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Research Article

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Effect of methanolic extract of *Buccholzia coriacea* (wonderful kola) seed on the liver cells of an alloxan – Induced diabetic wistar rats

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Abstract

The effect of methanolic extract of wonderful kola seed on the liver cells of an alloxan induced-diabetic wistar rat. The study was aimed to investigate the effect of methanolic extract of wonderful cola seed on the liver cells of adult albino wistar rats by the use of H & E stain. Diabetes was induced with a single dose of freshly prepared alloxan monohydrate 1ml/kg per body weight was injected intraperitoneally in the rats. After 24hours, rats that had blood glucose level above 200mg/l were considered diabetic and selected for the study. The extract were orally administered at a dose range of 0.5ml, 1ml and 1.5ml/kg per day for 14 days . 20 albino wistar rats were chosen at random and were divided into six groups. After 14days period, the animals were fasted for 12 hours anesthetized with chloroform and then sacrificed. Blood glucose level was checked, the extract decreases the respective fasting blood glucose values of 6.61±0.66, 5.12±0.83 and 3.84±1.34mmol L^{-1} when compared to the initial value of 14.77±1.69, 13.28±1.52 and 17.42±2.5. The liver was surgically removed and carryout histological examination. At the end of study the result shows normal central vein, dilated sinusoid with hepatocytes, hence wonderful kola extract with it antioxidant effect shows a significant tissue architecture particularly to the central vein of the liver which been covered by the diabetis mellitus.

Keywords: diabetis mellitus, alloxan induced, *Buccholzia coriacea.*

Introduction

About 1-7% of Nigerian population is affected by diabetes mellitus (Wokoma, 2002, Fabiye *et al.*, 2002). According to the work of (Wild *et al.*, 2004) in 2030 about 5.6% and 14% of world and Nigerian population respectively will be affected by diabetes mellitus. It becomes imperative to carry out studies that can see to the reduction, or possibly halt the geometric increase in the figures above.

The uses of plants in traditional medical practice have a long drawn history and remain the mainstay of primary health care in most of the third world (Prescott-Allen, 1982), several plants have been studied and were found to possess anti-diabetic and anti-oxidant properties (Al-Hader *et al.*, 1993; Al-Enazi, 2007).

Among these plant seeds are the seeds of *Bulcholzia coriacea* popularly known as wonderful kola. The plant is documented to possess diverse medicinal potentials. According to (Adisa *et al.*, 2010), the seeds are used traditionally for treating diabetes, hypertension, rheumatism, cold, cough and catarrh. It prevents premature aging and has the ability to migraine headache on the fore head for about ten (10) minutes. The stem and barks of the tree exhibit high concentration dependent antibacterial and antifungal activity when subject to ethanol extract (Ayaiyeoba *et al.*, 2003).

Aim

This study was designed to investigate the effect of methanolic extracts of wonderful cola seed on the liver of an alloxan-induced diabetic Wistar rat.

Materials and Methods

Preparation of the plants materials

The seeds were washed thoroughly with distilled water to remove adhering particles after which they were sliced and properly sun-dried. The dried pieces were grounded to fine powder using manual grinder. 250g of pulverized plant materials was soaked in 500mls of methanol and intermittently shaken. The mixture was kept for 72 hours after which it was filtered with whattman No.2 filter paper. The methanolic extract obtained was concentrated to a blue residue plate and evaporated to dryness on the sun. The percentage yield was 8.4%. The filtrate was stored in a refrigerator until required for use.

Experimental animal

The animal were acclimatized to the condition of the animal housing facility with ambient temperature 26-28^{0}c and adequate ventilation for two weeks and feed with growers mash (Vita feed Nig (TI)) and clean water and ad.libitum. They were used in accordance with National Institute of Health (NIH) guide for the care and use of laboratory animals.

