectation of nutrient metabolism may manifest as weight loss. This is the explanation for the recorded weight loss that occurred in all the groups that had acutely injured liver but were not administered the plant extract. The positive weight balance recorded in the counterpart groups ie those that received the plant extract is thus highly suggestive of the *M. indica* leaf extract being able to reverse the negative effect of the injury occasioned by the CCl4.

The relative organ weight is a function of the body weight thus in a situation where the body weight is low, this parameter will be high. This serves as a plausible explanation for the relative liver weight of the non-extract mild and moderate liver injured groups being considerably higher than their corresponding extract groups. However, it was observed that the relative liver weight of the non-extract severe liver injured group was similar to its extract counterpart. It can thus be inferred that a linear relationship exists between the dose of CCl4 and the severity of the organ injury.

In a pathological liver, protein synthesis is very likely to be depressed. The total protein levels of all the experimental groups with the exception of the non-extract mild liver injured group were significantly lower than that of the control. However, the total protein levels of the moderate and severe liver injured groups that had the plant extract were higher than their non-extract corresponding groups. This is another support for the beneficial role of *M. indica* leaf extract in CCl4 induced liver injury.

The aminotransferases (ALT and AST) are normally contained within the cytoplasm of the hepatocyte. Thus their serum levels are highly indicative of the extent of hepatocyte necrosis. In this study, the serum levels of alanine aminotransferase (ALT) of all the groups were markedly elevated. Also the ALT levels of the extract mild and moderate liver injured groups (ALIME & ALIDE) were lower than their respective non-extract group (ALIM & ALID) while this was the opposite for the severe liver injury groups (ALIS & ALISE). Arising from this trend in the serum level of ALT, it could be deduced that the extract of *M. indica* leaf is beneficent in mild to moderate liver injury but not in severe liver injury. Levels of alkaline phosphatase in patients with primary biliary cirrhosis have been documented to accurately predict progression to full blown liver cirrhosis in such patients as those with persistently elevated levels will invariably developed cirrhosis which is an end stage liver disease [11]. In this study the ALP levels in all the liver injured groups were markedly elevated, however, those that received the plant extract had significantly lower ALP levels when compared with their non-extract counterpart. It can thus be concluded that *M. indica* leaf extract is beneficent in chemical induced liver injury.

One of the established pathway of cellular injury in the liver by CCl4 is by production of reactive oxygen species that ultimately results in oxidative stress. The results of glutathione peroxidase in this study showed the occurrence of oxidation stress. However, the administration of *M.indica* leaf extract brought about its reversal. This further justified the earlier made assertion that the plant extract is beneficent in acute liver injury.

In the histopathological assessment of the liver, it was observed that the parenchymal distortion seen in all the injured groups that did not receive the extract was not observed in their corresponding extract groups. Arising from this structural observation, it is clear that CCl4 is hepatotoxic and the aqueous extract of *M. indica* leaf is capable of restoring the structural distortion. The liver has a very high regenerating ability. Provided the exposure to the injurious agent is not continuous or overwhelming, structural recovery may be complete or with some degree of fibrosis. In this study, there was no evidence of fibroplasia in all the three liver injured groups that had the plant extract. Thus the extract of *M. indica leaf* is beneficent in acute liver injury. Fatty infiltration of the cytoplasm is one of the features of acute liver injury. This was prominent in the liver injured groups but very sparse in their extract counterparts. This observation reinforces the earlier assertion that *M. indica* leaf extract is beneficent in acute live injury. Karyorhexis which is fragmentation of nuclear content and suggestive of cellular necrosis was another feature seen in the moderate and severe liver injured groups thus the earlier inference that CCl4 is toxic to the liver is further strengthened.

Administration of CCl4 to rodents results in liver fibrosis and the resolution of the fibrosis is promoted by macrophages[12]. The primary interstitial collagenase in rodents is matrix metalloproteinase13. This has been observed to the deficient in rodents with liver fibrosis [13]. However, macrophages associated with the tissue (liver) scar are said to be responsible for the spontaneous resolution of the fibrosis [13]. Thus the mechanism through which *M. indica* promotes tissue repair in CCl4 induced liver fibrosis could be stimulation of the production of scar associated macrophages.

Although the liver has a highly remarkable regenerative capability via the matured hepatocytes and cholangiocytes, these cells are usually mitotically dormant but resume division once there is insult to the liver [14]. However, if the extent of the liver insult is severe, the regenerative response of the hepatocytes and cholangiocytes may become inadequate and in this circumstance, the hepatic stem cells will become activated [15,16]. However, certain mesenchymal cells are capable of undergoing transformation to epithelial cells and eventually differentiate into either hepatocytes or cholangiocytes [17]. From the foregoing, it is thus obvious that multiple cell types modulate the outcome of liver injury. The severity of the liver injury following the initial insult is exacerbated by additional mechanisms such as inhibition of transporters, mitochondrial injury, oxidative stress and proinflammatory cytokines [18].

In one of our interventional animal based study, extract of *M. indica* plant was used as wound dressing material in rats with induced diabetes mellitus, the extract accelerated wound healing [19]. Also in another diabetic wound healing study, the fruit and rind of unripe M. indica were used to manage the cutaneous wounds and the wound healing process was excellent [20]. From these two referenced studies and in combination with the results of this study, it can thus be categorically stated that the *M. indica* plant be it the bark, fruit, rind or leaf potentiates healing be it the skin or the liver.

**Conclusion**-Oral administration of the aqueous extract of *M. indica* leaf is beneficent following acute liver injury of varying magnitude. This is supported by the restoration of the body weight, arrest of necrosis of hepatocytes as evidenced by restoration of the levels of the liver enzymes; reversal of the oxidative stress and preservation of the liver architecture.

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