Available online on 30.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

To Study Analgesic, Hypoglycemic and Hepatoprotective Activity of *Moringa olefera* Leaf Extract in Albino Wistar Rats

Aboobecker S.P.A.[1], Anurag Bajpai[1]*, Srivastava R.K. [1], Reshu Tewari [2], Hooda M.S. [3]

[1] Department of Pharmacology, NC Medical College & Hospital, Israna, Panipat-132107, Haryana, India

[2] Department of Biochemistry, NC Medical College & Hospital, Israna, Panipat-132107, Haryana, India

[3] Department of Pharmacy, Janta College of Pharmacy, Guhana, Haryana, India

ABSTRACT

Introduction: *Moringa oleifera* is widely found in Asian subcontinent and it has been used as an Analgesic, Hypoglycemic and hepatoprotective in Indian folklore medicine. In this study we compared the Analgesic, Hypoglycemic and hepatoprotective effects of *Moringa oleifera* ethanolic extracts with other standard drug in Albino Wistar Rats.

Methods: Male Wistar albino rats were divided into 5 groups and administered placebo (saline), diclofenac and 3 groups of *Moringa Oleifera* using 100mg/Kg, 200mg/Kg and 400mg/kg doses for Analgesic Activity. On the other hand, 30 Albino Wistar rats were divided into 5 groups of six each and administered placebo (saline), Liv-52 (standard) and 3 groups of Moringa Oleifera using 100mg/Kg, 200mg/Kg and 400mg/kg doses for hepatoprotective activity. In addition, the test leaf extracts preparations of *Moringa oleifera* [100, 200 and 400 mg], were administered for 21 days orally to the rats of respective groups by using oral feeding tube for Hypoglycemic activity.

Results: The highest Tail flick latency period was observed in Group 2 and Group 5 at 120 min. At all-time of point, the tail-flick latency period differed significantly between the extract and Aspirin treated Groups being greater in the Group 2. Comparing different doses of the extract revealed that there is positive relationship between reaction time and increase dose of the extract in which, protection against heat application with 400 mg /kg was significant compared to all doses of the extract. Whereas, Rats treated with ethanolic Leaf extract of *Moringa Oleifera* (100/200/400 mg/kg, orally once daily) for 21days, the SGOT values (242.66 ± 11.63 IU/L, 242.66 ± 11.63 IU/L, 242.66 ± 11.63 IU/L) were significantly lower (P<0.05), (P<0.05), (P<0.01) when compared to SGOT levels in control rats (265 ± 4.75 IU/dl). Rats treated with ethanolic Leaf extract of Moringa Oleifera (100/200/400 mg/kg, orally once daily) for 21days,

Conclusion: Ethanolic extracts of Moringa Oleifera leaves exhibits significant Analgesic, Hypoglycemic and hepatoprotective activity in a dose dependent manner.

Keywords: Analgesic, Hypoglycemic, Hepatoprotective, *Moringa oleifera*

Article Info: Received 20 June 2019; Review Completed 08 Aug 2019; Accepted 17 Aug 2019; Available online 30 Aug 2019

Cite this article as:

Aboobecker SPA, Bajpai A, Srivastava RK, Tewari R, Hooda MS, To Study Analgesic, Hypoglycemic and Hepatoprotective Activity of *Moringa olefera* Leaf Extract in Albino Wistar Rats, Journal of Drug Delivery and Therapeutics. 2019; 9(4-A):730-736 http://dx.doi.org/10.22270/jddt.v9i4-A.3566

***Address for Correspondence:**

Anurag Bajpai, Associate Professor, Department of Pharmacology, NC Medical College and Hospital, Israna, Panipat-132107, Haryana, India

INTRODUCTION:

In India, the sources of medicinal plants have been used as medicines since the days of historic Vedic glory. Several medicinal plants and herbs form part of our diet as spices, vegetables and fruits. Plants are being used in traditional medicine since history of mankind. [1] The knowledge of these medicinal plants has accrued in the course of many centuries leading to medicinal systems in India such as Ayurveda, Unani and Siddha. According to WHO reports that about 25% of modern medicines have been derived from plants which were first used traditionally. [2]