Table 1: Average weight of the animals after acclimatization and after 14 days of treatment with the extract are shown in the table below

Average weight after 14 days of acclimatization	Average weight after 14 days of treatment with extract
GROUP 1	
153g	159g
GROUP 2	
180g	185g
GROUP 3	
167g	172g
GROUP 4	
170g	175g
GROUP 5	
167g	170g
GROUP 6	
155g	157g

Experimental design

The twenty albino wistar rats of both sex were divided into six groups of three rats per groups and two was kept as backup for any unaccepted mortality. Animals were grouped and labelled a follows:

Group 1: Non-diabetic control (not induced with alloxan and B. coricea).
Group 2: Diabetic control (induced with alloxan only 1ml).
Group 3: Non-diabetic treated received B.coriacea extract low dose (not induced with alloxan but treated with B.coricea 1ml).
Group 4: Diabetic treated received B. coriacea extract low dose (0.5ml).
Group 5: Diabetic treated received Alloxan and B. coriacea extract medium dose (1.0ml).
Group 6: Diabetic received and B. coriacea high dose (1.5ml).
All the animals in the group received food and grounded pelletes ad. Libitum.

Induction of diabetes mellitus

A single dose of freshly prepared Alloxan monohydrate 1ml/kg body weight was injected intraperitoneal into the rats, blood samples collected by tail vein tapping were monitored for glucose level using a glucometer. After 24hours rats that had blood glucose level above 200mg were considered diabetic and selected for the study.

The single dose administration of alloxan maintained Diabetis mellitus in the rats for the whole duration of the study.

Oral administration of extract

The rates were randomly divided into 6 group of 3 rats each and labelled numerically using a permanent marker and average weight between and within groups did not exceed 20% of the average weight of the sample population. Rats in group 1 served as control and were given distilled water. Rats in group 3-6 served as the treatment groups were orally treated or administered with 0.5m, 1ml and 1.5mlkg/day of the extract respectively for 14days. The appreciate quantity of the methanolic extract that given orally by carefully insertion of the syringe between the tongue and the roof of the mouth until a free space is served for the extract to be delivered into the stomach.

Collection and analysis of sample

At the end of the 14 days period, the animals were fasted for 12 hours, anaesthetized with chloroform and then sacrificed. The liver was surgically removed and was immediately blotted using filter paper to remove traces of blood and then weighed with digital analytical balance.

Histopathology of tissue

The liver was fixed in 10% formal saline for historical examination before they were processed using the automated tissue processor. The essence of fixing the tissue was to prevent autolysis and putrefaction. After fixing, the tissues with the use of ascending grades of alcohol (70%, 95%, and 100% alcohol). The tissues were removed from the dehydrate agent after 2hours and moved into clearing agent. After clearing, the tissues were impregnated in molten paraffin wax. Then, the tissue was embedded in mould.

Finally, it was sectioned using rotary microtome at 5um thickness, stained with haematoxylin and eosin staining to demonstrate the general structure of the tissues.

Procedures for haematoxylin and eosin staining

The liver was dewaxed in xylene 1 and II. The tissue was hydrated in descending grades of alcohol 100%, 95% and 90%. After hydration, the tissuewere stained in Erhlich haematoxylin for 5-15mintues. The tissue was then washed in water. Then differentiated in 1% acid alcohol with a fast dip. The tissue was washed in water. Then blued in Scott's tap water for 6minutes. After blueing counter stained with 1% eosin for 1minutes. The tissue was washed in water. Then dehydrated in ascending grades of alcohol (90%, 95% and 100%). The tissue was cleared in xylene 1 and II. After clearing, the tissue was mounted in DPX (Dibutyphthalate polysterene xylene). It also aids the optical differentiation of the tissue constitution. After mounting them the stained tissue was viewed microscopically to detect histological disorders.

Results

Extraction of the plant material *Buchholzia coriacea* seed extract was brownish in colour with oily consistency. The total solids recovered from extracts were 8.4%. The seed extract of B. coriacea did not cause any mortality up to a dose of 1500mg/kg^{-1} in the rats treated orally with varying doses, (500, 100, 1500mg/kg^{-1}) of the crude extract. The extract was well tolerated by the rats with mild signs of toxicity (dizziness) at 1500mg/kg^{-1}.

