CIBTech Journal of Bio-Protocols ISSN: 2319 – 3840
An Open Access, Online Journal Available at http://www.cibtech.org/cjbp.htm
2018 Vol. 7(2) May-August/ pp.4-12/Okwari et al.
Research Article

EFFECT OF *MORINGA OLEIFERA* ON GASTRIC, HAEMATOLOGICAL AND HAEMOSTATIC PARAMETERS OF DIET FOLLOWING ISONIAZID -INDUCED TOXICITY IN FEMALE WISTAR RAT

[1]Okwari, O O, [2]Nwankwo A A, [1]Nkanu E.E, [3]Dasofunjo K[1] and Ededem E E
[1]Department of Physiology, Faculty of Basic Medical Sciences, Cross River University of Technology, Okuku, Nigeria
[2]Department of Human Physiology, School of Medicine and Applied Sciences, Abia State University, Uturu, Nigeria
[3]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Cross River University of Technology, Okuku, Nigeria
**Author for Correspondence:dasokay22@gmail.com*

ABSTRACT
The effect of *Moringa oleifera* diet on some gastric, haematological and hemostatic markers was studied in rats following INH- induced toxicity. The transit time, basal acid output (BAO) as well as peak acid output (PAO) in INH (LD) + MO - diet, and INH (HD) + MO – diet groups were significantly reduced compared with INH group. Ulcer scores and stomach mucus content increased ($P < 0.05$) significantly compared with INH or control. Red blood and white blood cell counts haemoglobin content, haematocrit and mean cell volume in the groups administered MO – diet were significantly ($P<0.05$)increased compared with INH group. Bleeding time, clotting time and prothrombin time in *Moringa oleifera* group were significantly decreased while platelet count were increased. *Moringa oleifera* diet might be useful in diarrheal state. Increased red blood cell, white blood cell and platelet count point to the diets suggest its haematopoietic effect .

Key words*: Isoniazid, Moringa oleifera, Transit time, BAO, FBC.*

INTRODUCTION
Tuberculosis (TB), is one of the most prevalent communicable disease, is transmitted by coughing and sneezing (Namdar and Peloquin, 2000).TB flourishes where there is poverty, large urban dwellers, chronic debilitating illnesses and in those having close contact with patients with active pulmonary TB (Peloquin, 2003). The reemergence of TB has been accompanied by a marked increase in drug resistant forms particularly in the developing countries where it is closely linked to inadequate treatment. TB cure is possible but it may be prolonged with less effective expensive therapies thus driving people in rural areas to resort to plant medicine.

It has been reported that Isoniazid (INH), a first line action TB drug is well absorbed when administered orally but intestinal absorption may be longer or incomplete when taken with food or anti acid (Walter *et al.,* 2014). The absorptive capacity of the intestinal mucosa sometimes alters in some clinical cases and INH is among other drugs that show reduced intestinal absorption in man. Extra pulmonary TB, common with HIV-positive individuals can present a wide range of symptoms and signs and can affect any part of the gastro intestinal tract. INH-induced hepatotoxicity has been reported by Lewis and Satya, (2006) in human. The effect of syngonuim leaf aqueous extract on oxidative stress and hepatic biomarkers in INH-induced toxicity in rat has been shown by Shushank et al,(2014) while Pal *et al*., (2006) reported the effect of garlic on INH and Rif induced hepatic injury in rat.

MO has enormous medical potentials (Mbikey, 2012, Farooq, 2007, Fahey 2005), Chemical compounds isolated from *Moringa oleifera* contain useful pharmacological properties with protective medicinal application (Anwar *et al*., 2007; Guevara *et al*., 1999; Caceres *et al.*, 1991).Therefore, since there are dearth of information on the effect of *Moringa oleifera* based diet on gastric, haemostatic and

haematological parameters of rat following Isoniazid induced toxicity. This work examines the effect of *Moringa oleifera*-diet following Isoniazid (INH) co-administration in the rat.

MATERIALS AND METHODS

Plant Material and Preparation of Diet

MO leaves were obtained from farms in Okuku in Yala Local Government Area, Cross River State Nigeria. The leaves were washed to remove sand and debris and air dried for 14 days to constant weight.They were subsequently pulverized to snuff-like powder. Ten (10)g of the powder were mixed with 90 grams of Rat chow Pfizer, (Aba, Imo state Nigeria-) to make *Moringa oleifera* diet (MO-diet).

