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RESEARCH ARTICLE

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Histological and Biochemical Effects of *Azadirachta indica* and Melatonin in Streptozotocin-induced Diabetic Wistar rats

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ABSTRACT

This study was aimed at evaluating the histological and biochemical effects of ethanol leave extract of Azadirachta indica and melatonin in streptozotocin-induced diabetic Wistar rats. To achieve this, forty five Wistar rats were used in the study. The animals were assigned into the following groups: Group 1: Normal control + normal saline orally; Groups 2: Normal + A. Indica (200mg/kg b w) orally; Group 3: Normal + melatonin (10 mg/kg b w) IP; Group 4: Normal + melatonin (10 mg/kg b w) IP + A. Indica (200 mg/kg b w) orally; Group 5: Diabetic control + normal saline orally; Group 6: Diabetic + A. Indica (200 mg/kg b w) orally; Group 7: Diabetic + melatonin (10 mg/kg b w) IP; Group 8: Diabetic + A. Indica (200 mg/kg b w) orally + melatonin (10 mg/kg b w) IP; Group 9: Diabetic + metformin (500 mg/kg b w) orally. The extract and drug were administered once daily for a period of three (3) weeks respectively. After the last day of treatment, the animals were sacrificed and the liver tissues were collected and processed histologically and specially stained with PAS for glycogen, Gordon and Sweet for reticular fibers and HVG for collagen fibers. The serum liver enzyme activities were also determined. The results showed regeneration of liver collagen and reticular fibers and improved hepatic glycogen stores in all extract and melatonin treated diabetic groups and as well as the extract and melatonin when combined as compared to diabetic control group. On the other hand there was a significantly ($p<0.05$) reduced levels of liver enzyme activities in the extract and melatonin treated diabetic groups as well as in the extract and melatonin when combined as compared to diabetic control group.

Key words: Liver, melatonin, Liver enzymes, *Azadirachta Indica*, Streptozotocin, Wistar Rats

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INTRODUCTION

Diabetes mellitus (DM) is a medical condition with devastating complications. It has global distribution and all ages are affected. The International Diabetes Federation stated that “every ten seconds, two people are diagnosed with diabetes somewhere in this world,” and given the current trend, it is estimated that more people will have diabetes by 2025 than the current populations of the United States, Canada and Australia combined [1]. Various hypoglycemic drugs, such as sulfonylurea, metformin are being used for the treatment of diabetes but their use is restricted by their limited action and accompanying side effects such as hypoglycaemic shock and weight gain. Plants and plant products continue to play a dominant role in traditional remedies against ailments from antiquity [2]. The search for natural anti oxidative agents that will ameliorate the harmful effects associated with

hyperglycaemia still continues in spite of considerable progress in the management of diabetes mellitus by synthetic drugs. Over 400 traditional plants have been used in the treatments of diabetes [3]. Opinion about the mechanism of action of plants include: presence of insulin-like substances, interference with carbohydrate absorption, inhibition of insulinase activity and increase in beta cells in the pancreas [4]. *Azadirachta indica* of the family- *Meliaceae melioideae,* is a medium-sized tree that is found throughout the South Asian region, Africa and in Northern Nigeria. It is one of the most versatile medicinal plants having a wide spectrum of biological activities. Previous studies have reported the beneficial effect of *Azadirachta indica* leaves in the management of diabetes mellitus and the amelioration of the oxidative stress associated with the disease [5]. This was explained by the presence of terpenoids and saponins which have been found to be potentially useful for the treatment of hyperglycaemia. Flavonoid, a known antioxidant is present in *Azadiracta indica* leaves and its extract is currently included in the poly-herbal anti diabetic drugs being subjected to controlled clinical trials in Man. Melatonin has been shown to be a major scavenger of both oxygen and nitrogen based radicals, including peroxynitrite anion ($ONOO^-$) [6]-[8]. Melatonin may influence diabetes and associated metabolic disturbances not only by regulating insulin secretion, but also by providing protection against reactive oxygen species, since pancreatic β-cells are very susceptible to oxidative stress because they possess only low-antioxidative capacity [9]. In developing countries adequate treatment measures for diabetes mellitus are often unavailable or too expensive hence the need to test for the viability of *Azadirachta indica* ethanolic leaves extract and melatonin a known potent antioxidant as alternatives to conventional antidiabetic drugs. However, the effects of melatonin and ethanolic extract of *Azadiracta indica* leaves on liver of diabetic subjects are yet to be reported. The present study aims at evaluating the histological and biochemical effects of ethanol leave extract of *Azadirachta indica* and melatonin in streptozotocin-induced diabetic Wistar rats.

MATERIALS AND METHODS

Chemicals and drugs used

Melatonin M5250-1G and Streptozocin SP0130 were purchased from Sigma chemicals (St. Louis USA) and One Touch Ultra 2 Glucometer, (Lifescan, CA, USA) was obtained from a reputable pharmaceutical company in Kaduna State, Nigeria.

Plant material collection and extraction

Leaves of fresh *Azadirachta indica* were harvested from Ahmadu Bello University Faculty of Medicine Zaria in the month of April 2012 and authenticated at the Department of Biological Sciences, Ahmadu Bello University Zaria, where a voucher specimen number 900151 was deposited. The Fresh leaves of *A. indica* were air dried, minced and powdered using laboratory mortar. 1000g of the powdered leaves was extracted in 1.5 liters of 80% ethanol using a Soxhlet extractor. This was filtered using a Whatman filter paper (24mm). The filtrate was dried in a laboratory water bath set at 67°C and total yield of 46.8g was obtained per 1000g of the powdered leaves and kept in an air tight sample bottle, labeled and stored in a desiccator until it was reconstituted in appropriate volume of distilled water for administration.

Animals

Forty five (45) adult Wistar rats, weighing approximately 140g each were obtained from the Faculty of Pharmaceutical Sciences of Ahmadu Bello University, Zaria. They were kept in plastic cages and allowed to acclimatize for 2 weeks in the Faculty of Pharmaceutical Sciences Animal house before the experiment, and maintained under laboratory conditions of temperature, humidity and light. They were allowed free access to water and standard pellet diet obtained from Grand Cereals Ltd, Jos Plateau State.

Induction of experimental diabetes mellitus

Experimental diabetes mellitus was induced by single intra-peritoneal injection of 55 mg/kg b w dose of streptozotocin dissolved in 0.1 ml fresh cold citrate buffer pH 4.5 which served as the vehicle into 18 hrs fasted rats. Blood samples were collected after 72 hours after Streptozotocin injection and blood glucose level was determined using a One Touch Ultra 2 Glucometer, (Lifescan, CA, USA). Streptozotocin treated wistar rats with fasting blood glucose level at 200mg/dl and above were considered diabetic and included in the study [10].

Experimental protocol

Twenty five (25) diabetic animals and twenty (20) normoglycaemic were randomly selected and divided into nine groups of five (5) animals each as follows:

Group 1: Normal control + normal saline orally

Groups 2: Normal + *A.Indica* (200mg/kg bw) orally
Group 3: Normal + Melatonin (10 mg/kg bw) IP
Group 4: Normal + Melatonin (10 mg/kg b w) IP + *A. Indica* (200 mg/kg b w) orally
Group 5: Diabetic control + normal saline orally
Group 6: Diabetic + *A. Indica* 200 mg/kg b w orally
Group 7: Diabetic + Melatonin (10 mg/kg b w) IP
Group 8: Diabetic + *A. Indica* 200 mg/kg b w orally + Melatonin (10 mg/kg b w) IP
Group 9: Diabetic + Metformin (500 mg/kg b w) orally

The extract and drug were administered once daily for a period of three (3) weeks respectively.

Collection of blood and preparation of sera samples

After the last treatment was given to animals, they were euthanized by exposing them to over dose of chloroform soaked in cotton wool placed in anesthetic box covered with lid. Blood was collected from each animal from all groups through cardiac puncture after they have been fasted for 16 hours into plain sterilized centrifuged bottles and allowed to clot. The serum was separated by centrifugation at 1,968 × g for 15 minutes and collected in sample bottles for determination of serum liver enzymes activities.

Determination of serum liver enzymes activities

The serum enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) were determined spectrophotometrically, using enzymatic colometric assay kits according to the laboratory procedures of Randox Laboratories Limited kits, United kingdom.

Histological preparation of liver tissue

The liver tissues were excised after the animal had been euthanized and fixed in buffered 10% neutral formal saline and embedded in paraffin wax. They were sectioned with Leica rotary Microtome to produce serial sections of 5µ thickness. The liver sections were stained for reticular fibers using Gordon and Sweet while Heamatoxylin Van Giesien Stain was used to demonstrate collagen fibers to study the micro-cytoarchitecture of the liver sections. Periodic acid Schiff (PAS) with and without Diastase digestion was used to stain glycogen in the liver. All the tissues were stained for general histological studies with Haematoxylin and Eosin. Photomicrographs were obtained using a microscope eye piece attached to a computer monitor and observation made.

