EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 3
77-83
Year: 2024

HISTOLOGICAL EFFECTS OF METHANOL EXTRACTS OF OCIMUM IRVINEI LEAF (CURRY LEAF) ON THE KIDNEY AND PANCREAS OF ADULT WISTAR RATS

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Article Received on 15/01/2024 **Article Revised on 05/02/2024** **Article Accepted on 25/02/2024**

ABSTRACT

Some reports have shown that diabetes and renal diseases which stern from malfunctioning of the cells of the pancreas and kidney respectively have resulted in many deaths in recent years globally. Since Ocimum irvinei is a good medicinal plant which is richly embedded with nutritional and phytochemical constituents that confers on it the ability to maintain health and wellbeing, the study sought to evaluate the effect of administration of its methanolic leaf extract on the histology of the kidney and pancreas of both male and female albino wistar rats. 40 adult wistar rats (20 males and 20 females) were used for the study and were divided into four groups of five animals each for both the males and females. Group I in both cases served as the Control which received normal water daily while the Group II, III and IV animals also in both cases served as the Test groups which received 50mg/kg, 100mg/kg and 150mg/kg of the extract respectively by oral gavage once daily for 14 days. The animals were sacrificed at the end of the study; the kidney and pancreas were harvested, weighed and fixed with formaldehyde in preparation of histological examination. The Hematoxylin and Eosin (H&E) tissue staining techniques were used. The result obtained for the kidney revealed that the administration of methanolic leaf extract of O. irvinei showed normal kidney sections in all animals except for those in group III which showed presence of abnormal cells. The effect of the methanolic extract of O. irvinei on the histology of the pancreas showed similar result as with the kidney. At 100mg/kg, the extract was seen to result in a cell which was seen to be histologically abnormal in both males and females. The result also showed the administration of the extract did not affect renal and pancreatic functions at the doses of 50 and 150mg/kg. Further studies is recommended to elucidate the possible mechanism if its action to evaluate the plant potential value.

KEYWORDS: Wistar Rats, Histological, Phytochemical, Kidney and *Ocimum Irvinei.*

INTRODUCTION

Several systems of medicine practiced in the world today all make use of a large number of plants due to their curative properties for the treatment of human ailments (Argal, Kumar, Choudhary, Thakkar, Verma & Seniya, 2011). This is because man has been dependent on nature of which herbal medicine has proved efficacious and potent for curing various chronic diseases that orthodox medicine cannot cure (Salomi & Manimekalai, 2016). This study is to evaluate the effects of methanol leave extract of *Ocimum irvinei* on the histology of Pancreas and Kidney of male and female Wistar rats. This has since spurred a lot of studies seeking to identify plants (fruits and vegetables) which are naturally enriched with phytochemicals and bioactive compounds which serve as the essential substance needed for the treatment of several human diseases (Khaki, Fathiazad & Nouri, 2010; Sarojini & Manavalan, 2012; Neethu & Neethu, 2016). These chemical compounds which are synthesized by plants are regarded to as secondary metabolites and includes; amino acids, sugars, proteins, alkaloids, flavonoids, vitamins, tannins, terpenoids, phenolic compounds and so on (Gupta & Prakash, 2009; Krishnaiah, Devi, Bono & Sarbatly, 2009), confers on plants several beneficial effects on long-term health and the ability to perform several important biological functions as well as defend the body against attack from bacteria, fungi, parasites, pests and insects (Salomi & Manimekalai, 2016). A popularly known plant which has been used since antiquity as a herbal therapy with a wide range of medicinal purpose in the traditional system of medicine is *Ocimum irvinei* due to its rich phytochemical content with significant nutritional as well as antioxidant capabilities and health benefits (Yayasinghe, Gotoh, Aoki & Wada, 2003). It is an aromatic deciduous small herb which grows in several regions of the world and has a slight minty lemon flavour; hence it is commonly known as Lemon Basil (Klima´nkova, Holadová, Hajšlová, Čajka, Poustka & Koudela, 2008; Al-Ghurabi, 2014). It is used in medicine, cosmetology, landscape design as an ornamental scent plant, production of essential oils (Makri & Kintzios 2008; Nurzyńska-