Experimental Design

Seventy five (75) albino Wister rat weighing 200-250kg were obtained from the Animal House of the Department of Physiology, Faculty of Basic Medical Sciences Cross River University of Technology Okuku. After 7 days acclimatization and Clearance obtained from the Faculty Ethical Committee, the animals were weighed and randomly assigned to five (5) groups of fifteen rats each. They were house under standard conditions, maintenance on 12h light /dark cycle at $28^{o}C \pm 2^{o}C$ and humidity, and had free access to food and water. Animals in Group A received normal feed and water served as control, Group B animals received normal feed and water with Isoniazid (1mg/100g), Group C animals received 1mg/100g INH (LD), Group D animals received 1.2 mg/100g INH (HD) MO-diet. Group E received MO-diet only. All drug administration was done orally for 28 days. The animals were fasted 12h before time experiment.

Sources of Isoniazid

Isoniazid was purchased from BEZ Pharmaceutical Store in Calabar, Cross River State, Nigeria.

Gastric Acid Secretion

Albino Wistar rats (200-250g) were prepared for the measurement of gastric acid secretion by perfusion method described by Ghosh and Schild (1958) and modified by Osim *et al*, (1991). Briefly, rats were starved for 24 hours preceding the start of the experiment but were allowed free access to water. 6ml/kg of 25% solution of urethane (Sigma, UK) given interperitoneally to anaesthetize the rat. The trachea was located and cannumulated. An infusion tube 75cm and 3mmdiameter connected a 60ml syringe carried by a pump was passed to the stomach through the oesophagus. A ligature to stop back flow was made round the oesophagus in the neck. The abdomen was opened along the liana alba to minimize bleeding. The small intestine was reached and a semi-transection of 1-2cm away from the pylorus was made and a fistula 8cm passed into the stomach through the pyloric sphincter and knotted. The stomach lumen was perfused saline (pH 7) $37^{o}C$ at the rate of 1ml/min using a perfusion. The perfusate was collected every 10minute interval and titrated with 0.01N NaOH (AR, England) solution in a 25ml burette using phenolphthalein as indicator with pink coloration for the end point. The pH of saline was maintained by passing the perfusion tube through a water bath kept at temperature of $37^{o}C$. Also a low wattage bulb was placed above the animal to warm it and the body temperature monitored. A rectal thermometer was inserted via the anus to ensure that the body temperature was at $37^{o}C$. After a steady basal acid secretion had been obtained, histamine was administered 100mg/kg and titre values were collected for six consecutive times. The basal and peak acid secretions were recorded. Phenolphthalein was used.

Gastric output was measured by titrimetric analysis. The calculation of acid in millimole per litre per hour follows the principle that states that a gram equivalent of acid balances a gram equivalent of base at neutralization. That means that: $N_A V_A = N_B V_B$ (Osim, *et al.*, 1991)

The Ethanol Induced Gastric Ulcer Model

Gastric ulceration was induced in rats as described by Alarcon de la Lastra et al, 1997, by oral instillation of 1ml/100g of 60% (v/v) ethanol in distilled water after an overnight fast. The test groups received 1ml/100g body weight of INH given orally while the control group received the same volume of normal saline. Thirty minutes after pre-treatment with saline, the animals were administered ethanol orally. One

hour later, the animals were sacrificed and their stomach were removed and opened along the greater curvature.

Assessment of ulcer. After carefully washing the stomach with saline in other to remove the content and blood clots, the ulcers were scored macroscopically according to the method described by Elegbe (1978) by using a hand lens of x2 magnification. The ulcer grading was as follows: ulcer score 0, normal stomach; ulcer score 0.5, punctated haemorrhage or pin – point ulcer; ulcer score 1.0, haemorrhagic of 1-3mm diameter; ulcer score 2.0, ulcer greater than 3mm in diameter.

Intestinal Transit

The rats were deprived of food but allowed water 24hours before the experiment. The marker was prepared by a modified method of Uwagboe and Oriminike (1995). An aqueous suspension (95 mls) of 10% charcoal (BDH, England) was mixed with 5 ml of 0.15% Leishman stain (BDH, England). Thirty minutes before administration of marker, 1ml/100g body weight of water was administered. All the rats were later administered1.5mls of marker orally and kept without food for 1hour before the determination of the intestinal transit. At the end of time, the rats were sacrificed by cervical dislocation. The peritoneum was opened and the entire length of the small intestine was carefully stretched out and cut open and the distance of the maker from the pyloric junction measured with a meter rule.