Table 1: Effects of ethanol leave extract of *Azadirachta indica* and melatonin on serum liver enzyme activities of normoglycaemic and streptozotocin-induced diabetic wistar rats

GROUPS (n=5)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
NC	17.60±1.288^{a}	30.80±1.463^{a}	59.20 ± 1.463^{a}
N+AI	17.68±1.414^{a}	39.40±2.502^{b}	69.00 ± 1.703^{b}
N+ML	18.40±1.166^{a}	42.00±1.304^{b}	68.60 ± 2.909^{b}
NAI+ML	18.20±1.625^{a}	41.00±2.098^{b}	71.20 ± 2.990^{b}
DC	27.20± 0.490^{e}	48.20±0.490^{c}	79.40 ± 2.441^{c}
D+AI	23.20±1.020^{c}	42.80±1.744^{b}	68.80 ± 2.267^{b}
D+ML	21.20±1.200^{a}	39.20±2.059^{b}	67.60 ± 1.400^{b}
DAI+ML	23.80±1.356^{c}	37.80±1.241^{b}	68.20 ± 3.362^{b}
D+MF	25.00±0.837^{c}	42.20±0.970^{b}	69.20 ± 0.583^{b}

Values are expressed as mean ±SD

Values with different superscripts in a column are significantly different ($p < 0.05$)

NC = Normal control + normal saline orally; N+AI= Normal + Azadirachta indica (200 mg/kg b w); N+ML = Normal + Melatonin (10 mg/kg b w); NAI+ML= Normal + Azadirachta indica (200 mg/kg bw) + Melatonin (10 mg/kg b w); DC = Diabetic control+normal saline; D+AI=Diabetic+Azadirachta indica (200 mg/kg b w); D+ML=Diabetic +Melatonin (10 mg/kg b w); DAI+ML=Diabetic+Azadirachta indica (200 mg/kg b w)+Melatonin (10 mg/kg b w); D+MF=Diabetic + Metformin (500 mg/kg b w)

RESULTS

Results in table 1 showed that the diabetic control group (DC) that received no treatment expressed significantly ($p<0.05$) elevated levels of AST, ALT and ALP when compared to normal control group. Treatment of diabetic animals with *Azadirachta indica* (200 mg/kg b w) leaves extract and melatonin (10 mg/kg b w) separately (D+AI and D+ML) and when they are combined (DAI+ML) significantly ($p<0.05$) decreased AST, ALT and ALP levels when compared with diabetic control group. The Effects of *Azadirachta indica* leaves extract (200 mg/kg b w) and

melatonin (10 mg/kg b w) were similar to that obtained with diabetic metformin (500 mg/kg b w) treated animals when compared.

Microscopic findings of effects of ethanol leave extract of *Azadirachta indica* and melatonin on the histology of liver tissue of normoglycaemic and streptozotocin-induced diabetic wistar rats.

Results of histological observations of the liver section of normal control (NC) group (Plate I) showed normal cyto-architecture of liver tissue of hepatocytes and sinusoids. Histological observation revealed no observable pathological change in the liver section. The liver sections of streptozotocin-induced diabetic group control (DC) (Plate II) showed necrotic changes including nucleus and cytoplamismic vacoulation, hepatocytes and sinusoids fragmentation and fatty change. However, *A. indica* (200 mg/kg b w) treated group (D+AI), melatonin (10 mg/kg b w) treated group (D+ML), combined treatment *A. indica* (200 mg/kg b w) + (10 mg/kg b w) (DAI+ML) and metformin (500 mg/kg b w) (D+MF) treated group (Plate III-VI) revealed a significant restoration of the liver cyto-architecture. Non significant effect was observed in the metformin (500mg/kg b w) treated group (Plate VI). Plate VII-VIII are liver sections of *A. indica* (200 mg/kg b w) (NA+I) and melatonin (10 mg/kg b w) (N+ML) treated normoglycaemic rats, showing normal liver hepatocytes and sinusoids indicating that the extract and melatonin on their own are not causing any cellular damage. Liver section stained with Periodic acid Schiff (PAS) showed glycogen depletion in the diabetic control group (DC) (Plate X) and glycogen restoration in *A. indica* (200 mg/kg b w) (D+AI) (Plate XI) and melatonin (10 mg/kg b w) (D+ML) (Plate XIV) treated groups. The metformin (500 mg/kg b w) (D+MF) treated group showed the least amount of glycogen restoration (Plate XIII). The liver sections stained with Heamatoxylin Van Gieson (HVG) stain demonstrated that in the diabetic control group (DC) that received no treatment showed fragmentation of reticular and collagen fibers, causing congestion of the central vein (Plate XVI). Liver sections of *A. indica* (200 mg/kg b w) (D+AI) and melatonin (10 mg/kg b w) (D+ML) treated diabetic groups showed total and partial restoration of these fibres (Plate XVII-XVIII). Diabetic metformin (500 mg/kg b w) (D+MF) treated group showed the least connective tissue restoration (Plate XX).

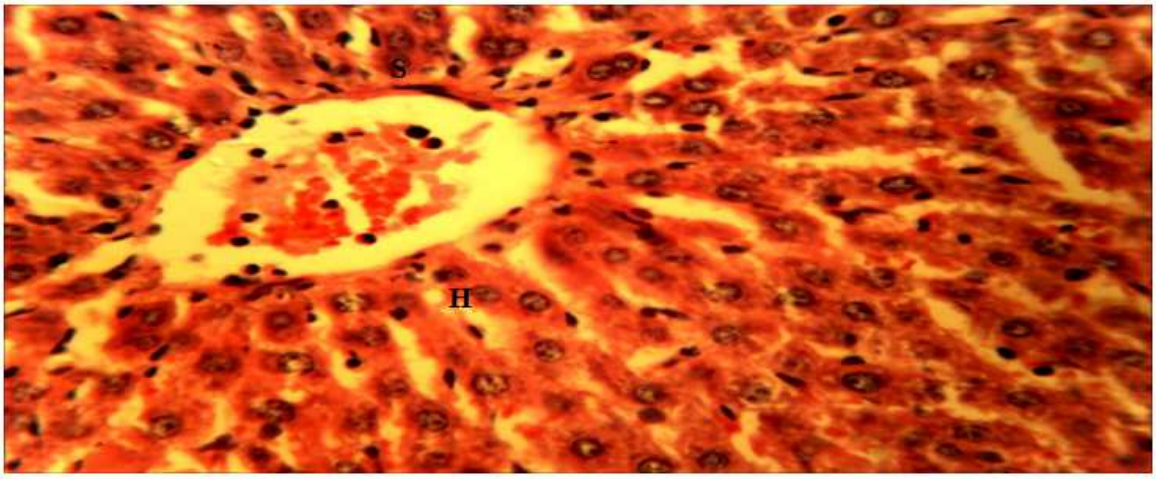


Plate I: Photomicrograph of liver section in normal control group treated with normal saline (NC) showing normal hepatocytes (H) and sinosuids (S) (H&E ×250).

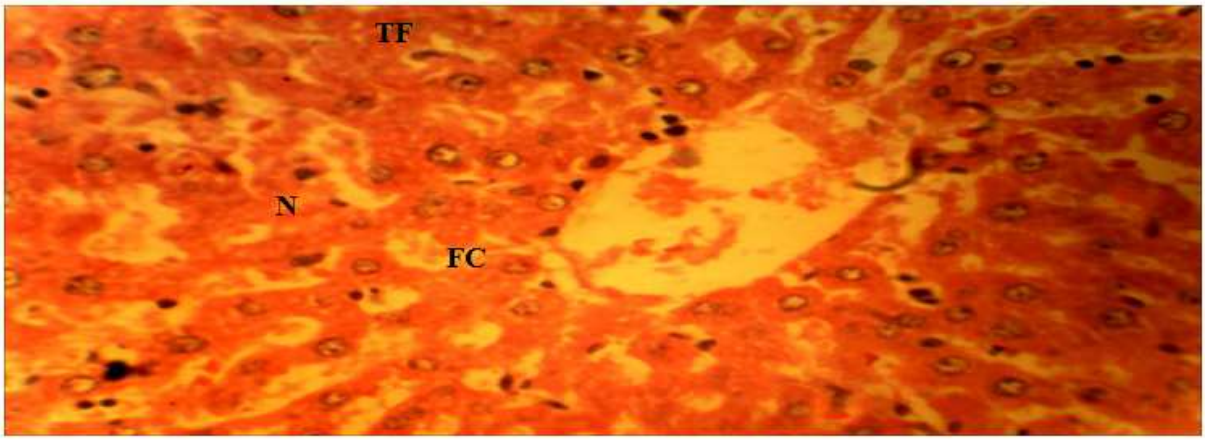


Plate II: Section of liver from Diabetic control group (DC) showing (N) necrosis, fatty change (FC) vacoulation (V) and tissue fragmentations (TF) (H&E × 250)

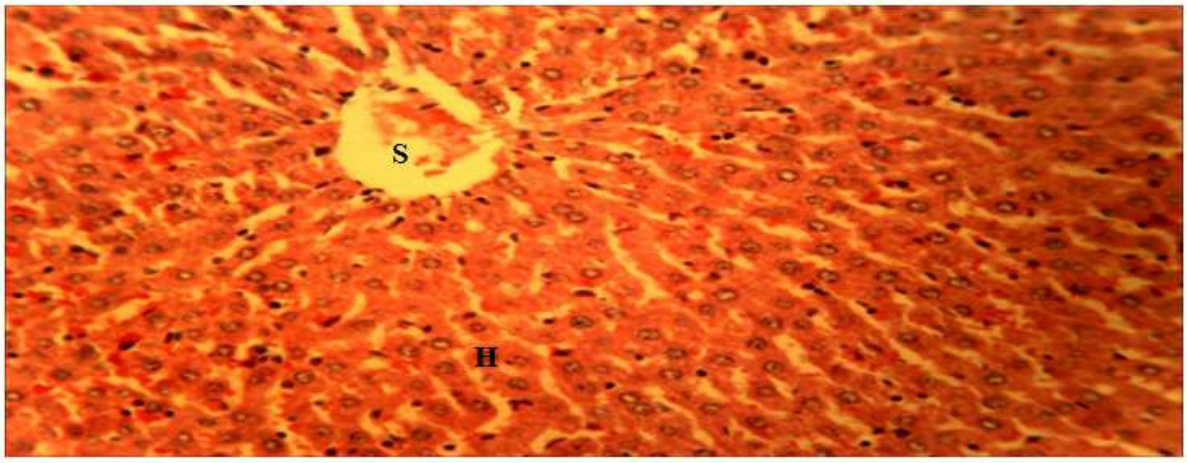


Plate III: Section of liver from *Azadrachta indica* (200 mg/kg b w) treated diabetic group (D+AI) showing restored micro-cytoarchitecture (H&E × 250).

