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Original Article

Analysis of Kras gene from induced pancreatic cancer rats administered with *Momordicacharantia* and *Ocimumbasilicum* leaf extracts

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ABSTRACT

Objective: To analyze K-ras gene from induced pancreatic cancer rats administered with *Momordicacharantia* and *Ocimumbasilicum* leaf extracts.
Methods: Twenty-five (25) adult rats weighing between 90–120 g were divided into 5 groups namely RA, RB, RC, NC and PC, each group had 5 rats. The PC which served as the control was fed with normal fish meal and water ad libitum; the NC which is the negative control received 20 mg/ml/week of Nitrosamines only while other groups received different concentrations of aqueous extract of both *M. charantia* and *O. basilicum* (200 mg, 100 mg, 50 mg) and Nitrosamine. Qualitative phytochemical screening of the aqueous extract of both *M. charantia* and *O. basilicum* was carried out. The extraction of DNA was done using Jena Bioscience DNA preparation kit and the protocol was based on the spin column based genomic DNA purification from blood, animal and plant cells. Agarose gel electrophoresis was used to analyze the K-ras gene extracted from the pancreas tissues of experimental rats while hematoxylinand eosin staining was used for histological assay.
Results: Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, saponins and glycosides in *M. charantia* while saponins, tannins and glycosides were discovered in *O. basilicum*. Significant reduction in the weight of rats treated with 200 mg of aqueous extracts of *M. charantia* and *O. basilicum* while rats that were dosed with nitrosamines only showed a slight increase in weight in the first three weeks when compared to the positive control. Histological studies revealed that there is both enlargement and reduction in the islet cell size, with one of the sections showing a normal islet cell size. While the agarose gel electrophoresis revealed that there may be possibility of prevention of damage to k-ras gene as a result of the effect of plants extract.
Conclusion: This work has shown that the leaf extracts of both *M. charantia* and *O. basilicum* will serve as a measure against induced pancreatic cancer in rats.

1. Introduction

Pancreatic cancer is one of the deadliest cancers affecting the Western world. Pancreatic cancers are more likely to exist in men than in women, and among African-Americans than among whites.[1] Smoking cigarettes increases one's risk of pancreatic cancer by a factor of 2 or 3. Even smokeless tobacco has been noted as a risk factor. Diet and obesity have also been linked to cancers of the pancreas. People who do not exercise much and who are obese are more likely to develop pancreatic cancer.[2] In addition, those who eat diets low in vegetables and fruits and high in red meat and fat are more likely to be diagnosed with the disease.[3] Alcohol consumption is also considered a risk factor for pancreatic cancer. Long term, heavy drinking leads to chronic pancreatitis, which is a known risk factor for pancreatic cancer.[4]

The disease is highly metastatic and difficult to diagnose until late stages, the 5-year survival rate is around 5%.[5] In the United States, the rate of mortality is nearly equal to the rate of new diagnoses.[6] Early disease detection is rare, and of those patients diagnosed with early-stage disease, only 20% are candidates for surgical resection. Approximately 50% of patients develop highly metastatic disease, for which current treatment regimens provide

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little increase in longevity.[7] Pancreatic cancer carries an extremely poor prognosis, with 90% of pancreatic cancers being malignant and the 5-year survival rate after diagnosis hovering at 5%. Less than 20% of patients are candidates for surgical resection; therefore, chemotherapy and radiation therapy (RT) remain the only other treatment options.[8] In the United States each year, over 30,000 people are diagnosed with pancreatic cancer. Europe sees more than 60,000 diagnoses each year. Because pancreatic cancer is usually diagnosed late into its development, the five-year survival rate after diagnosis is less than 5%.

Pancreatic cancer and cancer related diseases have been treated using surgery, chemotherapy, and radiation therapy, or a combination of these. But despite these options, cancer remains associated with high mortality.[9] This is basically due to difficulties in early diagnosis, exorbitant cost of treatment. Owing to these several shortcomings, there is need for other therapeutic options which will increase the chances of survival of pancreatic cancer patients with minimal or no side effects of treatment.