Research studies evaluating the safety, efficacy and appropriateness of the benefits of "*Moringa oleifera"* include an expanding list of promising medicinal and nutritional uses. Its English name is "*Drumstick*" and the local name in Marathi is "*Shevaga*" Although it may yet be too early to provide a significant number of claims about the precise benefits derived from *Moringa oleifera*, the breadth of current research appears to shed considerable light on many of the potential applications for products containing the primary ingredient of this plant. [3]

All the parts of the tree *(Moringa oleifera)* are used in folk medicine practices for the treatment of various diseases such

as UTI, External sores and Ulcers, Diabetes, Cancer, Gastritis, Diarrhea, Liver Diseases etc. The plant is reported to possess Anti-inflammatory, Antioxidant, Antiulcer, Anticancer, And Antihyperlipidaemic and Cardiotonic properties. [4] The studies on various extracts of its parts, i.e. coat, pulp and seed, leaves revealed more pronounced results indicating that its activity is widely distributed. [5]

Pain can be defined as, unpleasant sensation, usually evoked by an external or internal noxious stimulus. "Analgesic decrease the pain by acting on the CNS or peripheral pain mechanisms, without altering consciousness". So, analgesic activity means capacity of a substance to neutralize the pain sensation. The environment has provided a vast collection of remedies to cure all ailments of mankind. [6]

In recent decades, many scientific studies using the Extracts of Leaves, Barks, Seeds and Roots of *"Moringa oleifera"* are being carried out to confirm many potential uses. However very few studies have been done on the Leaf Extracts. Taking this background into consideration we conducted the study to investigate the presence of Phytochemical Constituents and to evaluate the Analgesic Activity of Ethanolic Leaf Extract of *Moringa oleifera*. [7]

Diabetes Mellitus (DM) is a metabolic disorder with significant morbidity and mortality. Diabetic patients present symptoms of chronic hyperglycemia along with glucose tolerance impairment. [8] There is oxidative stress concordantly which occurs with hyperglycemia and causes pathogenesis in many organs, leading to complications such as vasculopathies, neuropathies, nephropathies and ophthalmopathies. The diabetic patients require currently used drugs to control their blood glucose level and to improve blood glucose tolerance. [9] Recently, the use of herbal products has gained more interest for remedy of diabetes and other ailments. In the present review, an attempt has been made to investigate the hypoglycemic activity of *"Moringa oleifera"* Leaf Extract. [10]

Liver is the most important organ, which plays important role in regulating various physiological processes in the body. It performs various vital functions, like metabolism, secretion and storage. It is capable to detoxicate toxic substances and synthesize useful substances. Therefore, hepatoprotective agents having protective action on liver against hepatotoxic agents is of importance to be studied. [11] Conventional drugs used in the treatment of liver diseases are often inadequate and thus, it is therefore necessary to search for alternative drugs for the treatment of liver disease having better efficacy and safety. Thus this study was undertaken to investigate the Hepatoprotective Nature of *"Moringa oleifera"* on induction of Hepatotoxicity by Carbon Tetrachloride (CCl4) known to cause Liver damage in Albino Wistar Rats. [12]

The major aim of the present study is therefore to investigate the ameliorating potentials of the leaves extract of this plant on carbon tetrachloride (CCl4) mediated liver damage in Albino Wistar Rats.

The present study therefore attempts to prove scientifically the traditional claim that "*Moringa oleifera"* possess the Analgesic, Hypoglycemic and Hepatoprotective. The aim of our research is to find out new drug preparation from "*Moringa oleifera"* which are proposed to be potent and nontoxic agents and possess the various activities. Normally herbal drugs are free from side effects /adverse effects and these are low cost medicines which will be beneficial for the people of our country.

MATERIAL AND METHODS:

Experimental Animals

Male Albino Wistar Rats weighing 150-200g were used for the present study. The animals were maintained under controlled conditions of temperature (23 ± 2C), humidity (50 ± 5%) and 12-hour light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of nonspecific stress. The present study was conducted at Dept. of Pharmacology, NC Medical College and Hospital. Studies conducted were approved by the Institutional Animal Ethical Committee (IAEC).