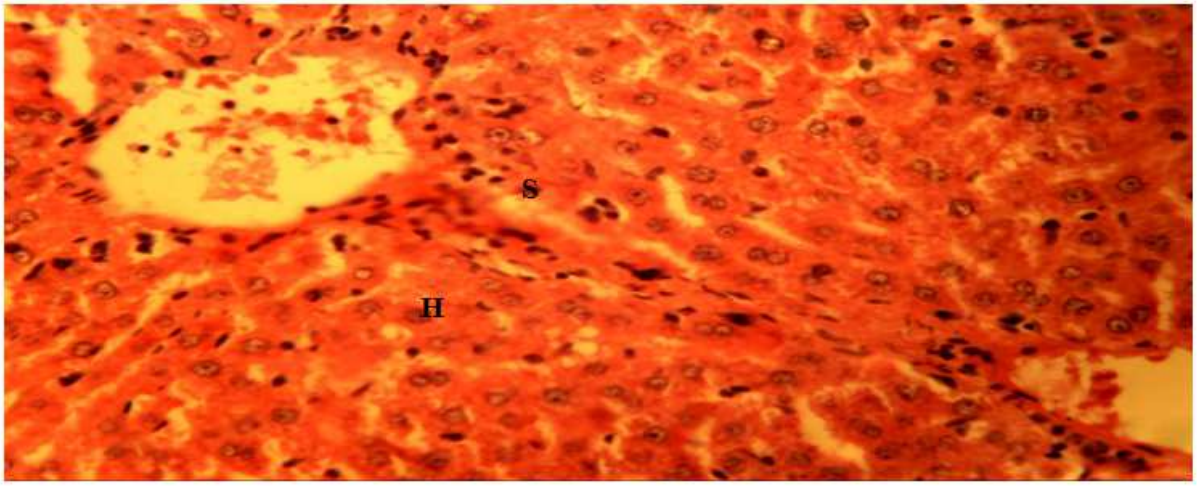


Plate IV Section of liver from melatonin (10 mg/kg b w) treated diabetic group (D+ML) showing restoration of livercyto-architecture indicating restored hepatocyes H) and sinusoids (S) and no fatty change (H&E × 250).

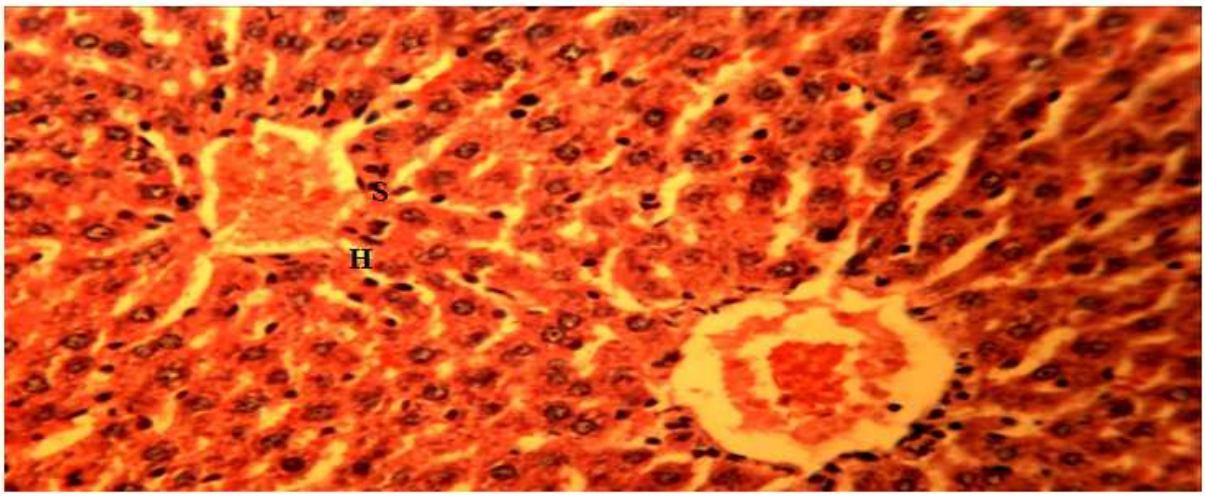


Plate V: Section of liver from *Azadirachta indica* (200 mg/kg b w) and melatonin (10 mg/kg b w) (DAI+ML) treated diabetic group showing restored hepatocytes and sinusoids, no observable fatty change (H&E × 250).

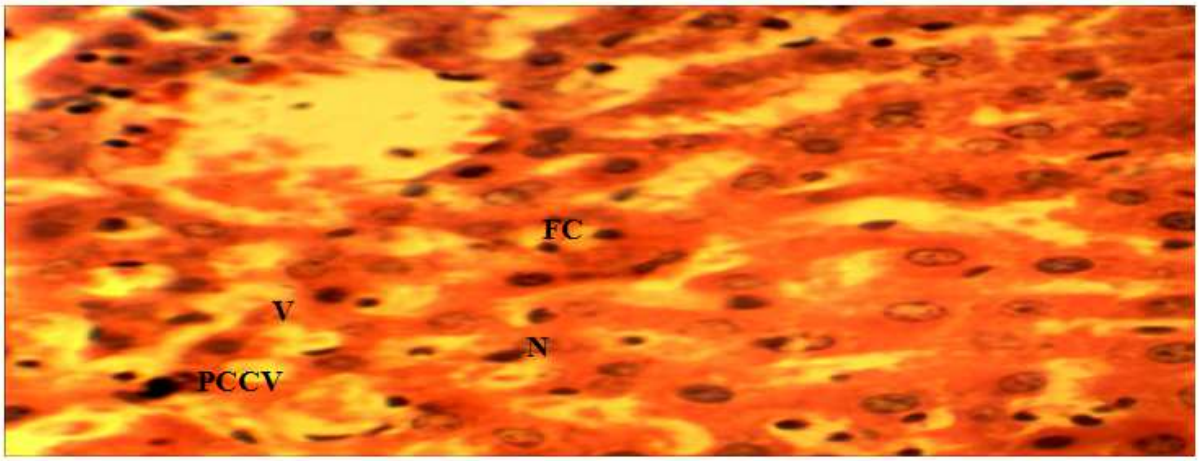


Plate VI: Section of liver from metformin (500 mg/kg b w) treated diabetic (D+MF) group showing necrosis of hepatocyte and sinusoids, fatty change (FC), vacoulation (V) and partial congestion of central vein (PCCV).There is poor restoration of liver cyto-architechture (H&E × 250)