Cancer of the pancreas is a genetic disease. Sporadic cancers of the pancreas are frequently associated with the activation of an oncogene, K-ras, and the inactivation of multiple tumor suppressor genes, including p53, DPC4, p16, and BRCA2.[10] Inactivation with a variety of tumor-suppressor genes such as p53, p16, and DPC4, and genome-maintenance genes such as BRCA2, coupled with the activation of oncogenes such as K-ras, are a few of the mutations that trigger the growth of cancerous cells. The genetic profile of pancreatic cancer has reshaped the nomenclature describing histological progression in pancreatic ductal tumorigenesis. K-ras mutations frequently occur early, whereas changes in the expression and genetic integrity of the p16 gene appear in intermediate lesions, and the inactivation of the p53 and DPC4 genes and activation of telomerase occur late in the neoplastic progression. Although the majority of pancreatic cancers occur sporadically, a minority has been shown to aggregate in families and has aided our understanding of pancreatic tumorigenesis.

The RAS gene family is among the most studied and best characterized of the known cancer-related genes. Of the three human ras isoforms, KRAS is the most frequently altered gene, with mutations occurring in 17–25% of all cancers. KRAS was initially identified in a human lung cancer cell in 1982 and, since then has been shown to be mutated in 35–50% of all non-small cell lung cancers.[11] Although a common mutation in cancer, KRAS has been difficult to therapeutically target. A better understanding of this gene, as well as its interactions with other genes and mutations, has recently revealed its potential prognostic and predictive roles in tumor aggressiveness and patient outcomes.

RAS is the name given to a family of related genes that encode a class of 21 kD membrane-bound proteins that bind guanine nucleotides and have intrinsic GTPase activity. The first two RAS genes, HRAS and KRAS, were identified in 1975 from studies of two cancer-causing viruses, the Harvey sarcoma virus and Kirsten sarcoma virus, by Scolnick et al. at the National Institutes of Health [NIH].[12] The human analog of this gene was subsequently discovered in 1982 and has been intensely studied and implicated in the pathogenesis of many cancers. KRAS is most frequently mutated in cancer of the three known human RAS genes.[13]

The KRAS gene encodes a 188 amino acid protein that has inherent catalytic activity. Post-translation modification of this protein facilitates its localization to the cell membrane. Normally, ras proteins exist in an inactive state in any given cell. All members of the ras family become activated when a nearby transmembrane receptor (e.g., growth factor receptors, G-protein coupled receptors, toll-like receptors, etc.) is bound by its corresponding ligand. The subsequent intracellular signal cascade involves guanine exchange factors (GEF) which facilitate the activation of ras by replacing the inactive GDP with GTP. Once activated, ras leads to the downstream activation of a wide variety of effectors including serine/threonine kinases, GTPase-activating proteins (GAPs), phosphoinositide 3-kinase (PI3K), and GEFs. Ras is deactivated when the GTP molecule is converted back to a GDP molecule. If KRAS is mutated, it remains in the GTP state. Therefore, KRAS remains in a constitutive GTP-bound state and, thus, regulation of downstream functions is lost. For example, the dysregulated GTP-bound activation of mutant-derived KRAS protein leads to unregulated downstream cell-growth.

Traditional medicine which involves the use of herbs has been used to treat various types of cancer and this has been found to be effective and the same time presenting minimal or no side effect.[14] Plants have produced many anticancer drugs such as taxanes and vincristine and still serve as a veritable source of new products through the use of standard bioassay methods.[9] Examples of plants and herbs use for cancer treatment includes *Momordica charantia*, *Ocimum basilicum*, *Annona muricata*, Pawpaw leaf, *Caspium frutescens*, *Ananas comosus* (Pineapple), *Allium cepa* (Onion), *Allium sativum* (Garlic), *Chenopodium ambrosioides* (Worm wood), *Bryophyllum pinnatum* (Resurection plant or Life plant), *Vernonia amygdalina* (Bitter leaf) and others.[15] However, the anti-cancer properties of *M. charantia* and *O. basilicum* is not yet fully elucidated scientifically.