MATERIALS:

A) Chemicals: -

Table 1. Chemicals used for study of *Moringa oleifera*

Chemicals	Manufacturer
Alloxan Monohydrate	Ozone Chemicals, Mumbai
Ethanol	Dipa Chemicals, Mumbai
Petroleum Ether	Dipa Chemicals, Mumbai
CCl_4	Gujarat Alkalies and Chemicals Limited

Drugs

Table 2. Drugs Used in Study of *Moringa oleifera*

DRUG	MANUFACTURER	BRAND
Glibencainamide	Sanofi Aventis India	[Daonil]
Diclofenac	Novartis India	[Voveron]
Liv 52	Himalaya Herbals India	Liv52[Compositioncapparis Spinosa (34 Mg), Cichorium Intybus (34 Mg), Solanum Nigrum (16 Mg), Terminalia Arjuna (16 Mg), Achillea Millefolium (8 Mg), Tamarix Gallica (8 Mg) And Cassia Occidentalis (8 Mg)]
Aspirin	Reckitt & Benckiser (India) Ltd.	Dispirin

1 A) Collection and authentication of the plant material

The leaves of *Moringa oleifera* were collected from local area of Panipat, Haryana, India, during July 2018.

1B) Authentication of the plant: The Plant Herbarium was submitted for identification and authentication at the Department of Botany, Janta college of pharmacy, Bhutana, Gohana, Haryana, India. The Voucher specimen (Number 1032) was the authentication number of the Herbarium after validation.

1C) Drying:

The leaves of "*Moringa oleifera*" plant was shade dried at room temperature.

1D) Preparation of Plant Extract:

The extraction includes the process of yielding of the extracts from plants species. This depends on the solvent polarity which determines qualitatively and quantitatively the quality of the extracted compounds. Ethanol is one of the most widely used solvent for the extraction because of its low toxicity and high extraction yield with the advantage of modulating the polarity of the solvent by using mixtures at different ratios. The plant Leaves were shade dried to control temperature, humidity and damage of active constituents. Then dried plant material of leaf was powdered individually by using grinder and defatted with petroleum ether. Defatted 500 gm of each powder was extracted by 95% ethanol in a soxhlet apparatus for 72hours as by cold maceration followed by concentrated in a rotator evaporator under reduced pressure at temperature 40-50°C and then lyophilized to get a dry residue. Some part of the total extract was used for qualitative and quantitative phytochemical investigation and rest of the extract was used for preliminary pharmacological screening.

EVALUATION OF ANALGESIC ACTIVITY

1. Tail-flick method [13]

Procedure

Tail-flick latency was assessed by the Analgesiometer. The strength of the current passing through the naked nichrome wire was kept constant at 5 ampere. The distance between heat source and the tail was 1.5 cm and the application site of the heat on the tail was maintained within 2 cm, measured from the root of the tail. To avoid any tissue injury during the process the cutoff reaction time was taken as 10 sec. The time taken by Rat to withdraw (flick) the tail was taken as the reaction time. The animals were subjected to the same test procedure at 0 before and 30, 60, and 120 min after administration of treatment as described in the grouping and dosing section.

Table 3. Groups classification for the Tail Flick Method Study

Group	Description	Drug /Extract dose
1	Control	Normal saline 10 ml orally
2	Standard	Aspirin 20 mg/kg orally
3	Ethanolic extract moringa oleifera	100 mg/ kg orally
4	Ethanolic extract moringa oleifera	200 mg/kg orally
5	Ethanolic extract moringa oleifera	400 mg/kg orally

EVALUATION OF HYPOGLYCEMIC ACTIVITY

Alloxan Induced Diabetes in Rats [14]

Induction of diabetes in rats:

Induction of diabetes: Overnight fasted rats were made diabetic by single intra peritoneal injection of freshly prepared solution of alloxan monohydrate in normal saline at a dose of 120 mg/kg body weight. Alloxan is capable of producing hypoglycemia as a result of massive pancreatic insulin release, in order to starve off the hypoglycemia during the first day of alloxan administration rats were treated with 20% glucose solution (5-10 mL) orally after 6 hours of alloxan administration for the next 24 hours. After 5 days blood samples were collected and blood glucose levels were determined to confirm diabetes. The Rats with blood glucose level > 140mg/d L were considered to be diabetic and were used in experiment.