The intestinal transit was calculated and expressed in percentage thus;

$$\%MI = \frac{\text{length traveled by marker}}{\text{Total length of small intestine}} \times 100$$

Collection of Blood

After an overnight fast, the animals were anaesthetized and sacrificed and blood was collected by cardiac puncture into EDTA and EDTA/Formalin sample bottles for the various haematological tests. The blood was analyzed using Automated Haematology Analyzer BC – 3200 Haematology.

Platelet Aggregation Ratio

Platelet aggregation was done on whole blood based on the principle described by (Wu and Hoak, 1974). Circulating platelet aggregates are fixed when exposed to a mixture of formalin and EDTA. The fixed platelet aggregates settle down on centrifugation, leaving platelet rich plasma. The platelet aggregation is 1 in the absence of aggregation (Ogunlade and Fasanmade, 2001). Two milliliters of blood was gently dispensed into EDTA tube and EDTA/Formalin tube labeled according to their code. 0.1ml of both EDTA and EDTA/Formalin sample was pipette to cleaned plain tubes and mixed with 1.9ml of 1% ammonium oxalate to lyse the red blood cell. The samples were allowed to stand at room temperature. Platelet counts were determined microscopically in the EDTA and EDTA/Formalin samples using Improved Neubauer Counting Chamber (England). Platelets appeared as refractive particles when viewed with the condenser racked down. Platelet count under $1mm^2$ was recorded.

Bleeding Time, Clotting and PTT were determined by the method described Ghai, (2007)

Statistical Analysis

Data are expressed as mean ±SEM. Data were analyzed using one way of ANOVA followed with post hoc (LSD) test for significant values. P-values of less than 0.05 were considered statistically significant.

RESULTS

Gastrointestinal indices

Table 1 shows the results of gastrointestinal indices. Transit time, Basal acid output (BAO) in test groups were significantly ($p<0.05$) decreased compared with control. Similarly ulcer indices in INH-induced toxicity groups were significantly increased but in the MO group had no differences. Mucus secretion in test groups were significantly ($p<0.05$) increased compared with control.

Table 1: Effect of MO based diet on gastro intestinal parameters following INH induced toxicity in rats

Variables	Control	INH only	INH(LD)+MO-Diet	INH(HD)+MO-Diet	MO-Diet only
Transit time (%)	84.83±0.11	75.10±0.21*	78.60±0.21*	75.75±0.41*	75.42±0.21*
Ulcer score(mm^2)	23.60±0.46	27.50±0.03*	37.00±1.22*	30.40±1.82*	23.70±0.05
Mucus(g)	0.16±0.02	0.18±0.02*	0.18±0.02*	0.30±0.01*	0.42±0.04*
BAO(mEq/l)	0.46±0.03	0.30±0.02*	0.20±0.03*	0.20±0.02*	0.23±0.01*
PAO(mEq/l)	1.46±0.02	1.64±0.02*	1.35±0.02*	1.25±0.02*	1.20±0.02*

*Values expressed as mean ± SEM *p<0.05, n=5*

Haematological Indices

Table 2 shows the result of haematological indices in test and control animals. Red blood cells (RBC) and white blood cells (WBC) counts were significantly (p<0.001) higher in INH (LD) + MO -diet, INH (HD) + MO and MO groups compared with INH or control. There was significant reduction (p<0.001) in RBC and WBC in INH group compared with control. Haematocrit (Hct) was significantly decreased in the INH – induced toxicity groups but it was significantly increased in MO group. Mean Cell Volume (MCV) was significantly increased (p<0.001) in INH (LD) + MO INH (HD) + MO and MO groups. Mean Cell Haemoglobin was significantly (p<0.001) higher in *Moringa oleifera* group. There was no statistically significant difference in the Mean Cell Haemoglobin Concentration (MCHC).