M. charantia, known as bitter melon, bitter gourd, bitter squash, or balsam-pearis a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit, which is extremely bitter. This *herbaceous*, tendril-bearing vine grows up to 5 m (16 ft) in length. It bears simple, alternate leaves 4–12 cm (1.6–4.7 in) across, with three to seven deeply separated lobes. Each plant bears separate yellow male and female flowers. *M. charantia* has a number of purported uses including cancer prevention, treatment of diabetes, fever, HIV and AIDS, and infections.

Basil Ocimum is a common name for the culinary herb *O. basilicum* of the family Lamiaceae. It is a half-hardy annual plant, best known as a culinary herb. Scientific studies *in vitro* have established that compounds in basil oil have potent antioxidant, antiviral, and antimicrobial properties, and potential for use in treating cancer.[16–19]

In order to proffer a possible better, cheaper means of preventing pancreatic cancer in individual who are liable to suffering the disease in their life time, this work was carried out with the aim of evaluating the chemo preventive effect of extract of *M. charantia* and *O. basilicum* leaves on Nitrosamines induced pancreatic cancer in rat.

This study was therefore carried out with the following specific objectives: -

1. To evaluate the qualitative phytochemical component of aqueous leaf extract of *M. charantia* and *O. basilicum*.
2. To evaluate the effects of an aqueous leaf extract *M. charantia* and *O. basilicum* on the weight trend of experimental animal over a period of six weeks.
3. To study the effects, the phytochemicals of these leaf extracts can have on the chemoprevention of pancreatic cancer.
4. To analyze the Kras gene by PCR-RFLP from pancreas tissues of Nitrosamines induced pancreatic rat.

2. Materials and methods

2.1. Animal and experimental design

The twenty-five (25) adult rats used for this study were obtained from Nigerian Institute of Medical Research, Lagos, Nigeria. The

animals were housed in standard clean rat cages at 25 °C, fed with standard pellet and tap water *ad libitum*. They were maintained under uniform conditions of natural photo period (12 h' light/dark cycle), and humidity (61–95%). Experiment were carried out in the animal house of the University of Lagos, Lagos, Nigeria in accordance with the rules in Nigeria governing the use of Laboratory animals as acceptable internationally (WMA Helsinki Declaration, 2008).

At the commencement of the experiment, twenty-five (25) adult rats weighing between 90–120 g were divided into 5 groups namely RA, RB, RC, NC and PC, each group had 5 rats. The experimental groups received different concentrations of aqueous extract of both *M. charantia* and *O. basilicum* prepared with respect to the LD_{50} result as documented by Arthur et al.[20] The rats were given increasing doses of the extracts.

Group RA: Rats that received 20 mg/ml/week of Nitrosamines + 200 mg/day of extract.

Group RB: Rats that received 20 mg/ml/week of Nitrosamines + 100 mg/day of extract.

Group RC: Rats that received 20 mg/ml/week of Nitrosamines + 50 mg/day of extract.

Group NC (Negative Control): Rats that received 20 mg/ml/week of Nitrosamines only.

Group PC (Positive Control): Rats that received distilled water only.

The Nitrosamines and extracts were administered intragastrically by gavage using a cannula fitted to a feeding needle. Treatment of animals lasted for 6 weeks. The experimental and control animals were carefully checked daily and weight taken weekly. The rats were sacrificed at the end of the sixth week by cervical dislocation. The pancreas tissues from each animal were sliced off using a surgical blade. They were fixed in ethanol for agarose gel electrophoresis.

2.2. Plant preparation and extraction

M. charantia and *O. basilicum* leaves were washed in a running tap water and sun dried. The dried leaves were later blended using electric blender. The leaves extract was obtained using Crude Extraction Protocol. About 100 g of both *M. charantia* and *O. basilicum* were soaked in distill water for 72 h. This was later filtered using a sieve. The filtrate was collected and store in refrigerator. This filtrate was used for experiment and phytochemical screening.