Antidiabetic activity of *Buchholzia coriacea* seed exerted significant ($p < 0.05$) reduction in fasting blood glucose level of diabetic rats at day 14 of treatment. The varying doses (500, 1000, 1500mg/kg^{-1}) of the extract produced fasting blood glucose values of 6.61±2.14, 5.12±1.01 and 3.84±0.81mmol L^{-1} compared to control which had 14.5±5.12 m mol^{-1} as shown in the table below;

Table 4.1: The effect of methanolic extract of *B. Coriacea* on fasting blood glucose of Alloxan – Induced diabetic rats

Day	Normal (Non-diabetic control)	Diabetic control	500mg/kg^{-1}	1000mg/kg^{-1}	1500mg/kg^{1}
Before induction	5.02±0.05mmol L$^{-}_{1}$	5.72±3.72mmol L^{-1}	5.40±1.01mmol L^{-1}	5.70±0.66 mmol L^{-1}	4.82±1.06 mmol L^{-1}
After induction	5.40±0.06mmol L$^{-}_{1}$	21.15±0.73mmol L^{-1}	14.77±1.69 mmol L$^{-}_{1}$	13.28±1.52 mmol L$^{-}_{1}$	17.42±2.5 mmol L^{-1}
14	5.15±0.01mmol L$^{-}_{1}$	14.15±1.28mmol L^{-1}	6.61±0.66 mmol L^{-1}	5.12±0.83 mmol L^{-1}	3.84±1.34 mmol L^{-1}

Significant decreases at ($p < 0.05$) compared to negative control values are Mean ± SEM, n=3 in each group. Sample was collected after the period of 14 days.

Histological slides

Micrograph of diabetic albino wistar rat kidney treated with methanolic extract of *Buchholzia coriacea* seed for 2weeks. Mag. 40x H & E Stain.

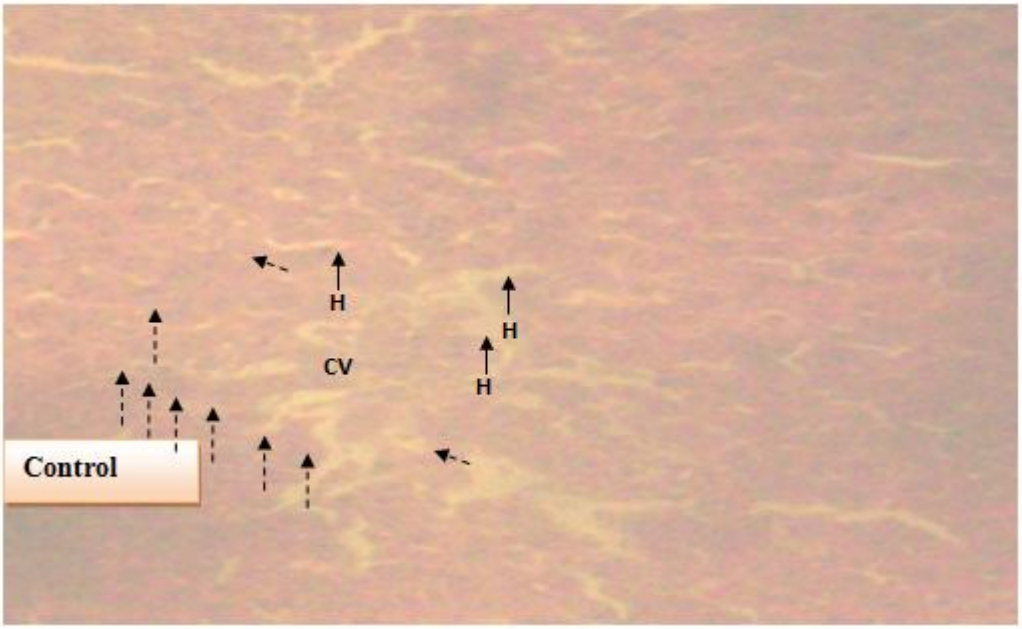

Fig. .1: Photomicrograph of liver (Control H&E x100):Shows normal central vein (**CV**), dilated sinusoids (**dash arrows**) with hepatocytes (**H**).