Table 2: Haematological Parameters in INH-induced toxicity in female rats

Variables	Control	INH only	INH(LD)+MO-Diet	INHHD)+MO-Diet	MO-Diet only
RBC ($x10^{12}$/L)	6.21±0.06	5.40±0.40*	7.62±0.04*	7.44 ± 0.07*	8.02 ± 0.01*
WBC ($x10^{9}$/L)	7.26 ± 0.22	6.20 ± 0.20*	9.57 ± 0.23*	7.92 ± 0.22*	8.60 ± o.12*
Hgb	15.72±0.02	13.67±0.03*	15.45±0.02*	14.47±0.02*	16.34±0.02*
Hct (%)	47.16 ± 0.34	40.70±0.36*	46.34 ± 0.09*	43.40 ± 0.40*	49.02 ± 0.48**
MCV (fl)	57.84 ± 0.02	58.50 ± 0.12*	60.28 ± 0.73*	60.10 ± 0.80*	60.82 ± 0.58*
MCH (pg)	18.45 ± 0.15	18.34 ± 0.22	18.78. ± 0.05	18.04 ± 0.10	19.35 ± 0.14*
MCHC (g/dl)	31.62 ± 0.07	31.38 ± 0.32	30.92 ± 0.44	32.10 ± 0.34	31.78 ± 0.12

*Values are expressed as mean ± SEM *p<0.05, n=5*

Table 3 also shows the result of bleeding time (BT) clotting time (CT) and prothrombin time (PTT). BT and CT were significantly (p<0.001) decreased in the group treated with *Moringa oleifera* only. Although the CT showed increase, these were not statistically significant. INH, INH (LD) + MO and MO only groups had significantly reduced PTT while INH (HD) + MO showed no significant difference.

Table 3: Effect of haemostatic biomarkers in INH-induced toxicity of female rats

Variables	Control	INH only	INH(LD)+MO-Diet	INH(HD)+MO-Diet	MO-Diet only
BT (s)	4.90 ± 0.20	4.70 ± 0.20	4.32 ± 0.28	4.30 ± 0.21	3.36 ± 0.16*
CT (s)	4.08 ± 0.24	4.10 ± 0.24	4.90 ± 0.20	4.70 ± 0.20	3.94 ± 0.10*
PTT (s)	12.40 ± 0.02	9.40 ± 0.06	11.80 ± 0.04	12.40 ± 0.04	10.60 ± 0.04*
Plt ($x10^9$/L)	70. 20 ± 0.24	55.21 ± 0.41*	72.58 ± 0.18*	78.00 ± 0.40*	80.92 ± 0.14*
Plt Agg Ratio	0.67 ± 0.04	0.73 ± 0.06*	0.78 ± 0.03*	0.55 ± 0.07*	0.76 ± 0.01*

*Values expressed as mean ± SEM, *p<0.05, n=5*

DISCUSSION

The results of the gastrointestinal markers of test and control groups provide very relevant information. Transit time was significantly decreased in INH, INH (LD) + MO, and MO compared with control. It is known that INH causes enteritis which may lead to diarrhea. The transit time result suggest that in INH – induced state of diarrhea *Moringa oleifera* may inhibit secretary and/or osmotic diarrhea in which water is pulled into the bowel by hyper osmotic content of the bowel. In a similar manner in congenital defects in which Cl^-/HCO_3^- well as Na^+/ H^+ exchangers found in the intestinal brush border that may give rise to altered Na^+ ion transport (Porth and Kunert, 2002). When hyper motility of the intestine is present, water and electrolytes may be delivered to the colon at a rate too fast to be absorbed, or when colonic bacteria that in mal-absorption ferment unabsorbed nutrients to produce toxins that increase motility, these may give rise to derangement in motility (Kane and Kurmar, 2006). Any wonder, *Moringa oleifera* had been found to be a wonderful plant in treatment of many disease conditions.

Basal acid output (BAO), were reduced in INH (LD) + MO, INH (HD) + MO and MO groups. Several studies have demonstrated in vivo and in vitro free radical scavenging ability of aqueous leaf extract of *Moringa oleifera* (Screelatha *et al*., 2011; Screelatha and Padma, 2010; Sing *et al*.,2009).The reduction in BAO observed might be caused by the MO-diet. In a wound healing study in the rat, Rathi *et al*., (2006), *Moringa oleifera* increased wound closure rate, skin breaking strength, granuloma dry weight and decreased scar area. Debnath *et al*. (2011) showed that *Moringa oleifera* offered protection with oral administration to aspirin-induced ulcers.