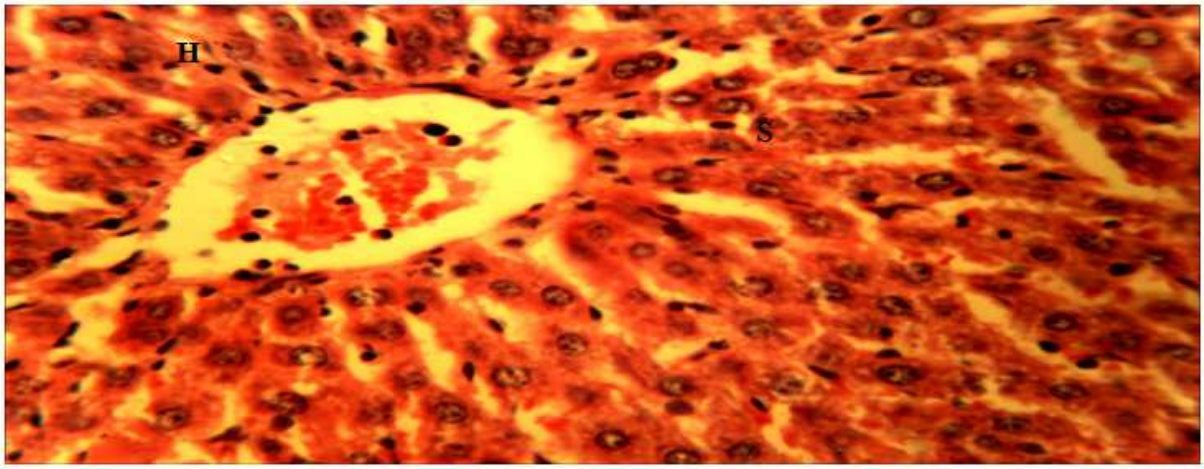
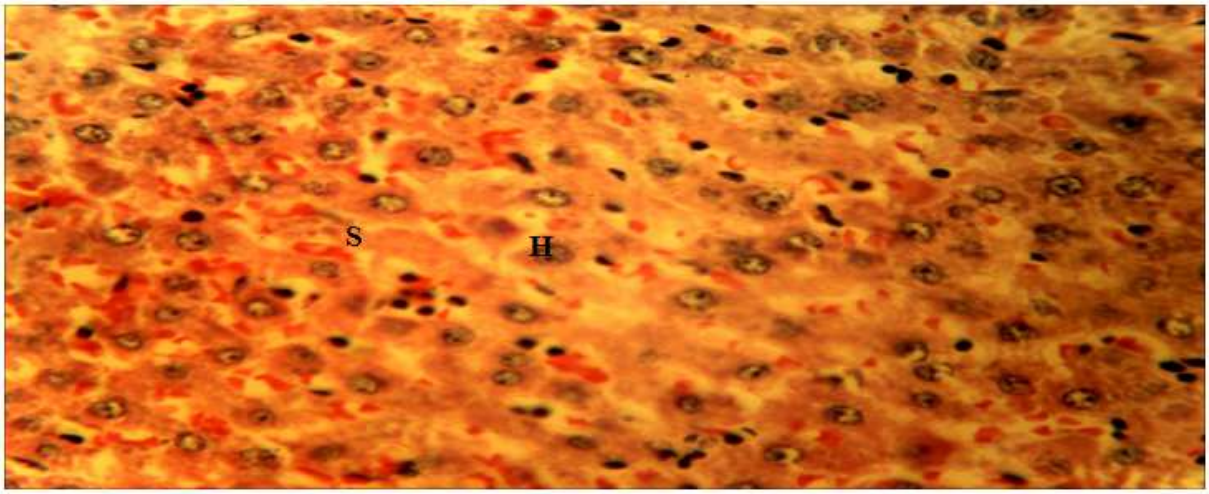


Plate VII: Section of liver from *Azadirachta indica* (200 mg/kg b w) treated normoglycaemic rat (N +AI) showing normal hepatocytes (H) and sinusoids (H & E ×250).



Plat VIII: Section of liver tissue from *Azadirachta indica* (200 mg/kg b w) and melatonin (10 mg/kg b w treated normoglycaemic rat (NAI + ML) showing normal hepatocytes and sinusoids (H&E × 250).

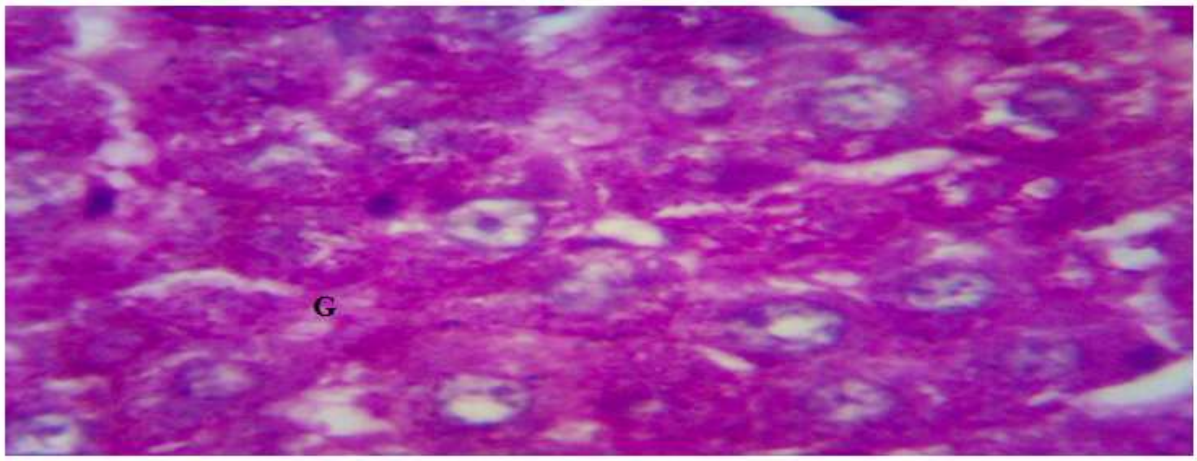


Plate IX: Section of liver from normal control group (NC) stained with periodic acid Schiff demonstrating glycogen (G) in liver hepatocytes cytoplasms (PAS × 400).

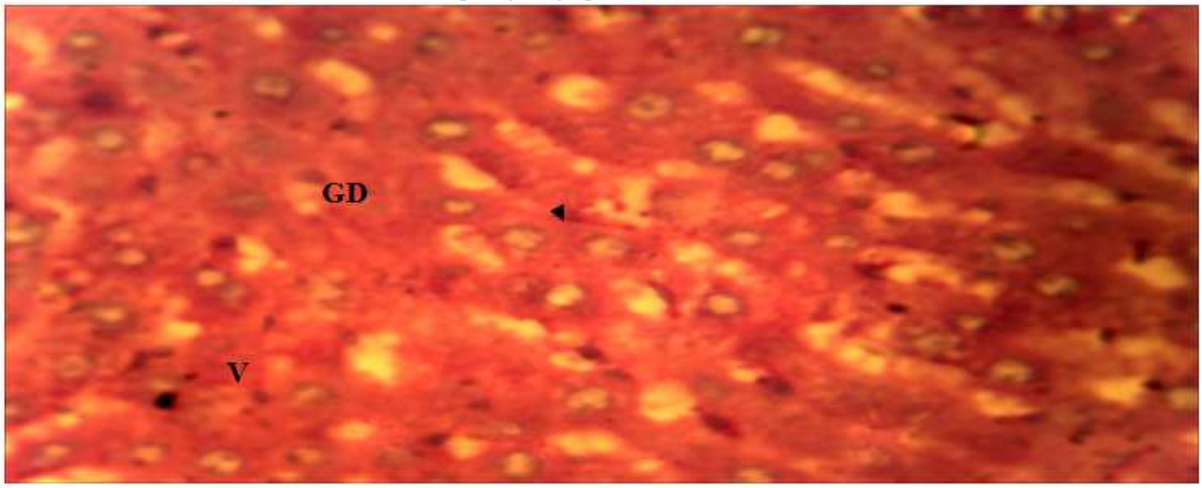


Plate X: Liver section of diabetic control group (DC) treated with normal saline showing depletion of glycogen (GD), fatty change, nuclear and cytoplasmic vacoulation (V) (PAS × 400).

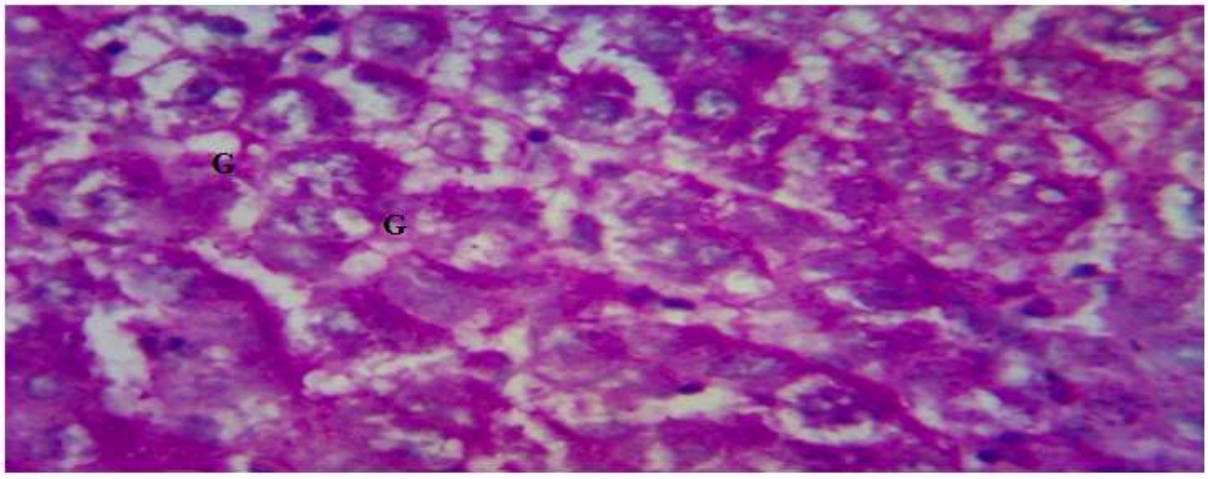


Plate XI: Section of liver from *Azadiracta indica* (200 mg/kg b w) treated diabetic group (D+AI) showing improved glycogen (G) stores in hepatocytes, (PAS × 400)