2.3. Primer

The primer sequence was obtained from the work of Salek et al.[21] The primer was designed for Kras and mutation in pancreatic cancer. The sequence of the forward and reverse primers is 5′-atgactgaatataaacttgtg-3′ and 5′-FL-[gc]-cctctattgttggatcatattc-3′, respectively.

2.4. Phytochemical composition analysis

The phytochemical screening of aqueous extract of *M. charantia* and *O. basilicum* leaves was carried out using standard procedure in accordance with.[22]

1. **Test for Flavonoids**: 5 ml of dilute ammonia was added to portion of an aqueous filtrate of the extract. Concentrated H_2SO_4 (1 ml) was later added. A yellow coloration that disappears on standing indicated the presence of flavonoids.
2. **Test for Saponins**: To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.
3. **Test for tannins**: About 0.5 g of the extract was boiled in 10 ml of water in a test tube and the filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.
4. **Test for alkaloids**: About 0.5 g of extract was diluted to 10 ml with acid, alcohol, boiled and filtered. To 5 ml of the filtrate was added to 2 ml of dilute ammonia, 5 ml of ammonia was added and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.
5. **Test for cardiac glycosides (Keller–Killiani test):** To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under laid with 1 ml of concentrated H_2SO_4 acid. A brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

2.5. Polymerase chain reaction (PCR)

Polymerase chain reaction was carried out to amplify the K-ras gene of the DNA using the primer pair K-ras 1 (5′-atgactgaatataaacttgtg-3′), K-ras 2 (5′-FL-[gc]-cctctattgttggatcatattc-3′) and KR up (aggcctgctgaaaatgactg), KR down (tcaaagaatggtcctggacc) and KR up′ (actgaatataaacttgtggtagttggacct). The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. PCR was performed in 20 μl of a reaction mixture, and the reaction concentration was brought down from 5x concentration to 1X concentration containing 1X Blend Master mix buffer Buffer (Solis Biodyne), 1.5 mM MgCl2, 200 μM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 20 pMol of each primer (Inqaba Biotech), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), Proofreading Enzyme, 5 μl of the extracted DNA, and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in an PTC 100 Peltier thermal cycler (MJ Research) for an initial denaturation of 95 °C for 5 min followed by 35 amplification cycles of 30 s at 95 °C; 1 min at 61.8 °C and 1 min 30 Seconds at 72 °C. This was followed by a final extension step of 10 min at 72 °C.

2.6. Electrophoretic analysis

The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80 V for 1 h 30 min. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder (Solis Biodyne) was used as DNA molecular weight marker.

2.7. Histological determination

For microscopic evaluation, pancreas tissues were fixed in a fixative (10% formal saline) and embedded in paraffin section at 4–5 μm and subsequently stained with hematoxylin/eosin. Sections were studied under the light microscope at 40 and 100 magnifications. Slides of all the treated groups were studied and photographed.

2.8. Statistical analysis

Statistical analysis was carried out using SPSS version 23. Tool used was One Way Analysis of Variance (ANOVA) and means were

compared. Significant differences between the treatment means were determined at 5% confidence level using the Duncan's Multiple Range Test (Duncan, 1955).[23]

3. Results

Table 1 shows the result obtained for the qualitative phytochemical screening of the aqueous extract of *M. charantia* leaves. The phytochemical screening revealed that alkaloids, tannins, flavonoids, saponins and glycosides were present. Table 2 shows the result obtained for the qualitative phytochemical screening of the aqueous extract of *O. basilicum* leaves. The phytochemical screening revealed that tannins, saponins and glycosides were present while alkaloids, flavonoids were absent.

Table 3 shows the effect of aqueous extract of *M. charantia* and *O. basilicum* leaves on the weight trend of experimental animals over a period of 6 weeks. There was a progressive increase in the weights of rats that were neither administered with the extract (PC) nor Nitrosamine after the first two weeks. Rats that were dosed with Nitrosamine only (NC) had a progressive increase in weight for the first three weeks but had a sharp decrease in weight afterwards. However, rats that were given 200 mg (RA) of extracts maintained approximately the same weight for the first three weeks and experienced decrease in weights afterwards. Rats dosed with 100 mg (RB) showed increase in weights in the first three weeks before decline in weights afterwards while the rats that received 50 mg of extract showed an uneven weight trend.