Similarly, peak acid output (PAO) was reduced in INH (LD) + MO, INH (HD) + MO, groups but was significantly increased in INH group. Several mechanisms serve to protect the gastric mucosa barrier (Brzozoski *et al.*, 1999; Tekuchi *et al.*, 2001) but damaging agents modify the characteristics of the gastric mucosa (Porth and Kunert, 2002; Abdel-Salem *et al.*, 1999). *Moringa oleifera* contains antiulcer agents: quercetin and kaempferol, known to lower acid secretion (Bajpai *et al*., 2005).

There was also significant increase in the production of mucus with corresponding decrease in acid secretion. Mucus and bicarbonate secretion by the mucosa play important role in the acid rich gastric juice. Prostaglandins stimulate mucus secretion while the bicarbonate is stimulated by local reflexes. Prostaglandins have been implicated in gastric mucosal protection (Allem and Flemstrom, 2005; Cho *et al*., 1994, Konturek *et al*., 1987). It is possible that the increase in mucus secretion was a direct effect of the stimulant action of prostaglandins or their action along with the MO-diet on the mucosa that caused the secretion of mucus which could not stop development of ulcer. The gastric mucosa damage requires severe degree of ischemia, high reduction in gastric mucosal blood flow that can greatly potentiate the effect of the mucosa damaging agent (Abdel-Salem *et al.*, 1999), such as alcohol. It is possible that ethanol potentiated INH-induced mucosal damage in the gut, thus the high ulcer scores. Alcoholic beverages may not be favorable to TB patients. Ogbunogufor *et al*., (2011) and Pal *et al*., (1995) showed that quercetin a flavonoid in *Moringa oleifera* has ulcer healing activity. Dednath and Guha, (2007) reported that *Moringa oleifera* reduced gastric acid secretion. The non-inhibition of gastric ulcers may also be due to the quantity of MO-diet consumed.

The full blood count of INH (LD) + MO, INH (HD) + MO and MO groups were significantly higher when compared with INH/or control groups. This indicates that *Moringa oleifera* diet (MO-diet) enhanced full blood cell count of INH–induced toxicity in the rat. Moyo *et al*., (2011) showed that *Moringa oleifera* contains mineral elements like iron necessary for the production of red blood cells and haemoglobin in the bone marrow as seen in INH (LD) + MO, INH (HD) + MO and MO groups. This result is similar to the findings of Okwari *et al.(*2015). These authors found that MO-diet improved haematologic and hemostatic indices in rats following ingestion of palm wine and local gin (Ogogoro) in the rat. Another study involving human dermal fibroblast Muhammud *et al*., (2013) showed that aqueous extract of *Moringa oleifera* significantly increased cell proliferation and viability of cells. Chemical compounds isolated from *Moringa oleifera* have been shown to contain useful physiological and pharmacological properties with protective medicinal application (Anwar *et al*., 2007; Guevara *et al*., 1999., Caceres *et al.,* 1991). Flavonoids in *Moringa oleifera* have been reported to increase white blood cell production because they cause increase intracellular vitamin C synthesis, leukocytosis, decreased capillary permeability, fragility (Lee *et al.,* 2003). As in the seed, flavonoids have been shown to exert protective effect on chemically induced haemolysis in G6PD deficient human and animal red blood cell, anti-inflammation and antipyretic activity (Braide and Vitrotio, 1989, Braide, 1990).

There haemoglobin content of INH (LD) + MO, INH (HD) + MO, and MO groups was higher compared with INH group. Increased red blood cells caused increased haemoglobin. The increased MCV in INH (LD) + MO, INH (HD) + MO and MO groups suggest defect in the absorption of vitamin B_{12} and folate and/or their utilization in the haemopoesis (Fantozzi et al., 1986). It is well known that increased MCV might as well be caused by liver disease and drugs (Walter *et al.,* 2014) In the MO group, both MCV and MCH were significantly increased indicating macrocytic anemia. The expression of macrocytosis in this group could as well be that folate and vitamin B_{12} are poorly absorbed and/or utilized in the process of cell production (Fantozzi *et al*., 1986) as earlier noted.