The Blood Samples were collected by tail-vein puncture method and samples were collected in fluoride bulbs and blood glucose levels were determined by using GOD POD METHOD.

Table 7. Hypoglycemic Activity Group Description

Group	Description	Drug /Extract dose
1	Control	Normal saline 10 ml orally
2	Standard	Glibenclamide (0.25 mg/kg) orally
3	Ethanolic extract *Moringa oleifera*	100 mg / kg orally
4	Ethanolic extract *Moringa oleifera*	200 mg / kg orally
5	Ethanolic extract *Moringa oleifera*	400 mg / kg orally

Administration of test samples:

The test leaf extracts preparations of *Moringa oleifera* [100, 200 and 400 mg], were administered for 21 days orally to the rats of respective groups by using oral feeding tube. The quantity of drug administered to each animal was calculated daily from its body weight

Administration of standard drug samples:

Glibencainamide was administered orally at a dose of 0.25 mg/kg using sterile oral feeding needle. The quantity of drug administered to each animal was calculated daily from its body weight.

Determination of blood glucose:

The blood glucose levels were estimated after the induction of diabetes. The blood was collected on 0, 7th ,14th day and 21th day after the induction of diabetes.

Evaluation of Hepatoprotective Activity [15]

Carbon tetrachloride was procured from Sigma Chemical Company, Olive Oil from Sasso, Italia. Liv 52, the standard drug was obtained from Himalaya Drug Company, India. All the reagents used were of analytical grade. The estimation of biochemical markers for liver conditions like Aspartate Serum Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (TB) were analyzed using Autoanalyzer. The following experimental The Rat Model was used for used for present study for induction of acute hepatic damage by CCl4(Carbon Tetrachloride). The Animals were divided into five groups of six rats each as given in the table. A suspension of carbon tetrachloride in olive oil (1:1) was given for induction of hepatic damage.

The rats were kept overnight fasting after 21 days and blood samples were collected by retro orbital puncture under ether anesthesia.

Table 4. Hepatoprotective Activity Group Description

Group	Description	Drug /Extract dose
1	Control	Normal saline
2	Standard	Liv52 0.216 ml/kg /day orally
3	Ethanolic extract *Moringa oleifera*	100 mg /kg orally
4	Ethanolic extract *Moringa oleifera*	200 mg /kg orally
5	Ethanolic extract *Moringa oleifera*	400 mg /kg orally

The blood was collected and allowed to stand for 30 min at 37°C and then centrifuged to separate the serum to estimate various biochemical parameters and the serum was used for the estimation of hepatic biochemical markers like serum SGOT, SGPT, Alkaline Phosphatase and Sr. BILURUBIN levels using Autoanalyser. The enzyme levels were estimated and the results were expressed as U/l.

Histopathological study:

The Albino Wistar Rat one from each group were euthanized by the intracardiac injection of inj. pentobarbitone. The liver was dissected following a midline incision. A portion of the median lobe of the liver was dissected and fixed in 10% neutral buffered formalin solution for 24 h. The washed tissue was dehydrated in descending grades of isopropanol and finally cleared in xylene. The tissue was then embedded in molten paraffin wax. Sections were cut at 5 µm thickness after deparaffinized and rehydrated tissues using standard techniques, and the sections were stained with Hematoxylin and Eosin (H& E). The sections were then viewed under light microscope for histopathological changes. The extent of CCl4 induced necrosis was evaluated by assessing morphological changes in liver sections using standard techniques.

RESULTS:

Herbal drugs play a restorative role in many diseases; most of them speed up the natural healing process. Previous literature indicates that a number of plant extracts and compounds isolated from various plant sources and minerals have shown activity against the various disease conditions. In the present investigation, we have studied the Analgesic, Hypoglycemic and Hepatoprotective Activity of Ethanolic Leaf Extracts of *Moringa oleifera* in Wistar Albino Rats.

ANALGESIC ACTIVITY

Tail Flick Method Evaluation

In Group 1 there was no Analgesic Effect seen at 0 ,30, 60 and 120 minutes. In group 2 the response to heat application was not seen at 0-minute interval but the Tail flick latency period was slightly increased after 30 minutes which gradually increased after 60 and 120 minutes. In Group 3 at 0 minute there was no analgesic effect seen, but Tail flick latency period increased at 60 and 120 min. In Group 4 the analgesic effect was Tail flick latency period increased between 60 and 120 minutes. In Group 5 the analgesic effect was not seen at 0 minutes but Tail flick latency period increased progressively from 30 minutes to 60 min and up to 120 min.