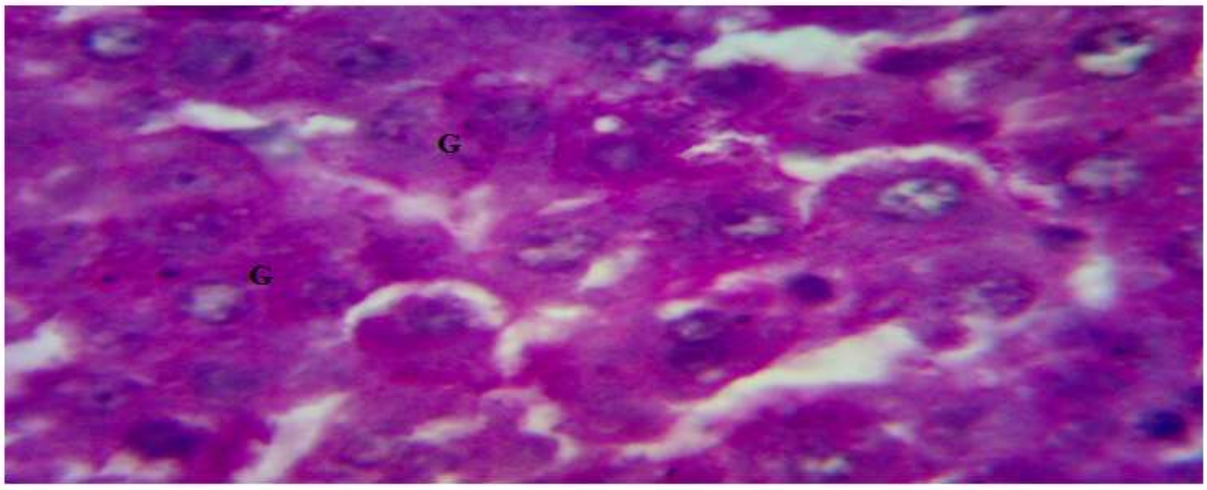


Plate XII: Section of liver from *Azadirachta indica* (200 mg/kg b w) and melatonin (10 mg/kg b w) treated group (DAI +ML) showing normal hepatocytes and sinusoids. There are improved cytoplasmic glycogen (G) stores, (PAS × 250).

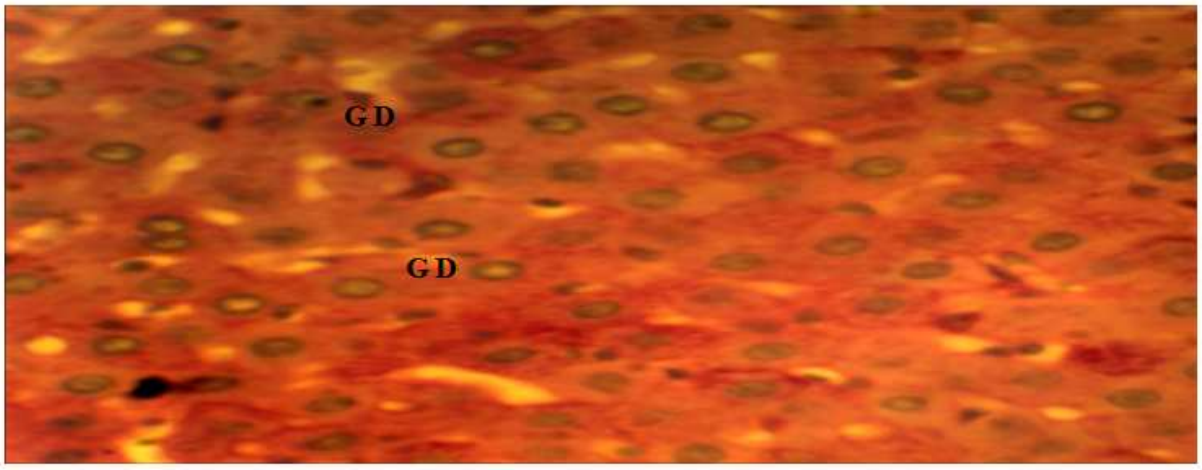


Plate XIII: Liver section of metformin (500 mg/kg b w) treated diabetic group (D+MF) showing reduced cytoplasmic glycogen (G) stores (PAS × 400).

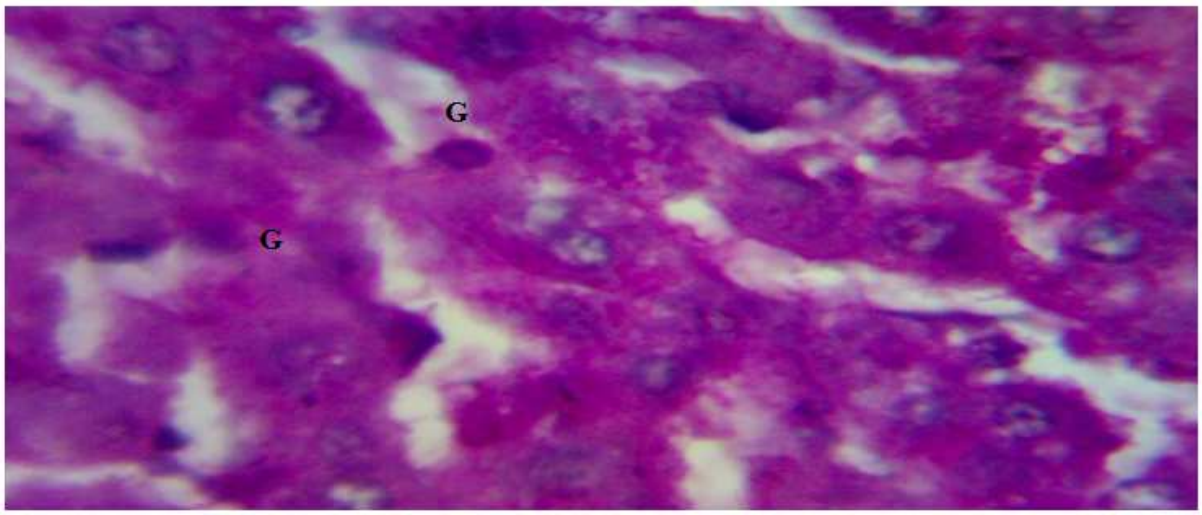


Plate XIV: Section of liver from melatonin (10 mg/kg b w) treated diabetic group (D+ML) showing improved glycogen (G) stores in hepatocytes cytoplasm (PAS × 250).

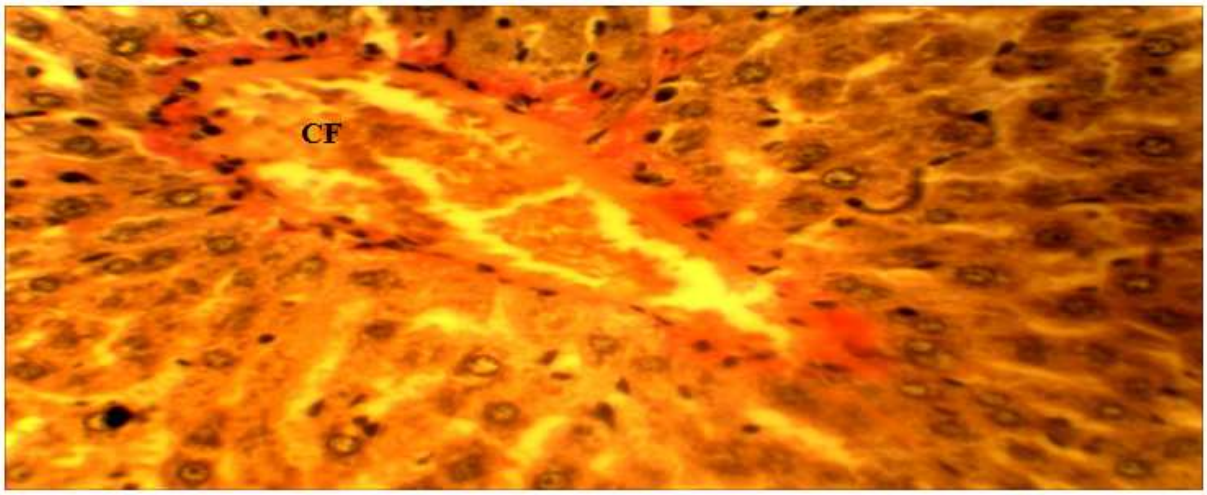


Plate XV: Section of liver from normal control group stained with HVG, showing collagen fibers (CF), (HVG × 250).

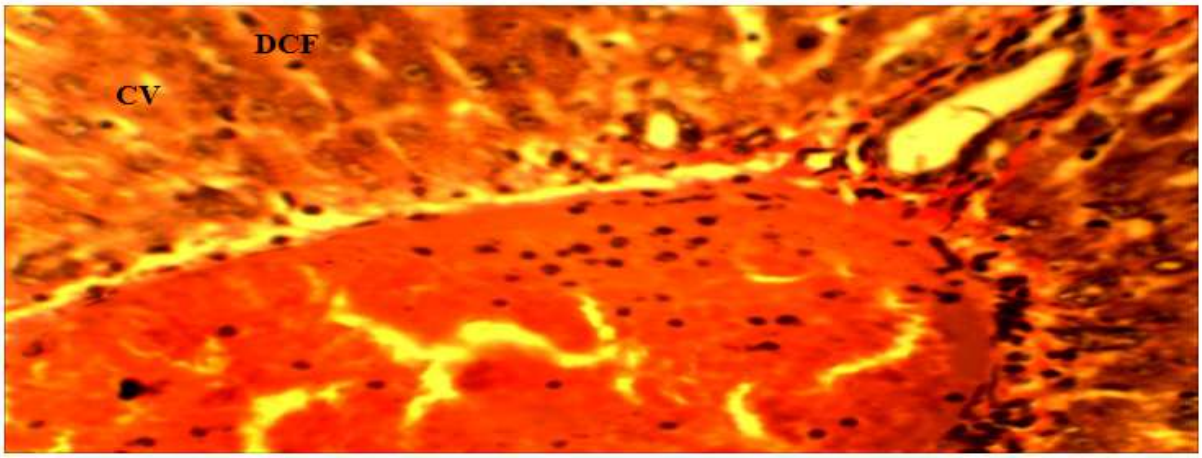


Plate XVI: Section of liver from diabetic control group (DC) showing degeneration of collagen (DCF) fibers and congestion of central vein (CV) (HVG ×250).