The bleeding time was significantly reduced in the *Moringa oleifera* group but was not statistically significant in INH–treated groups when compared with control. This suggests a protective effect. The in vivo and in vitro free radical scavenging activity involving different parts of *Moringa oleifera* extracts have been reported (Screelatha *et al.,* 2011; Screelatha and Padma, 2010; Sing *et al.,* 2009; Chumark *et al.*, 2008). The polyphenol fraction of *Moringa oleifera* has been shown to exhibit free radical scavenging activity in vitro. The polyphenol fraction of *Moringa oleifera* administered to rats has been reported to inhibited carbon tetrachloride-induced toxicity, hepatic lipid peroxidation while increasing glutathione, the primary antioxidant in the liver, as well as catalase and super oxide dismutase, a physiological and biochemical rationale for the antioxidant and chemo protectant effects (Screelatha *et al.*, 2011; Screelatha and Padma, 2010).

It is seen that ingestion of *Moringa oleifera* diet resulted in proliferation of blood cells. Large numbers of young platelets are thought to enhance hemostatic capabilities Sood, 2006). The slight decrease in bleeding time shown, may suggest that there might be some platelet abnormalities, deficiency of clotting factors (ii, iv, viii and xi), or vascular abnormalities (Mann *et al.,* 2003; Bouchard and Tracy, 2001) caused by INH – induced toxicity.

Clotting of blood by Wright's capillary method involves both intrinsic and extrinsic systems of clotting (Sood, 2006). The clotting time of *Moringa oleifera* group was significantly reduced compared with control while there were slight increases in clotting time of the INH–treated groups. Disturbance in the synthesis of vitamin K and absorption of dietary vitamin K resulting from INH–induced toxicity may affect clotting time. Although the mechanism is not clear, it has been thought that many of the coagulating factors (ii, vii, ix, and x) are post translationally modified by vitamin K dependent enzymes in the liver; therefore their synthesis may be impaired by vitamin K deficiency (Sitren,1991) and may alter the clotting time.

Prothrombin time in INH, INH (LD) + MO and MO groups were significantly reduced. This means that there was decrease level of factor ii (prothrombin) as well as factors v, vii and x that might be low or non-

functional. *Moringa oleifera* contains vitamin K (Vinodini *et al.*, 2014). It is possible that the effect on the rats could result from a reduced intake of the diet or reduced synthesis. Prothrombin time is prolong in vitamin K deficiency, and it has been reported that vitamin K stimulates the liver to produce prothrombin and factor x, factors of the extrinsic system (Stamatoyannopoulos, 2001).

In conclusion,*Moringa oleifera* diet reduced basal acid output, peak acid output and propthrombin time, but increased mucus concentration, red blood cells, white blood cells, platelets, hemoglobin content and platelet aggregation in INH-treated male rats.

REFERENCES

Abdel-Salem, OME. Szolesanyi J and Mozsik GY (1997). Capsacin and the stomach. *Journal of Physiology-Paris* **91** 151-171.

Allen A and Flemstom G (2005). Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. *American Journal of Physiology Cell Physiology* **288**(1) C1-C19.

Alphin RS and Ward JW (1967). Actions of hexopyronium bromide on gastric secretion in dogs and gastric secretion and ulceration in rats. *Archives Internationales de Pharmacodynamie et de Thérapie* **168** 82-100,

Anjorin TB and Ikoko P and Okolo S (2010). Mineral composition of *Moringa oleifera* leaves, pods from two regions in Abuja, Nigeria. *International Journal of Agriculture Biology* **12** 431 434.

Anwar FS, Latif M, Ashraf A and Gilani H (2007). *Moringa oleifera*. Food plant with multiple medicinal uses. *Phytotherapy Research* **21** 17-25.

Bajpai M, Paride A, Tewari SK and Prakash D (2005). Phenolic content and antioxidant of some food and medicinal plants. *International ournal of Food Science and Nutrition* **56** 287-291.

Bouchard B and Tracy P (2001). Platelets, leucocytes and coagulation. *Current Opinion in Haematology* **9** 263-269.

Braide VB and Vitrotio G (1989). Histological alterations by a diet containing seeds of Garcinia kola: effect on liver, kidney, and intestine in the rat. *Gegenbaurs Morphologisches Jahrbuch* **136**(1) 95- 101.

Braide VB (1990). Pharmacological effect of chronic ingestion of Garcina conruana seed in rats. *Fitoterpia* **4** 39-41.