Wierdak 2012; Aladekoyi & Orungbemi, 2016) and more essentially in the production of leaves as a culinary herb, condiment/spice in the food industry as aromatic, flavouring ingredients and food preservatives due to its strong and fragrant aroma (Javanmardi, Khalighi, Kashi, Bais & Vivanco, 2002; Muafia, Shaista & Nuzhat, 2011; Aladekoyi & Orungbemi, 2016; Takwa, Caleja, Barreira, Sokovic, Achour, Barros, & Ferreira, 2018) which is conferred on it by its rich phytochemical compounds which manifest mostly as an antioxidant due to presence of linalool, eugenol, methyl chavicol, methyl cinnamate, ferulate, methyl eugenol, triterpenoids and steroidal glycoside known to exhibit antioxidant activities (Pietschmann, Mehdipour, Atri, Hofmann, Hosseini-Asl, Scherneck & Peters, 2005; Siddiqui, Aslam, Ali, Begum & Khatoon, 2007; Zheljazkov, Callahan & Cantrell, 2008) in preventing many diseases brought about by detoxification of reactive oxygen species (ROS) (Suhaj 2006). The chemical composition of *Ocimum irvinei* is highly complex as it contains many nutrients and other biological active compounds which significantly vary with time, cultivation process and storage (Tewari, 2012).

MATERIALS AND METHODS

Sample collection

Freshly harvested leaves of Ocimum irvinei were bought on the 25th of May 2018, from Oyigbo Market. The identification was carried out at the Reference Herbarium for Research and Germplasm Conservation, Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt.

Ethical considerations

All animals used in this study were handled with the international, natural and institutional guidelines for care and use of laboratory animals in biomedical research as promulgated by the Canadian Council of Animal Care (CCAC, 1985). The procedures carried out on them were in accordance with the guidelines of Ethical Committee of the National Institutes of Health Guide for Care and use of Laboratory animals (Publication No 85-23 revised 1996).

Preparation of the ocimum irvinei leaf plant

The fresh leaves bought from the market were sorted out and left to dry until zero water content can be found in it. The dried leaves were then grounded into powdered form with the help of a blender (manual).

The fine powder was then soaked or dissolved in absolute methanol in an extraction vessel for about 72 hours during the period of maceration, and the extraction was done by the aid of a rotary evaporator. The extract was then dried in a water bath, after which it was diluted with DMSO (Dimethyl sulfoxide) before being administered to the test animals.

Collection of experimental animals

A total number of 40 rats (20 males and 20 females) weighing between 180-200g was used for this experimental research. The animals were procured from the Animal House of the Department of Physiology, in the Faculty of College of Health Science, University of Port Harcourt, and were used throughout the study.

Acclimatization of the animals

The animals were kept according to their sexes in clean and dry wire mesh cages in an environment of normal room temperature, where they were exposed to 12 hours light/dark cycle under the standard conditions, and left to adapt to their new environment for a week (7 days). During this period, they had access to water and food (standard finisher diet, Top feed, Nigeria) ad libitum, throughout the duration of the study.

The male and female rats were kept in separate cages to prevent them from mating and reproducing, since it's not part of the study being carried out.

Experimental design

After the period of acclimatization, the animals were divided into eight groups of male and female.

The extract administration was by oral gavage; carried out once daily for 14 days, while the dose of the extract used for this study was based on previous studies which used Methanol leave extract of Ocimum species in determination of several biological parameters in wistar rats. According to Saha, Ghosh, and Nathan (2012), the 100 mg/kg body weight dose is the non-toxic range; hence this study will employ ranges below the non-toxic level (50mg/kg) and above the non-toxic level (150mg/kg).