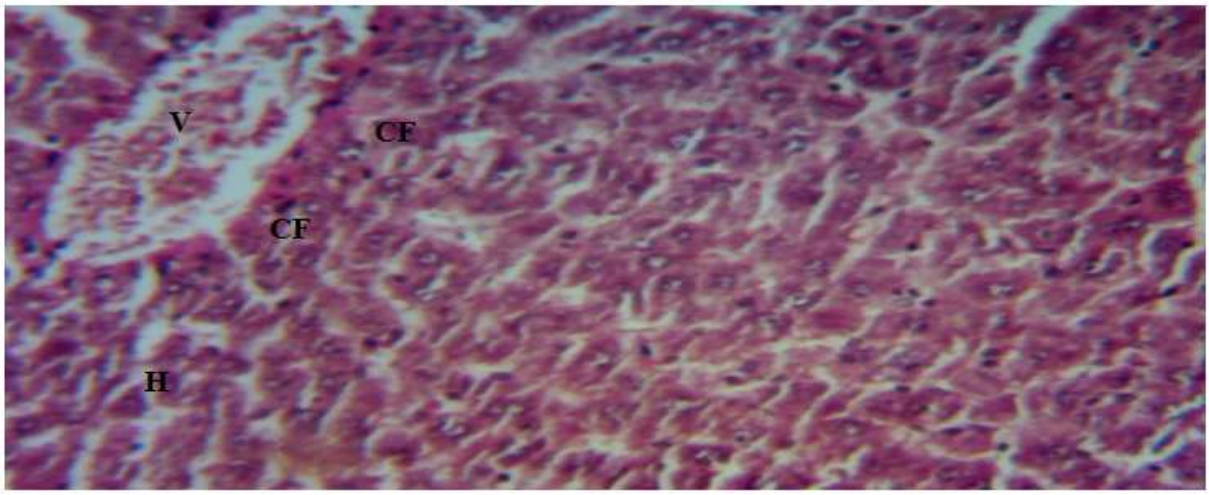


Plate XVII: Section of liver from melatonin (10 mg/kg b w) treated diabetic group (D+ML) showing restored collagen fibers (CF) around hepatocytes vessels (V) (HVG × 250).

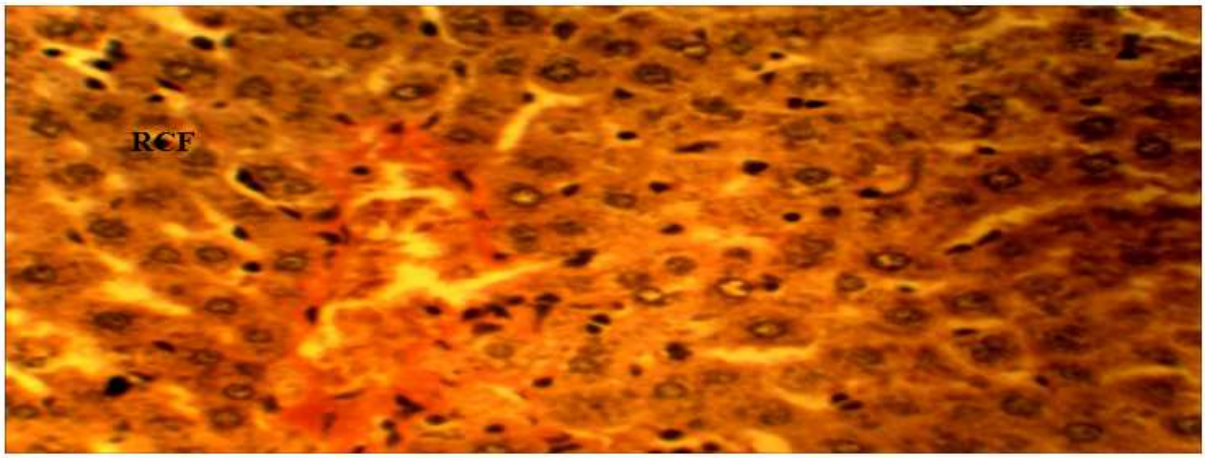


Plate XVIII: Section of liver from *Azadiracta indica* (200 mg/kg b w) treated diabetic group (D+AI) stained with HVG showing regenerated collagen fibers (CF-Arrowed) around liver hepatocytes, sinusoids and around the veins (HVG × 250).

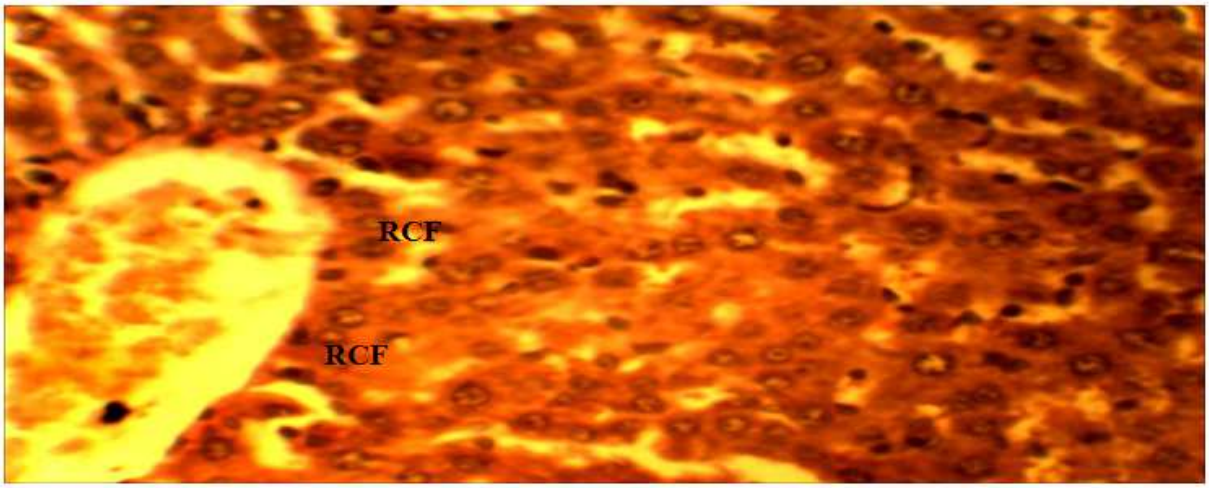


Plate XIX: Liver section of diabetic *A. indica* (200 mg/kg b w) and melatonin (10 mg/kg b w) treated group showing regenerated collagen fibers (RCF), no necrotic change, no fatty degeneration and restored hepatocytes and sinusoids (HVG ×250).

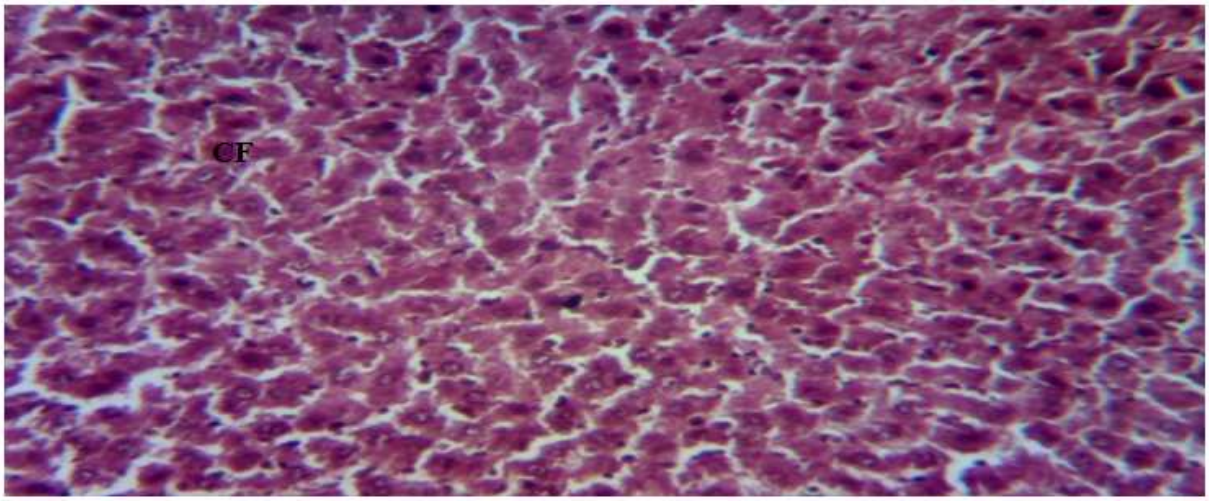


Plate XX: Section of liver from metformin (500 mg/kg b w) treated diabetic group (D+MF) stained with HVG showing poorly restored collagen fibers (CF) around the hepatocytes, sinusoids and vessels (HVG × 250).