Brzozoswski T, Kounturek PCH and Kounturek SJ (1999). The role of prostaglandin generated by cyclooxygenase1 and cyclooxygenase 2 in healing of ischemic reperfusion in rat. *European Journal of Pharmacy* **385** 47-51.

Caceres A, Cabrera O, Murales O, Mollinedo P and Mendia P (1998). Pharmacological properties of *Moringa oleifera*. 1 Preliminary screening for antimicrobial activity. *Journal of Ethnopharmacology* **33** 213-216.

Cho CH, Ko IK and Tang XL (1999). The differential mechanisms of mild irritants on adaptive cytoprotection. *European Journal of Gastroenterology and Hepatology* **4** (9)(Suppl 1) 24-27.

Cho EJ, Yokozawa DY, Rhyu SC, Kim NS and Park C.J(2003). Study on inhibitory effect of some Korean medicinal plants and their main compounds on the 1,1-diphenyl-2-picrylhydrazyl radical. *Phytomedicine* **10** 544-555.

Chumark P, Khunawat P and Sanvarinda Y (2008). The in vivo and ex vivo properties,hypolipidemic and antiathrosclrotic activities of water extract of *Moringa oleifera* Lam leaves. *Journal of Ethnopharmacology* 116 439-446.

Debnath and Guha D (2007). Role of *Moringa oleifera* on enterochromafin cell count and serotonin content experimental ulcer model. *Indian Journal of Experimental Biology* **45** 726-731.

Debnath S, Biswas D, Ray and Guha D (2011). *Moringa oleifera* induces potential of serotonin released by 5HT receptors in experimental ulcer model. *Phytomedicine* **15** 91-95.

Fahey JW (2005). *Moringa oleifera*. A review of medical evidence for its nutritional, therapeutic and prophylactic properties. Part 1. *Trees Life J*ournal **1** 1-15.

Fantozzi R, Caramelli L, Ledda F, Moroni F, Masini E and Blandina P (1986). Biological markers and their therapeutic outcome in alcoholic disease. A twelve year survey. Department of Preclinical and Clinical Pharmacology, University of Florence, Italy, Toxicological Unit, School of Medicine, Italy. *Klin Wochensche* 7-33.

Farooq A Sajid L, Muhammad A and Anward HG (2007). *Moringa oleifera*: A Food plant with multiple medicinal uses. *Phytotherapy Research* **21**(1)17-25.

Ghai C.L. (2007). A Textbook of practical physiology. 7th Ed Jaypee Brothers Medical Physiology Publishers (P) Ltd New Delhi P 117.

Ghosh MN and Shild HO (1958). Continuous recording of gastric acid secretion in rat. *Journal Pharmacol*ogy 13 54-67.

Guevara APC, Vargas H, Sakurai Y, Fujiwara K, Hashimoto T and Maoka M (1999). An antitumor promoter from *Moringa oleifera* Lam. *Mutation Research* **440** 181-188.

Kane AB and Kumar B (2006). Environmental and Nutritional Pathology. In *Kumar V*, Abba, AK. and Fauso N (eds) Robbins and Cortan, Pathological Basis of Disease. 7th ed Saunders Elsevier, Singapore.

Kounturek SJ, Stachura J, Radecki T, Drozdowicz D and Brzozowski T (1987). Cytoprotection are ulcer healing properties of prostaglandin EP 2, Colloidal bismuth and sucralfate in rat. *Digestion* **38** 102-113.

Lee KW, Kim YJ, Lee HJ and Lee CY (2003). Cocoa has more phenolic phytochemicals and high antioxidant capacity than tea and red wine. *Journal of Agriculture Food Chemistry* **51**(25) 7292-7295.

Lewis JH (2000). Drug –induced liver disease, *Medical Clinics of North America* **84** 12775-1211

Mann KG, Batenas S and Brummel K (2003). The dynamics of thrombin formation. *Atherosclerosis Thrombosis Vascular Biology* **23** 17-25

Moyo B, Masik PJ, Mar LJ, Hugo A and Muchenje V(2011). Nutritional characterization of *Moringa oleifera* (*Moringa oleifera* Lam) leaves. *African Journal of Biotecthnology* **10** 12925-12933.

Muhammud AA, Pauzi NAS and Arulselvan P (2013). *In vivo* wound healing potential and identification of bioactive compounds from *Moringa oleifera* Lam. *BioMed Research International*Doi: 10 1155/2013/974580.