The effects of aqueous extract of *M. charantia* and *O. basilicum* leaves on the survival rate of rats induced with pancreatic cancer using Nitrosamine is shown in Fig. 1. No death was recorded in any group in the first 4 weeks. The highest number of deaths was recorded in RA in the fifth week. Only one death was recorded in PC throughout the 6 weeks of this experiment.

Plate 1a and b are the electrophoretic band of the K-ras genes obtained from the pancreas tissue of Nitrosamine induced pancreatic cancer in rats the result as shown in Plate 1b indicate that there was no amplification. There is an amplification at sample number 4,9 and 11 at different DNA base pairs respectively, this amplification was seen on rat samples from groups NC, RA and RB induced with Nitrosamine and administered with 200 mg, 100 mg of *M. charantia* and *O. basilicum*. The histological section of pancreas tissue of induced pancreatic cancer of rats that were administered with 100 mg of extract is shown in Plate 5. The section reveals a normal sized Islet cells. Plate 6 shows the histological section of pancreas tissue of induced pancreatic cancer of rats that were administered with 50 mg of aqueous extract of *Momordica charantia* and *O. basilicum* which reveals a reduction an islets cell size (Plate 2–4).

4. Discussion

Cancer of the pancreas is a genetic disease. Sporadic cancers of the pancreas are frequently associated with the activation of an oncogene, K-ras, and the inactivation of multiple tumor suppressor genes, including p53, DPC4, p16, and BRCA2.[10] Although the majority of pancreatic cancers occur sporadically, a minority has been shown to aggregate in families and has aided our understanding of pancreatic tumorigenesis. An improved understanding of the genetics of pancreas cancer should lead to new tests to screen for this disease and novel rational gene-based therapies.[24] However, treatment with chemotherapeutic agent in preventing the development of the disease is clearly not an acceptable course of therapy because of the general nature of the agents used for this purpose. Because of bioactive compounds in certain plants and herbal mixtures, it is conceivable that such preparations could offer therapeutic benefits to patients if the efficacy and lack of toxicity are demonstrated. The concept put forward by researchers is the fact that anticancer activity of compounds which are typically present in plants at sub-pharmaceutical doses could synergize to delay or disrupt the development of aggressive disease.[25,26] The effect of aqueous extract of *M. charantia* and *O. basilicum* leaves extract on Nitrosamine induced pancreatic cancer in rats was evaluated. Nitrosamine is well-known potent carcinogen which is known to cause cancer of the pancreas.

Phytochemical analysis of the result presented in this study confirm the presence of constituents such as alkaloids, tannins, flavonoids, saponins and glycosides (Tables 1 and 2); which are known to exhibit remarkable as well as physiological activities showing specific mode of action. Studies have shown that tannins, glycosides and flavonoids possess anticancer activities.[27,28] The presence of tannin determined qualitatively could have been responsible for the preventive properties of aqueous extract of *M. charantia* and *O. basilicum* against induced pancreatic cancer. Flavonoid exerts its anticancer activity by inhibiting cyclo-oxygenase − 2 (COX2) in colon cancer cells.[29]

The active compound present in *M. charantia* extract responsible for its anticarcinogenic effect is known as charantin.[30] Charantin is a non-nitrogenous neutral substance with a melting point of 272 °C, which according to literature is a mixture of β sitosterol glucoside and 5, 25-stigmastadien-3-β-ol glucoside. The leaves are used to reduce the level of blood sugar and it also show effect on diabetics and likewise have antimicrobial activity. *O. basilicum* active ingredient is the essential oil which arise from a variety of components including linahol, 1, 8-cineole, estragole, and eugenol.[18,31,32] Essential oils have been used medicinally in history. Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purposes. Various mechanisms involved in cancer treatment are activation of detoxification enzymes, modulation of DNA repair signaling, antimetastasis, and antiangiogenesis. Multiple pathways are involved in the antiproliferative activity demonstrated by the essential oils in the cancer cells and essential oils are even effective in reduction of tumors in animal models.[33]

Nevertheless, the presence of alkaloids, tannins, flavonoids, saponins and glycosides confirms the earlier findings of Bakare et al[34] who reported the presence of these components in the Nutritional

Table 1
Qualitative analysis of aqueous extract of *Momordica charantia* leaves.