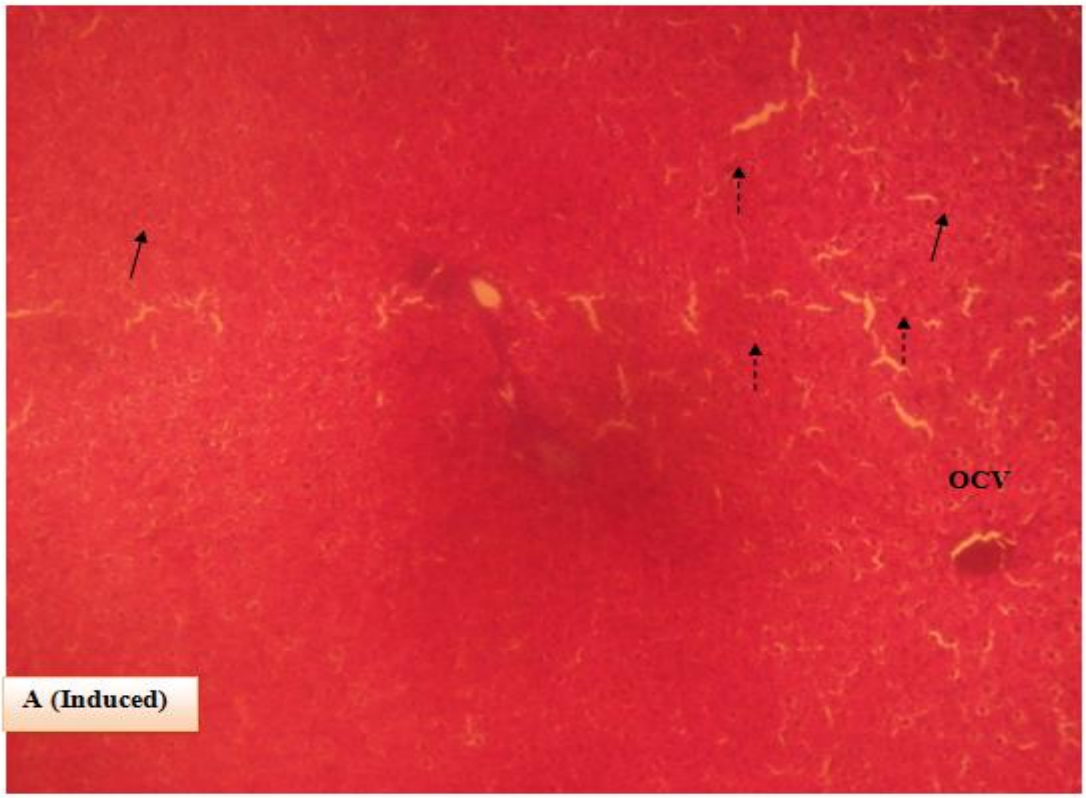

Fig 2: Photomicrograph of liver (H&E x100) induced with alloxan shows the occluded central vein (O**CV**), dilated sinusoids (**dash arrows**) with hepatocytes (**H**). Photomicrograph is not different from the control. No degeneration of hepatocytes