Namdar R, Ebert S and Peloquin CA (2006). Drugs for TB. ***In***: Piscitelli, SC. Rodvold, K.A. eds. Drug interaction in infectious diseases. 2nd ed. Totowa, NJ, Hamana Press, 191- 214.

Ogbonugaor H, Eneh F, Ozumba A, Igwo-Ezikpe M, Okpuzor T, Adenekan S and Onyekwelu, O (2011). Physio- chemical and antioxidant properties of *Moringa oleifera* seed oil. *Pakistan Journal of Nutrition* **10** 409-414.

Ogunlade OA and Fassanmade AA (2001). Abnormalities in thrombogenic and rheologic factors in Nigeria hypertensive patients. *African Journal of Medical Sciences* **30** 291-294.

Okwari OO, Kolawale TA, Wali C, Olatundun OB and Okon H (2016). *Moringa oleifera* diet improves the toxic effects of palm wine and local gin on haematologic and haemostatic parameters in albino rats. *Journal of Medical Sciences* **6**(8) 221-228.

Osim EE, Arthur SK and Etta KM (1991). The influence of cola nut (cola nitida) on in vivo secrtion of acid in rat. *International Journal of Pharmacognosy* **29** 215-220.

Owagboe PE and Orimilikwe SO. (1995). Effect of histamine H_2 receptor blocker on gastrointestinal transit in conscious albino rats. *Nigerian Journal of Physiological Sciences* **11** 56-58.

Pal SK. Mukherjee PK and Saha BP(1995). Studies on antiulcer activity of *Moringa oleifera* leave extract on gastric ulcer models in rats. *Phytotherapy Research* **9** 463- 465.

Porth CM. Kunert MP (2002). Pathophysiology: concept of altered health state. 6th ed Williams and Wilkins, London.

Rathi BS. Bodhankar SL and Baheti AM (2006). Evaluation of leaf extract of *Moringa oleifera* Lam for wound healing in albino rats. *Indian Journal of Experimental Biology* **44** 898-901.

Screelatha S and Padma PR (2010). Protective mechanism of *Moringa oleifera* against CCL_4 – induced oxidative stress in precision cut liver slices. *Forschende Komplementarmedizin* **17** 189-194.

Screelatha S. Jeyachitra A and Padma PR (2011). Antiproliferation and induction of apoptosis by *Moringa oleifera* leaf extract on human cancer cells. *Food Chemistry and Toxicology* **49** 1270-1279.
Singh BN, Singh BR, Singh RL, Prakash D, Dhakarey R, Upadhyay G and Singh HB (2009). Oxidative DNA damage, protective activity, antioxidant and antiquorum sensing potentials of *Moringa oleifera*. *Food Chemistry and Toxicology* **47** 1109-1116.
Sitren H (1991). Vitamin K; *In*: Baumgartner, T.G. Clinical guide to Parenteral Micronutrition. New York. Fujizawa. USA **6** 411- 431.
Sood R (2006). Text Book of Medical Laboratory. Jaypee Brothers Medical Publishers P Ltd. New Delhi.
Stamatoyannopoulos G (2001). The molecular basis of blood disease. 3rd ed. WB Saunders,
Takeuchi K, Araki H, Umeda M, Komokie Y and Zuzuki K (2001). Adaptive Cytoprotection is medicated by prostaglandin EPI receptor: A study using rats and knockout mice. *Pharmacological and Experimental Therapeutics* 297 1160-1165.
Takeuchi K, Araki H, Umeda M, Komokie Y and Suzuki K (2001). Adaptive cytoprotection is mediated by prostaglandin EP1 receptor. A study using rats and knockout mice. *Pharmacological and Experimental Therapeutics* **296** 1160-1165.
Vinodini NA Kumar P and Chatterjee AA (2014). Evaluation of liver function with *Moringa oleifera* leaf extract in cadmium induced Wistar rats. *International Journal of Plant Animal Environmental Science* 4(3) 1-4.
Walter BR, College NR, Ralson SH and Pennan ID (2014). Davidsons principles and practice of medicine. 22nd edition Churchill Livingstone, Elsevier, Philadelphia.
Wu KK and Hoak J C (1974). A new method of quantitative detection of platelets aggregation in patients with arterial insufficiency. *Lancet* 19 294-296.