S. No	Phytochemical components	Aqueous extract
1.	Alkaloids	+
2.	Tannins	+
3.	Flavonoids	+
4.	Saponins	+
5.	Glycosides	+
6.	Phenols	–

(+) = Presence of phytochemical; (−) = Absence of phytochemical.

Table 2
Qualitative analysis of aqueous extract of *Ocimum basilicum* leaves.

S. No	Phytochemica components	Aquoeus extract
1.	Alkaloids	–
2.	Tannins	+
3.	Flavonoids	–
4.	Saponins	+
5.	Glycosides	+
6.	Phenols	–

(+) = Presence of phytochemical; (−) = Absence of phytochemical.

Table 3
Effects of aqueous extract of *Momordica charantia* and *Ocimum basilicum* leaves on the weight trend of Nitrosamine induced pancreatic cancer in rats for six weeks.

Week	RA (g)	RB (g)	RC (g)	NC (g)	PC (g)
1	106.18 ± 5.60[a]	102.12 ± 3.71[a]	97.74 ± 3.75[a]	93.80 ± 4.22[a]	97.64 ± 2.24[a]
2	111.99 ± 5.45[b]	112.6 ± 1.12[b]	112.40 ± 4.96[b]	109.80 ± 5.39[b]	110.80 ± 2.69[b]
3	113.02 ± 5.36[c]	114.01 ± 1.20[c]	114.26 ± 5.18[b]	114.30 ± 0.34[c]	111.20 ± 2.75[b]
4	110.20 ± 3.00[b]	96.80 ± 1.53 [d]	101.20 ± 3.15[c]	107.20 ± 3.67[b]	104.20 ± 3.69[c]
5	107.67 ± 2.85[a]	93.00 ± 0.91[e]	100.50 ± 3.43[c]	102.50 ± 3.38[d]	103.50 ± 3.43[c]
6	105.50 ± 0.50[a]	90.00 ± 0.58[f]	97.25 ± 3.20[a]	102.33 ± 0.84[d]	103.25 ± 3.63[c]

Results are represented as Means ± SEM weight of animals.
Values with different superscript between groups p > 0.05 are considered significant.

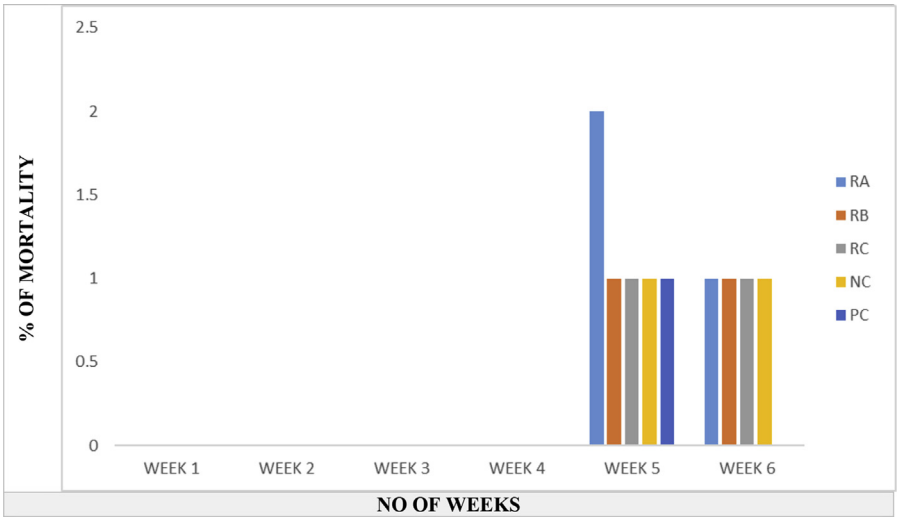

Fig. 1. Effects of aqueous extract of *Momordica charantia* and *Ocimum basilicum* leaves on the survival rate of Nitrosamine induced pancreatic cancer in rats.