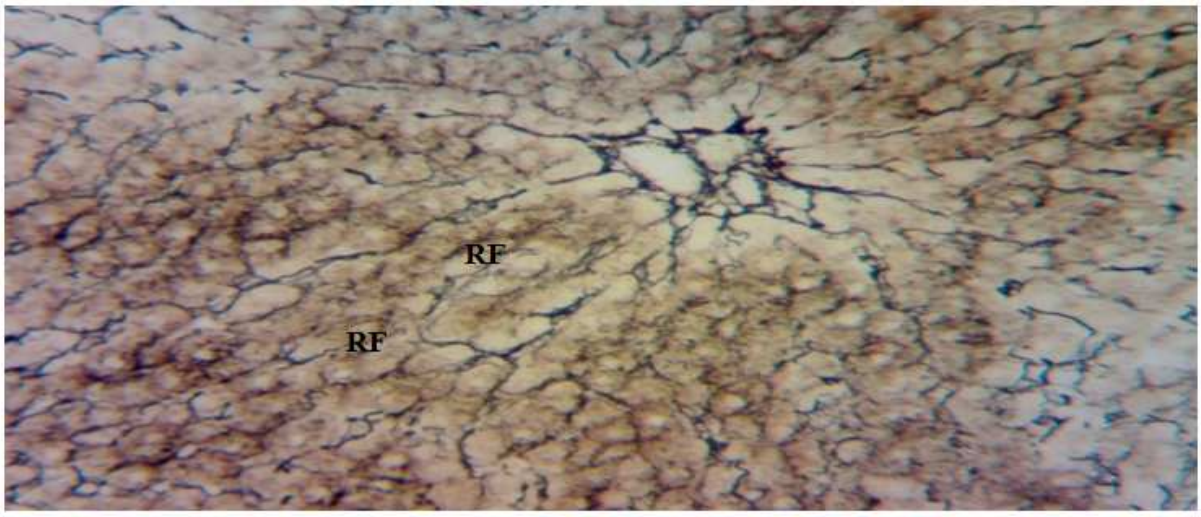


Plate XXI: Section of liver from Normal control group (NC) showing reticular fibers (RF), (Gordon and Sweet × 250).

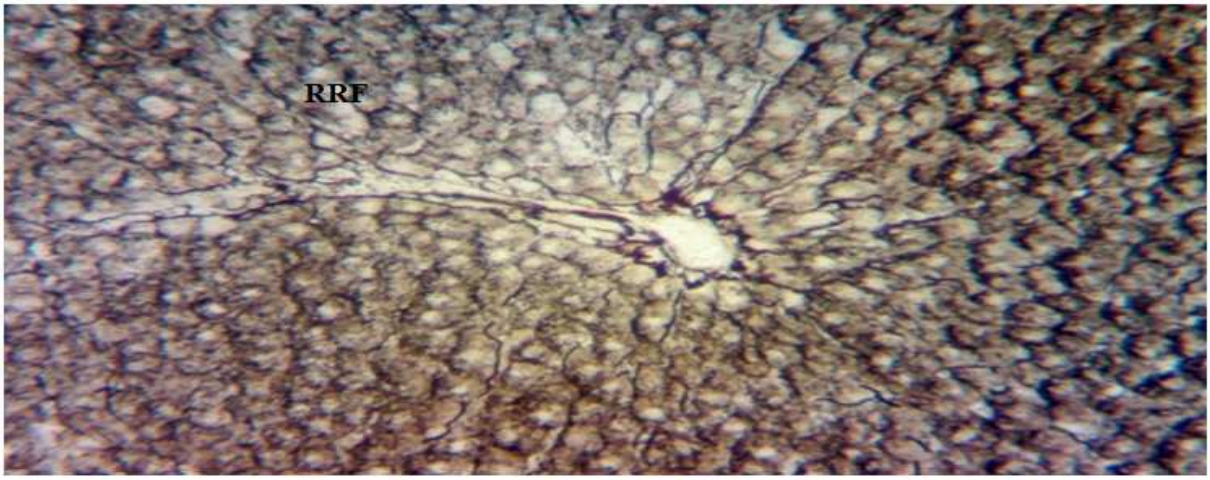


Plate XXII: Section of liver from *Azadiracta indica* (200 mg/kg b w) treated diabetic group (D+AI) showing regenerated reticular fiber (RRF) (Gordon and Sweet × 250).

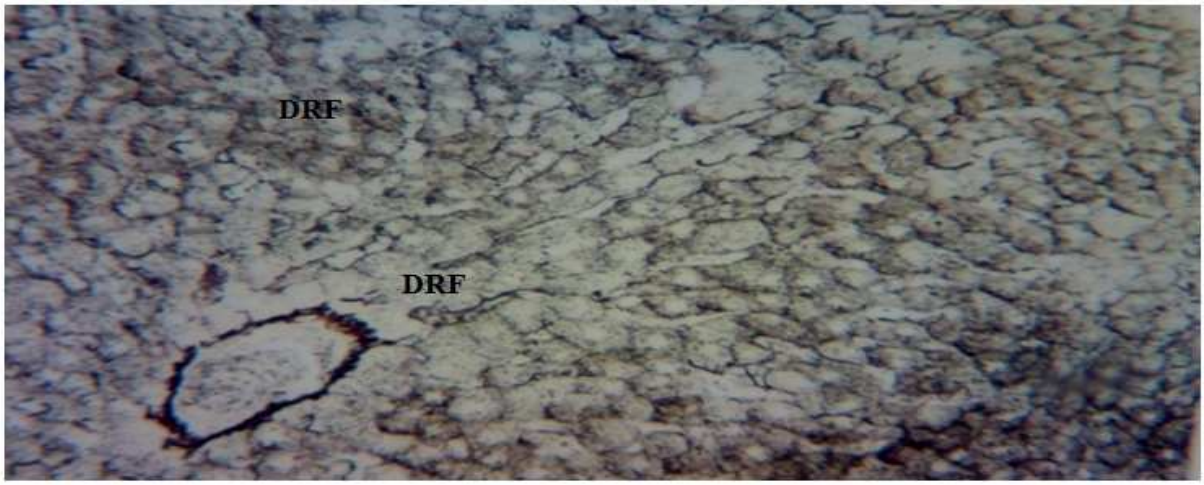


Plate XXIII: Section of liver from Diabetic control group (DC) demonstrating degenerated reticular fibers (DRF) (Gordon and Sweet × 250).

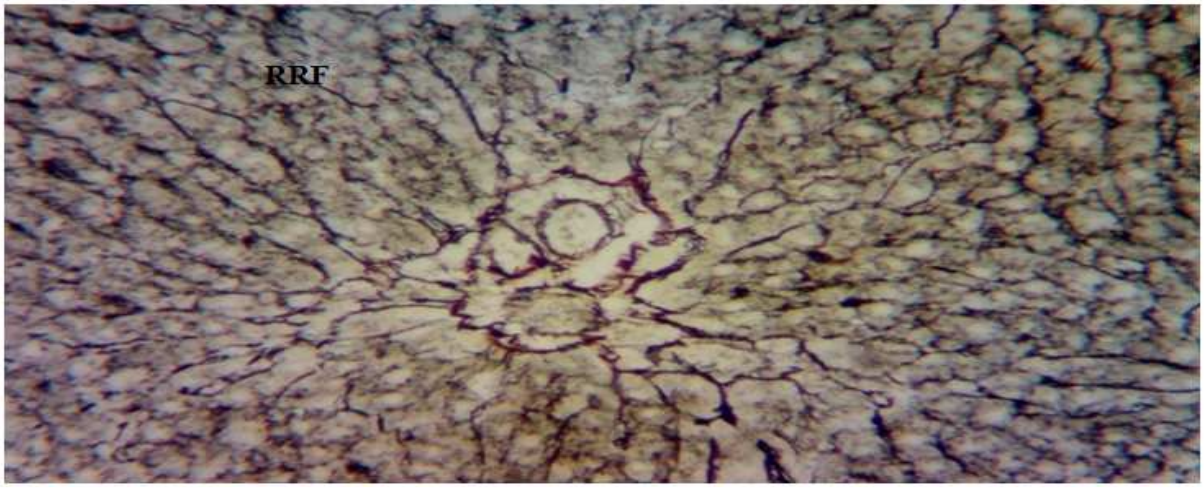


Plate XXIV: Section of liver from Melatonin (10 mg/kg b w) treated diabetic group (D+ML) showed restored reticular fibers (RRF), sinusoids and around blood vessels (Gordon and Sweet × 250).