Male rats

Group 1 (Control) – Consists of 5 rats weighing between 180-200g, received only feed and distilled water daily throughout the study.
Group 2 – Consists of 5 rats weighing between 180-200g, received 0.06ml of 50mg/kg of the extract orally once a day (morning) with feed and water.
Group 3 – Consists of 5 rats weighing between 180-200g, received 0.12ml of 100mg/kg of the extract orally once a day (morning) with feed and water.
Group 4 – Consists of 5 rats weighing between 180-200g, received 0.18ml of 150mg/kg of the extract orally once a day (morning) with feed and water.

Female rats

Group 1 (Control) – Consists of 5 rats weighing between 180-200g, received only feed and distilled water daily throughout the study.
Group 2 – Consists of 5 rats weighing between 180-200g, received 0.06ml of 50mg/kg of the extract orally once a day (morning) with feed and water.

Group 3 – Consists of 5 rats weighing between 180-200g, received 0.12ml of 100mg/kg of the extract orally once a day (morning) with feed and water.
Group 4 – Consists of 5 rats weighing between 180-200g, received 0.18ml of 150mg/kg of the extract orally once a day (morning) with feed and water.

Sample Collection and Determination
The administration of the Ocimum irvinei extract lasted a period of 2 weeks (14 days) immediately after acclimatization. And at the end of the experimental administration, the animals were anesthetized using chloroform and sacrificed humanely. The organs vital to this study (Kidney and Pancreas) were harvested, weighed and fixed in plain bottles with formaldehyde in preparation for histological examination.

Hematoxylin and Eosin tissue staining techniques
After fixing the tissues with 10% formalin, they were dehydrated in an ascending series of ethanol, cleared in xylene and finally embedded in molten paraffin (melting point: 50-58°C).

A 5µm – thickened sections were cut with the aid of rotary microtome and mounted on clean glass slides. The mounted sections were then stained with Ehrlich hematoxylin and counterstained with eosin (Lillie Fulmer, 1976).

Sections were later deparaffinized and endogenous peroxidase activity was blocked by H2O2 in methanol before heating them for 20 minutes in 0.01 mol/L citrate buffer using microwave pressure cooker. Slides were left to reach room temperature and nonspecific binding was blocked with normal horse serum for 20 minutes.

Image analysis
Analysis of the digital images was obtained via semi-quantitative scoring system (Image J software, Java based application for analyzing images) in high power fields at magnification of 400X. The images were then read and analyzed for possible deviations from the normal.

RESULTS AND DISCUSSION
This chapter presents the findings of the experimental study which is based on the histology effect of methanol leaf extract of *Ocimum irvinei* on the kidney and pancreas of adult male and female albino wistar rats.

Presentation of results

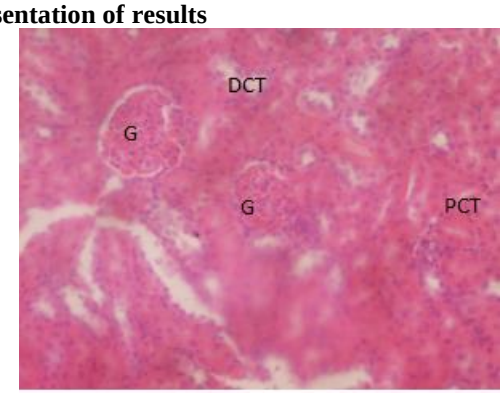

Plate 1 A: ***Histology of the Kidney of the control group (mag. x400).***

Plate 1A showed the histology of a normal (Control) adult female wistar rat kidney which is normal. The analysis shows that the glomeruli (G) contain capillaries and mesangial cells are histologically normal. The Bowman's capsule is patent while the renal tubules (DCT and PCT) are histologically normal.