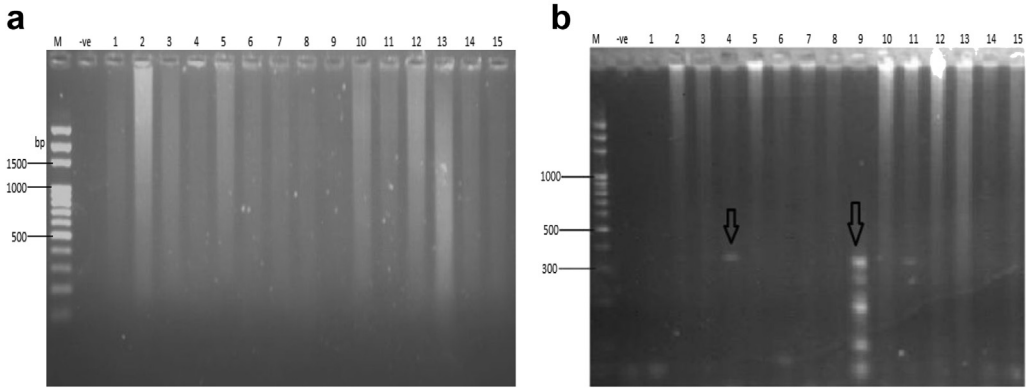

Plate 1. a: Electrophoretic bands of k-ras gene from the pancreas tissues of Nitrosamine induced pancreatic cancer in rats using Kras primer. b: Electrophoretic bands of k-ras gene from the pancreas tissues of Nitrosamine induced pancreatic cancer in rats using KR up primer.

and chemical evaluation of *M. charantia*. This is likewise in agreement with the study of Daniel et al[35] on the Phytochemical Analysis and Mineral Elements Composition of *O. basilicum*.

The results as shown in Plate 1b indicate that there was no amplification in the Kras gene located in the pancreas tissue of rats 1–3, 5–8, 10,12–15, this is in contrast with Upadhyaya et al (2009)[36] that nitrosamine-derived electrophiles react with nucleophilic centers in DNA to yield a variety of products including O^6-methylguanine which causes miscoding of DNA during replication, this miscoding can lead to damage of Kras gene, the cause of the non-DNA damage in the gene could be as a result of the chemopreventive potential of the leaves extracts of *M. charantia* and *O. basilicum*. The amplification at sample number 4,9 and 11 (Plate 1b) at different DNA base pairs respectively, from groups NC, RA and RB induced with Nitrosamine and administered with 200 mg, 100 mg of *M. charantia* and *O. basilicum*, might be due to damage to the DNA by the NNK, this supports the claim by Hecht et al[37] that exposure to NNK undergo a series of steps uptake, metabolic activation, and DNA and protein adduct formation which subsequently leads to altered growth kinetics in the target organ and development of neoplasia.

The reduction in the weight of animals that took 200 mg of extract (Table 3) indicates the possible toxicity of the leaf extracts. This extract may have indeed metabolized to a toxic end point

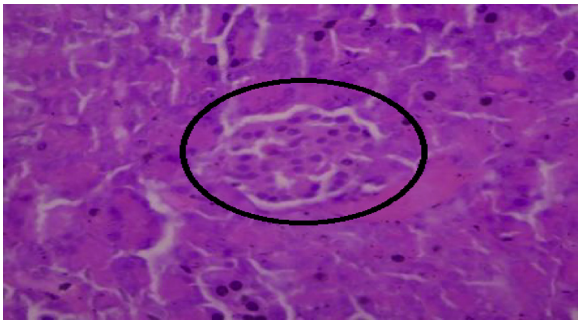

Plate 2. Histological section of pancreas tissue of induced pancreatic cancer in rats from PC. PC: Rats that received distilled water only.