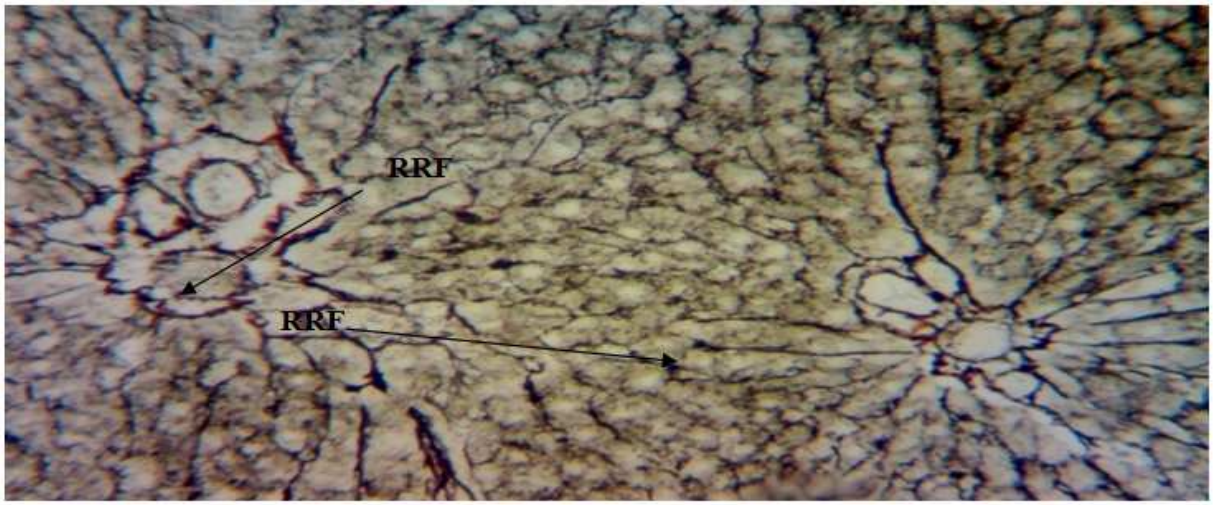


Plate XXV: Section of liver from *Azadirachta indica* (200 mg/kg b w) and melatonin (10 mg/kg b w) treated diabetic group with Gordon and Sweet stain showing regenerated reticular fibers around the hepatocytes,sinusoids and blood vessels (Gordon and Sweet × 250).

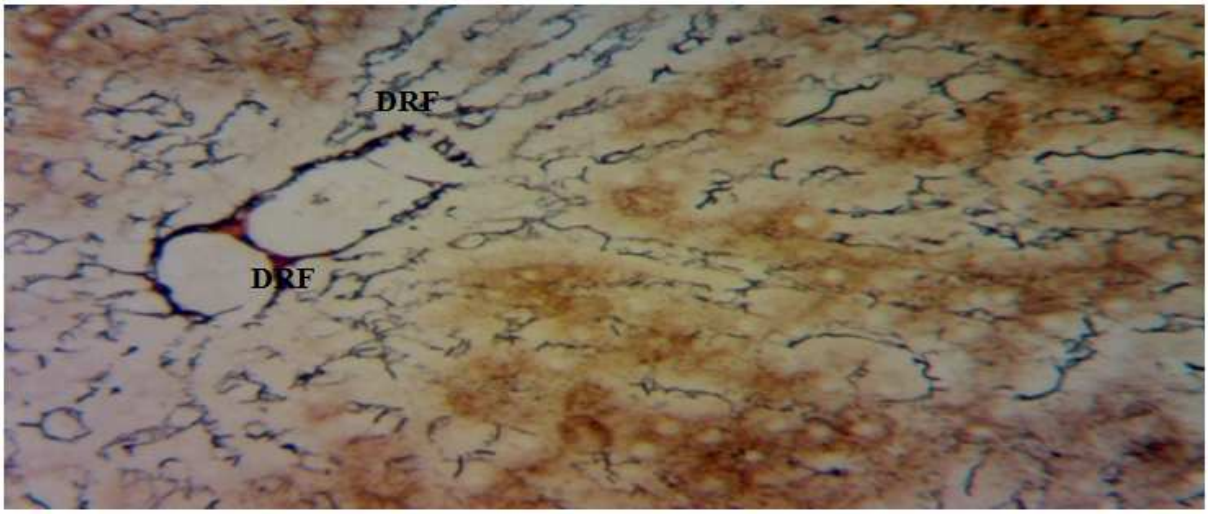


Plate XXVI :Section of liver from Metformin treated diabetic group showing fragmented reticular fibers (DRF) around the hepatocytes, sinusoids and blood vessels, reticular fibers are not fully regenerated (Gordonand Sweet × 250).

DISCUSSION

Liver enzymes Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are present in high concentration in the normal hepatocytes of the liver and the enzymes leaked into the circulation as a result of damage to cell membrane of hepatocytes [11]. Dufour *et al* [12] had reported that ALT is more specific for liver damage as compared to AST, and that AST activities persist longer than ALT. There were some levels of liver damage as seen in the results of the present study, where elevated levels of these enzymes were observed to be high in the diabetic control group as compared to the experimental treated groups. The reduced activities of these enzymes in groups treated with *Azadirachta indica* (200 mg/kg b w) and melatonin (10 mg/kg b w) indicated that *Azadirachta indica* leaves extract and melatonin possessed hepato-protective effects in streptozotocin-induced diabetic animals. The significantly decreased levels of liver enzymes (AST, ALT and ALP) observed in the present study when treated with *Azadirachta indica* (AI) and melatonin separately and when they are combined, showed that the extract and melatonin exhibited some therapeutic effects and hence have membrane stabilizing effects that prevented further leakages of intracellular enzymes. This is consistent with findings by Atangwo *et al* (2010) who demonstrated that A. *indica* and *Vernonia* amygdalina alone and when combined caused decrease in liver enzyme activities in diabetic animals. The diabetic control group that received no treatment, showed a significantly increased levels of AST and ALT and ALP when compared with the normal control group. However, treatment with A. *indica* only and when combined with melatonin showed a significant reduction in the activities of ALT and AST while melatonin treatment alone had a high significant reduction in the levels of these enzymes when compared the diabetic control group. Metformin (500 mg/kg b w) treatment showed the least reduction in liver enzymes activities when compared with diabetic control group. The central observation of the present study showed that *Azadrachta indica* (200 mg/kg b w) and melatonin (10 mg/kg b w) when administered to streptozotocin-induced diabetic Wistar rats, restored the degenerative changes associated with and diabetes mellitus by reducing fatty change, necrosis, hepatocyte vacoulation, glycogen depletion, connective tissues derangement and restoration of the cyto-architecture of the liver. The reticular tissue fibers restoration in the liver parenchyma of stretozotocin- induced diabetic treated groups with melatonin (10 mg/kg b w) and A. *indica* (200 mg/kg b w) was significantly improved. There were also improved restored depleted cytoplasmic glycogen stores in hepatocytes of *Azadrachta indica* (200 mg/kg b w) and melatonin (10 mg/kg b w) treated diabetic groups. This finding is in agreement with the studies of Bhanwra *et al*. [13] that showed that *Azadirachta indica* has hepato-protective properties based on liver histology in paracetamol induced hepatotoxicity in rats. Report by Ebong *et al*. [14] demonstrated the presence of phytochemicals with known antioxidant properties like flavanoids, polyphenols and micronutrients such as antioxidant vitamins and micronutrient in the leaves of A. *indica* extract and suggested their actions might have inhibited the free radical generation process and mopped up circulating free radicals causing histological lesions and complications of diabetes, thereby resulting in the regeneration of hepatic cells.

CONCLUSION

It can be concluded that administration of *Azadirachta indica* leave extract and melatonin separately and when they are combined, possess hepato-protective effects as evidenced by liver tissue regeneration and significantly reduced liver enzyme activities in streptozotocin-induced diabetes in wistar rats.

REFERENCES

[1] International Diabetes Federation, The IDF Consensus Worldwide Defination of Metabolic Syndrome: Bussels; IDFCommunication;*http:/www.idf.org/webdata/docs/IDF,def final, pdf.June, 15,* **2012**.
[2] DE Kelly, *Diabetes Review*, **1995**, 277(21), 18919 –18927.
[3] CJ Baily, C Day, *Diabetes Care* , **1989**, 12, 553 –564.
[4] GA Jelodar, M Maleki, MH Motadayen, S Sirus, *Indian Journal of Medical Science*:, **2005**, 59(2), 64 –69.
[5] R R Chattopadhyay, M Bandyopadhyay, *African Journal of Biomedical Research*, **2005**, 8, 101-104.
[6] G Baydas, H Canatan, A Turkoglu, *Journal of Pineal Reearch,* **2003**, 32, 225.
[7] RJ Reiter, *Journal of Pineal Research*, **2003**, 34-75.
[8] E A Lapshina, EJ Sudnikovich, JZ Maksimchik, SV Zabrodskaya, LB Zavodnik, *Life Science*, **2006**, 79, 1804.
[9] E Javier, JA Pariente, AB Rodríguez, Role of melatonin on diabetes-related metabolic disorders: *World Journal of Diabetes*, **2011**, 2 (6), 82-91.
[10] IJ Atangwo, EP Ebong, EE Godwin, MI Akpaso, EE Asuquo. *Research Journal of Agricultural and Biological Sciences*, **2010**, 6(4), 514-521.
[11] MR Ahsan, KM Islam, IJ Bulbul, MA Musaddik, E Haque, *European Journal of Science Research*, (**2009**) 37, 302-31.
[12] DR Dufour, JA Lott, FS Nolte, DR Gretch, RS Kff, LB Seeff, *Clinical Chemistry*, **2000**, 46, 2027-2049.
[13] S Bhanwra, J Singh, P Khosla, *Indian Journal of Physiology and Pharmacology*, **2000**, 44, 64-68.
[14] PE Ebong, Paper presented at international neem conference in Kunming, China, **2006**, 234-238.