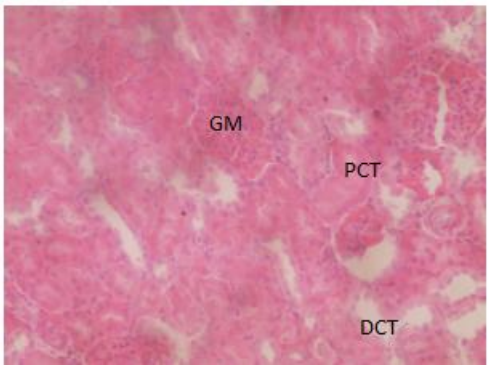

Plate 1B: ***Histology of the Kidney of group II animals (50mg/kg O. irvinei) (mag.x400).***

Plate 1B which showed that the kidney histology of the female animals administered with 50mg/kg of the methanol leaf extract of *Ocimum irvinei* were also seen to be histologically normal with presence of glomeruli (Gm) containing capillaries and mesangial cells. The Bowman's capsule was also patent while the renal tubules (DCT and PCT) were histologically good.

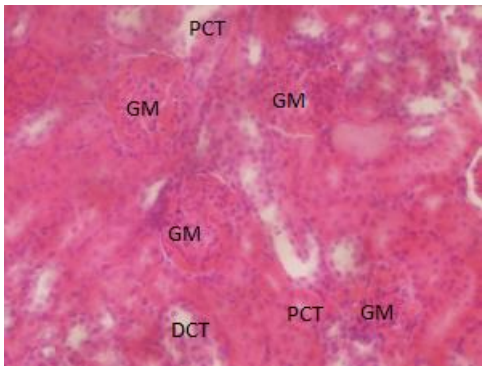

Plate 1C: ***Histology of the Kidney of group III animals (100mg/kg O. irvinei) (mag.x400).***

Plate 1C showed that the kidney histology of the female animals administered with 100mg/kg of the methanol leaf extract of *Ocimum irvinei* were histologically distorted with presence of Glomeruli (Gm) that has occluded Bowman's capsule but good renal tubules.

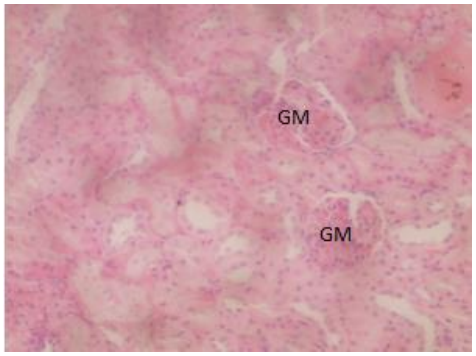

Plate 1D: ***Histology of the Kidney of group IV animals (150mg/kg O. irvinei) (mag.x400).***

Plate 1D showed that administration of 150mg/kg of the methanol leaf extract of *Ocimum irvinei* left the kidney in good shape with presence of histologically good glomeruli tuft, patent Bowman's capsule and renal tubules with good architecture.

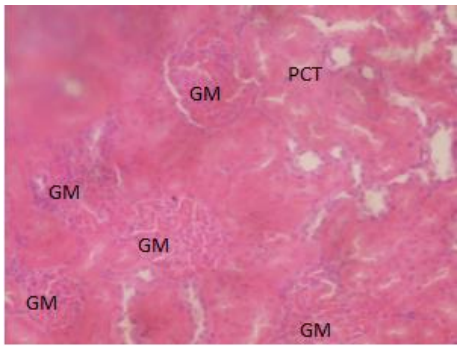

Plate 2A: ***Histology of the Kidney of the control group (mag.x400).***

Histological analysis of the Kidney of the male adult wistar rats involved in this study shows that animals in group I as seen in plate 2A has glomeruli (G) with histologically good tuft and normal renal tubules but occluded Bowman's capsule. Hence the kidney was said to be distorted.

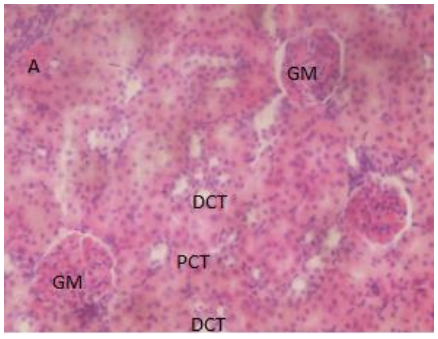

Plate 2B: ***Histology of the Kidney of group II animals (50mg/kg O. irvinei) (mag.x400).***

Animals in group II 50mg/kg of the methanolic leaf extract of *Ocimum irvinei*, on the other hand, expressed histologically normal glomeruli (Gm) with normal renal tubules (DCT and PCT) as seen in plate 2B. This conferred the section with the status of a histologically normal section.