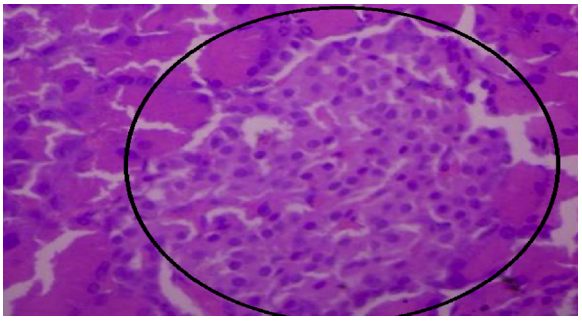

Plate 3. Histological section of the pancreas tissue induced with pancreatic cancer in rats from NC. NC (Negative Control): Rats that received 20 mg/ml/week of Nitrosamine only.

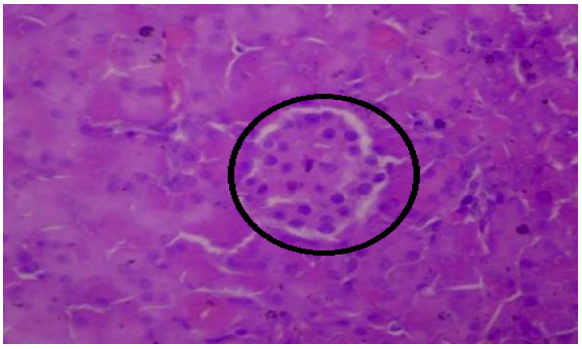

Plate 4. Histological section of the pancreas tissue of induced pancreatic cancer in rats from RA. RA: Rats that received 20 mg/ml/week of Nitrosamine + 200 mg/day of extract.

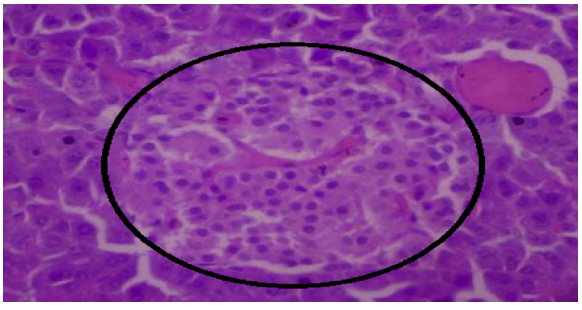

Plate 5. Histological section of the pancreas tissue of induced pancreatic cancer in rats from RB. RB: Rats that received 20 mg/ml/week of Nirosamine + 100 mg/day of extract.

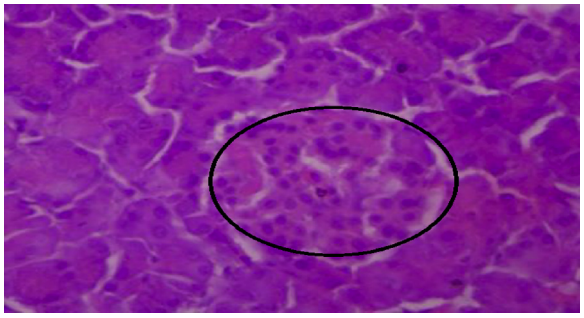

Plate 6. Histological section of pancreas tissue of induced pancreatic cancer in rats from RC. RC: Rats that received 20 mg/ml/week of Nitrosamine + 50 mg/day of extract.

product which thereby interfere with gastric function and decreased food conversion efficiency. The increase in weight in the negative control group within the first three weeks and subsequent decrease (Table 3), could be as a result of the action of the immune system. The immune system of the mice might have acted against the carcinogen within the first three weeks before it was finally overpowered.

The present study showed the aqueous extract of *M. charantia* and *O. basilicum* leaves can be used as a preventive measure against Nitrosamine induced pancreatic cancer in rats. Agarose gel electrophoresis showed that the plant extracts acted in such a way as to reduce Nitrosamine induced damage to Kras gene to some extent. Nevertheless, further studies and more research need to be done to optimize the quality of extracts, effective dose and its specificity on the Kras gene mutated in pancreatic cancer.

Conflict of interest

The author declares that there is no conflict of interest with any organization or individual on the work.

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