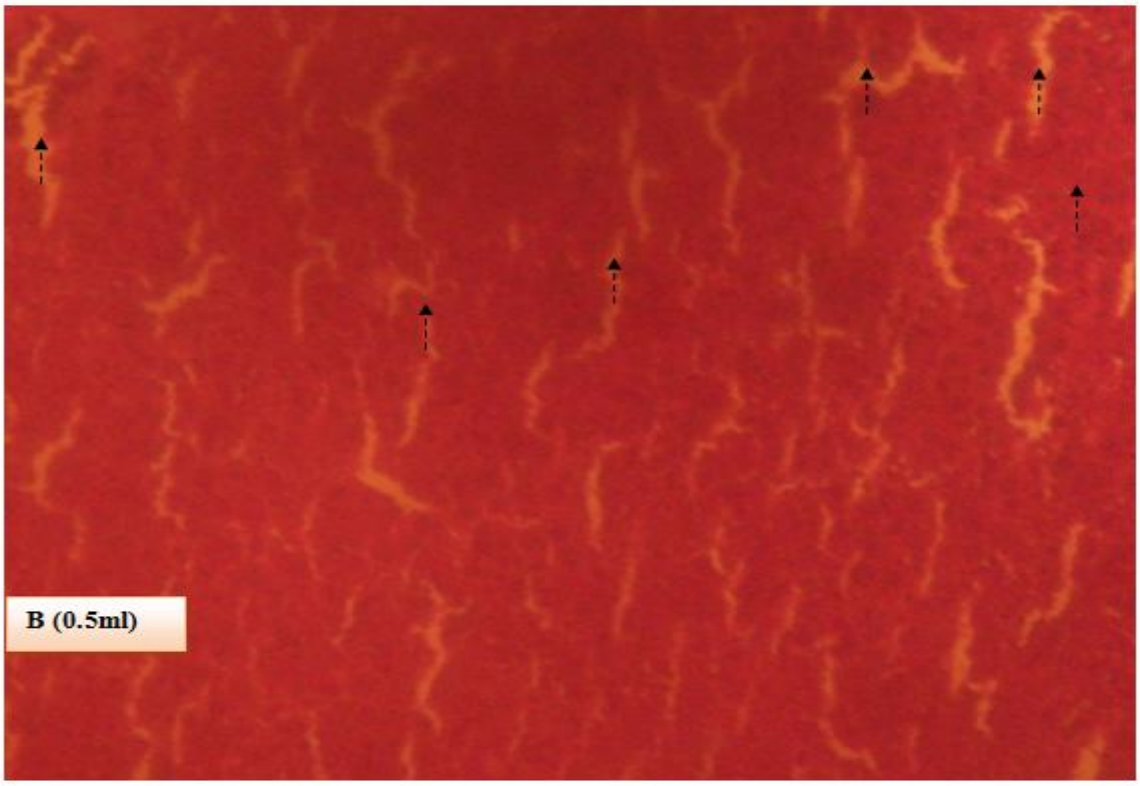

Fig .3: Photomicrograph of liver (H&E x100) treated with *B. coriacea* shows the central dilated sinusoids (**dash arrows**). Photomicrograph is not different from the control.

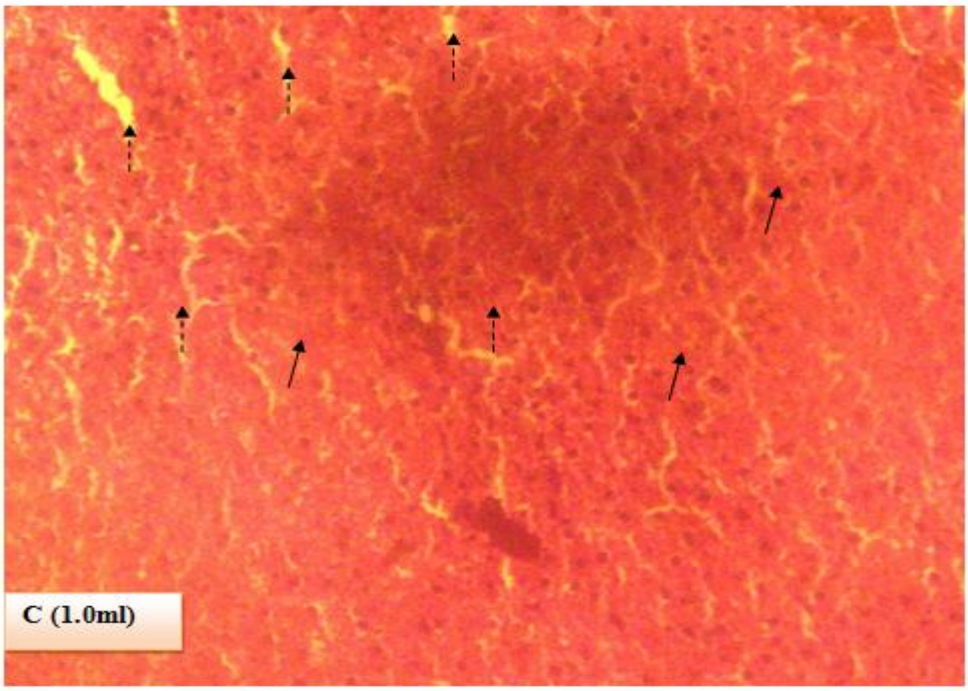

Fig 4: Photomicrograph of liver (H&E x100) treated with *B. coriacea* shows the cdilated sinusoids (**dash arrows**) with hepatocytes (**H**). Photomicrograph is not different from the control. No degeneration of hepatocytes