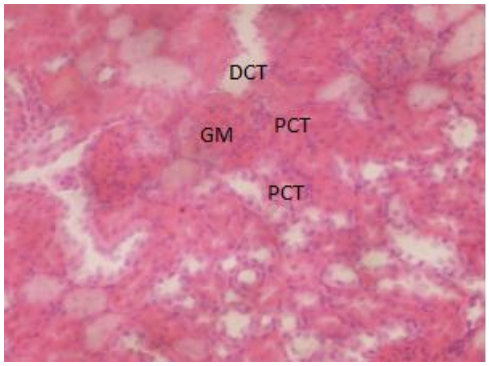

Plate 2C: ***Histology of the Kidney of group III animals (100mg/kg O. irvinei) (mag.x400).***

The histological representation of the Kidney of the animals in group III which received 100mg/kg of the methanolic leaf extract of *Ocimum irvinei* is such like a histologically destroyed section as it showed the presence of occluded Bowman's capsule but good renal tubules (Plate 2C).

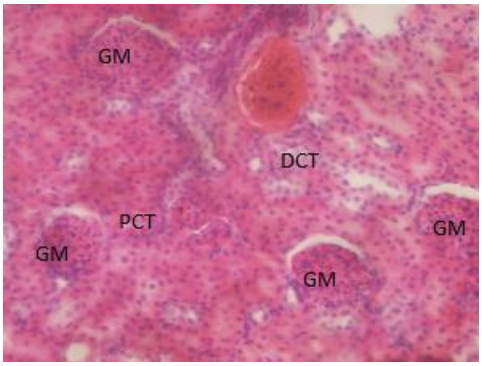

Plate 2D: ***Histology of the Kidney of group IV animals (150mg/kg O. irvinei) (mag.x400).***

According to Plate 2D which showed the histological sectionof the kidney of the male animals which received 150mg/kg of the methanolic leaf extract of *Ocimum irvinei* glomeruli (Gm) were histologically good with presence of normal renal tubules. This therefore infers that the section id histologically normal.

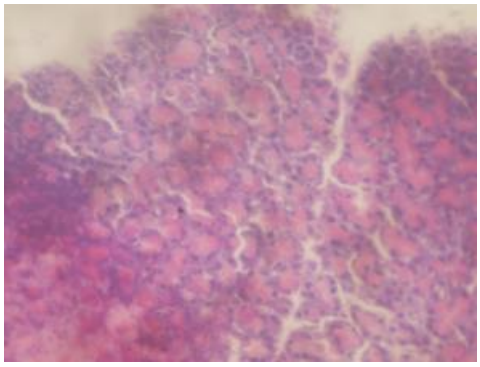

Plate: 3A Histology of the Pancreas of the control group (mag.x400).

According to the histological sectioning of the pancreas of the female animals used for the study, Plate 3A was not totally clear but the normal pancreatic acini cells were observed.

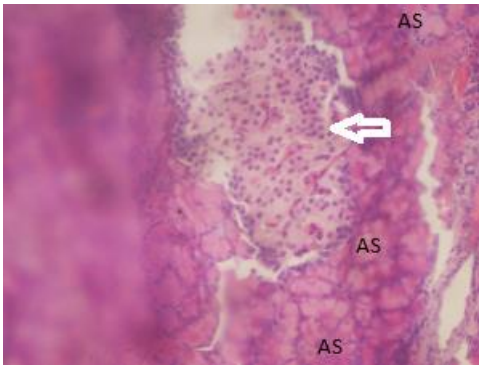

Plate 3B: *Histology of the Pancreas of group IV animals (50mg/kg O. irvinei) (mag.x400).*

Plate 3B showed presence of normal pancreatic cells (AS) and luxuriously rich pancreatic islet marked with white arrow. This goes to show that the histological section of the pancreas is normal.