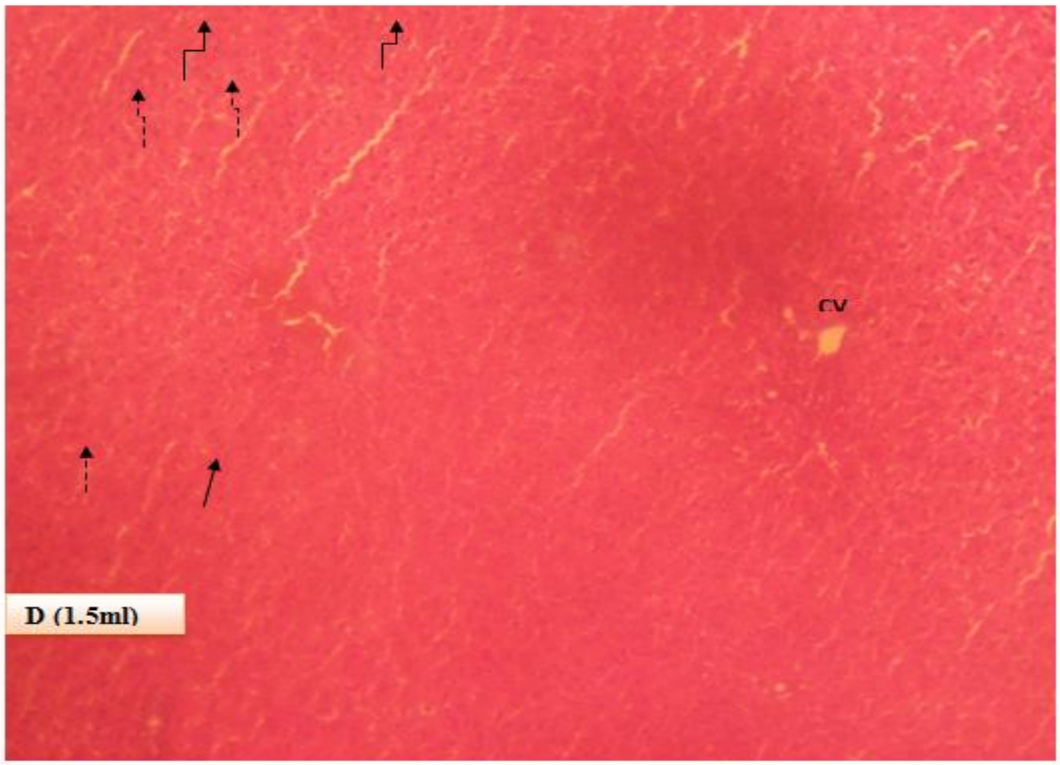

Fig 5: Photomicrograph of liver (H & E x 100) treated with *B. coriacea* Shows the central vein (**CV**), Dilated smusoids **(dash arrows**) with hepatocytes (**H**). Photomicrograph is not difference from the control. No degeneration of hepatocytes