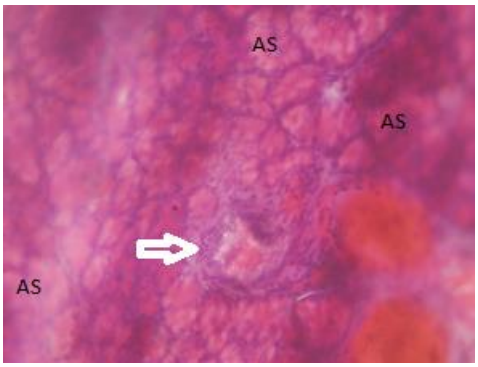

Plate 3C: *Histology of the Pancreas of group IV animals (100mg/kg O. irvinei) (mag.x400).*

Plate 3C on the other hand showed the histological presentation of the pancreas of the female animals which received 100mg.kg of the leaf extract. The analysis of the result shows that pancreatic acini cells (AS) are normal while the pancreatic islet as represented by the arrow are distorted. This is a representation of histologically distorted pancreas.

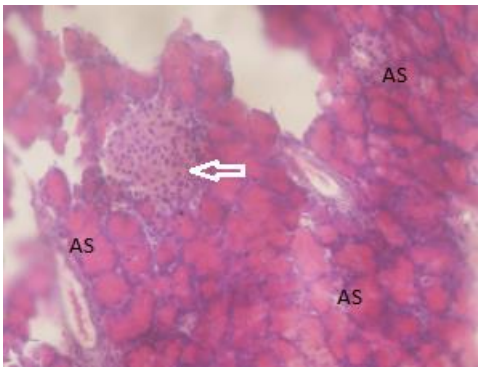

Plate 3D: *Histology of the Pancreas of group IV animals (150mg/kg O. irvinei) (mag.x400).*

From plate 4.3D, it was observed that upon administration of the 150mg/kg of the leaf extract, the animals showed normal pancreatic acini cells (AS) as well pancreatic islet (arrowed), all of which points to a histologically normal pancreatic section.

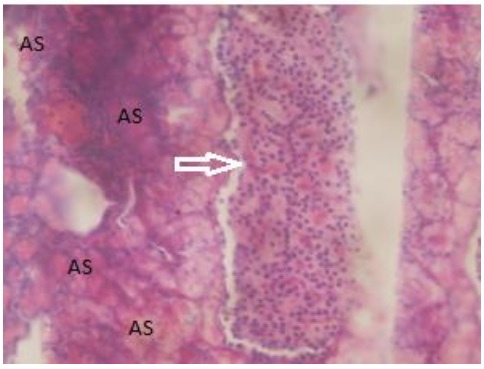

Plate 4A: *Histology of the Pancreas of the control group (mag.x400).*

On the side of the males, the histological sectioning of the pancreas as seen in Plate 4A shows a histological normal pancreatic section which has normal pancreatic acini (AS) as well as normal pancreatic islet represented with arrows.

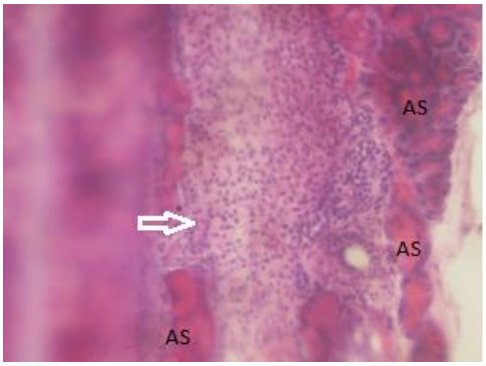

Plate 4B: *Histology of the Pancreas of group IV animals (50mg/kg O. irvinei) (mag.x400).*

In Plate 4B, the sectioning was also seen to represent a histologically normal pancreatic section with the presence of a normal pancreatic acini (AS) and normal pancreatic islets (arrowed).

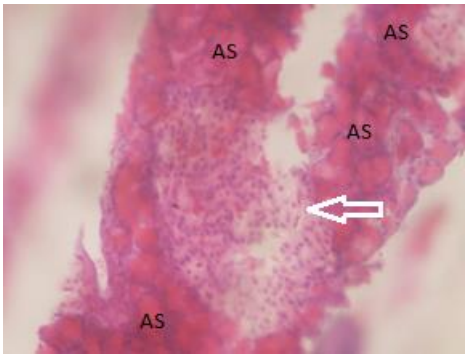

Plate 4C: ***Histology of the Pancreas of group IV animals (100mg/kg O. irvinei) (mag.x400).***

The plate 4C on the other hand showed a pancreatic section which has normal pancreatic acini (AS) and vacuolated pancreatic islet (arrowed) which makes them mildly distorted.

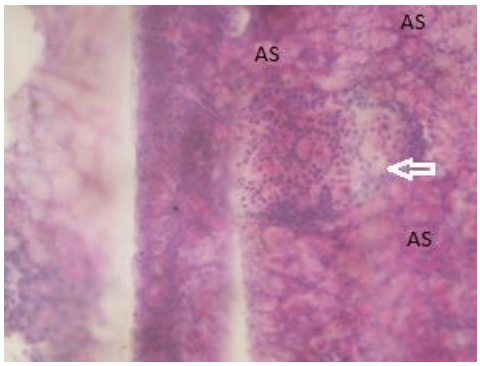

Plate 4D: ***Histology of the Pancreas of group IV animals (100mg/kg O. irvinei) (mag.x400).***

The last plate which belongs to the group of animals administered with 150mg.kg of the leaf extract shows the presence of normal pancreatic acini (AS) and pancreatic islet, thereby conferring them the status of a normal pancreatic section.

DISCUSSION

Various parts of *O. irvinei* are well-known for its application in a number of countries in the area of traditional, folk or ayurvedic medicine against several ailments, hence it has been enlisted as a medicinal plant according to the description of the World Health Organisation (WHO).

The results for the Hematoxylin & Eosin tissue staining techniques for the kidney revealed that the administration of methanolic leaf extract of *O. irvinei*, showed normal kidney sections in all animals except for those in group III which showed presence of abnormal cell. The methanol-fed group experienced kidney swelling and significantly reduced kidney function. Therefore, this further suggests that *O. irvinei*, may have little deleterious effect of the histology of the cells when administered at the dose if 100mg/kg. This deleterious effect can be caused by the presence of tiny blood vessels in the glomeruli in which the kidney can no longer remove waste and excess fluids efficiently because blood and protein cannot be filtered and are excreted in the urine. This conditions can be caused by disease, such as diabetes, lupus, infection, or drug use .This findings, is also in agreement to earlier research works done on urinary insufficiency which is a medical condition in which the kidneys fail to adequately filter waste products from the blood (Nurzynska-Wierdak & Laddha, K. 2012).

The effects of the methanolic extract of *O. irvinei* on the histology of the pancreas showed similar result as with the kidney. At 100mg/kg, the extract was seen to result in a cell which was seen to be histologically abnormal in both males and females. However, at other doses, the extract was seen to have increased the islet cells. This is formed by B cells which are responsible for producing insulin which when deficient as a result of the depletion of B cells will therefore lead to a disorder in carbohydrate, protein and fat metabolism with a resultant hyperglycaemia.

CONCLUSION

In conclusion, the present finding of the activity of the methanolic leaf extract of *Ocimum irvinei* revealed its beneficial effects in improving the pancreatic and renal histology of adult male and female rats. This lends support to the claims for its numerous applications in traditional medicine to cure various kinds of illness relating the renal system as well as its use as a hypoglycaemic agent. The result also showed that the administration of the extract did not affect renal and pancreatic function at the doses of 50 and 150mg/kg but had mild deleterious effect when the 100mg/kg of the extract was administered.

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