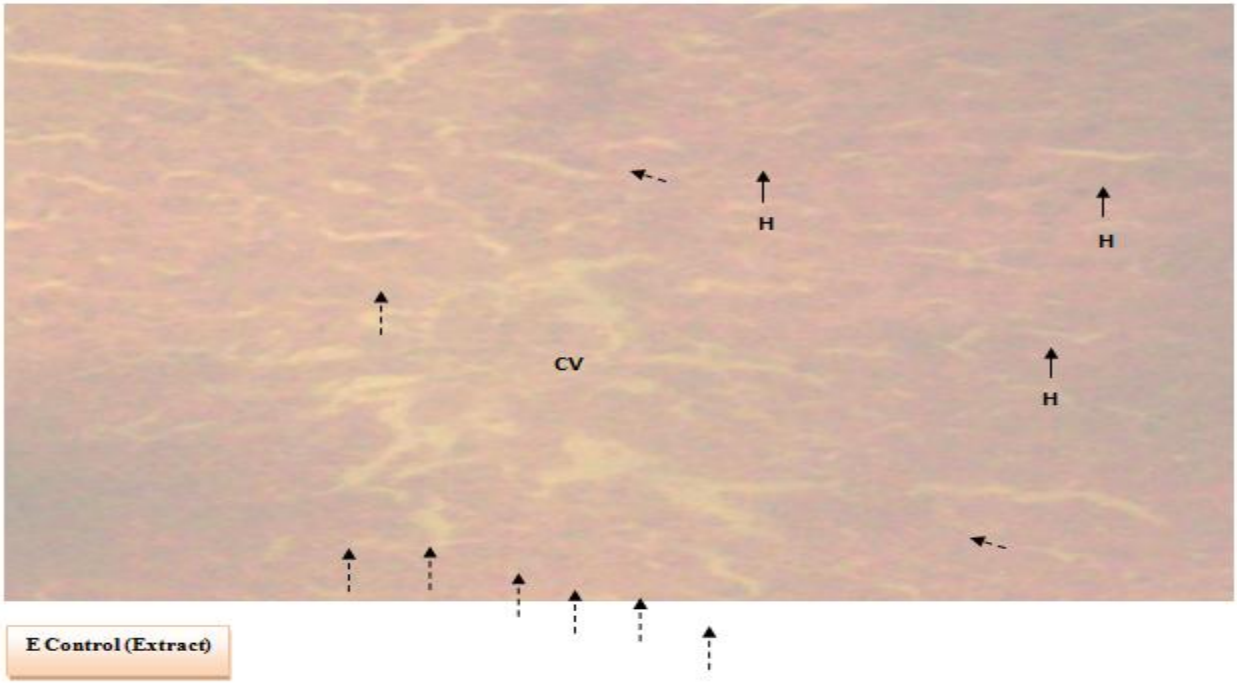

Fig 6: Photomicrograph of liver (Control extract 1ml of *B. coriacea* H&E x100):Shows normal central vein (**CV**), dilated sinusoids (**dash arrows**) with hepatocytes (**H**).

Discussion

The study sought to know the effect of methanolic extract of *Buchholzia coriacea* on liver in diabetic tissue using H & E stain. Result of the anti-diabetic activity of B. coriacea seed exerted significant ($P < 0.05$) reduction in fasting blood glucose level of diabetic rats. This findings agrees with (Chinaka *et al.*, 2012) he reported that treatment with the extract induced significant dose dependent decreases in the respective fasting blood glucose values from the initial mean value when compared with the negative control. After initiation of diabetic mellitus the histological study from the figures above shows a deposit of haematoxylin and eosinophilic materials in the micrograph.

The control (non-diabetic control) shows normal cell central vein, dilated sinusoids with hepatocytes. The induced (diabetic control) shows occluded central vein, dilated sinusoids with hepatocytes. The diabetic treated with 0.5ml of extract shows the central dilated sinusoids. Photomicrograph is not different from the control. The diabetic treated with 1.0ml of extract shows the dilated sinusoids with hepatocytes. Photomicrograph is not different from the control no degeneration of hepatocytes.

The diabetic treated with 1.5ml extract shows the central vein, dilated sinusoid with hepatocytes. Photomicrograph is not different from the control. No degeneration of hepatocytes. The control (extract 1ml of B.coriacea) shows normal cell central vein, dilated sinusoids with hepatopcytes.

Conclusion

This investigation revealed that methanolic extract of *B. coriacea* seed was tolerated by wistar rats and it possessed significant antidiabetic and antioxidant activities which effectively improve the central vein of the diabetic liver by opening the central vein.

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