The highest Tail flick latency period was observed in Group 2 and Group 5 at 120 min. At all-time of point, the tail-flick latency period differed significantly between the extract and Aspirin treated Groups being greater in the Group 2. Comparing different doses of the extract revealed that there is positive relationship between reaction time and increase dose of the extract in which, protection against heat application with 400 mg /kg was significant compared to all doses of the extract.

Table 5. Analgesic Activity by Tail Flick Method

Groups	0min	30min	60min	120min
Group 1[control]	5.33 ± 0.51ns	5.00 ±0.00 ns	5.83 ±0.75ns	6.66 ± 0.51ns
Group 2[std]	5.33 ± 0.51 ns	7.66 ± 1.36 **	10.50 ± 1.04**	13.00 ± 0.89**
Group3[mo100]	5.50 ± 0.54ns	6.00 ± 0.63*	7.66 ± 0.51**	8.66 ± 0.51**
Group4[mo200]	5.50 ± 0.54 ns	8.50 ± 0.54**	9.00 ± 0.63**	11.33 ± 0.81**
Group5 [mo400]	5.5 ± 0.54ns	7.5 ± 0.54 **	9.33 ± 1.03**	12.00 ± 0.89**

* p<0.01 – significant, **P<0.001 -- Highly significant, values are Tail Flick Latency time in Seconds

Hypoglycemic Activity:

Table 6. Effect of Ethanolic Leaf Extract of *Moringa oleifera* on Blood Glucose Levels Of Alloxan Induced Diabetic Albino Wistar Rats.

Groups	0day	7 th day	14 th day	21 h day
Group 1[control]	285.16 ± 31.42 ns	317.83 ±12.96 ns	309.5 ± 14.39 ns	294.83 ± 3.71 ns
Group 2[std]	294.33 ± 11.41 ns	160.83 ± 6.62**	118.66 ± 7.20**	98 ± 5.47**
Group3[mo100]	293.16± 14.4 ns	251 ± 10.78*	213 ± 3.28*	168 ± 6.56**
Group4[mo200]	277.33 ± 11.57 ns	181.5 ± 5.35**	178.16 ±10.43**	164.83 ± 8.47**
Group5[mo400]	264 ± 11.45 ns	161.16 ± 5.74 **	154.33 ± 5.38	145.50 ± 2.33**

Values are * p<0.01 – significant, **P<0.001 -- Highly Significant ns – non-significant

Hepatoprotective Activity

For Evaluation of Hepatoprotective activity carbon tetrachloride(CCL4) was used to induce hepatotoxicity. Prior to Induction by carbon Tetrachloride for 21 days in group 1(control) Normal saline was administered orally. In group 2 (standard) Liv 52, 0.216 ml/kg orally was administered.

In group 3 Ethanolic Extract of *Moringa oleifera* in dose of 100 mg/kg was administered orally. In group 4 Ethanolic Extract of *Moringa oleifera* in dose of 200 mg/kg was administered orally. In group 5 Ethanolic Extract of *Moringa oleifera* in dose of 400 mg/kg was administered orally.

After induction of hepatotoxicity in group 1(control) Normal saline was administered orally. In group 2 (standard) Liv 52 0.216 ml/kg orally was administered. In group 3 ethanolic extract of *Moringa oleifera* in dose of 100 mg/kg was administered orally. In group 4 ethanolic extract of *Moringa oleifera* in dose of 200 mg/kg was administered orally. In group 5 ethanolic extract of *Moringa oleifera* in dose of 400 mg/kg was administered orally for 21 days.

The results observed from serum biochemical parameters with respect to induction of hepatotoxicity using Carbon Tetrachloride (CCl4) are given in table below. A marked reduction in SGOT, SGPT, Serum Bilirubin and Alkaline Phosphatase Levels was observed in the group treated with ethanolic extracts of *Moringa oleifera* and Standard group ($P < 0.05$) when compared with the control group. Rats in group 1 (control) developed significant liver damage and it was well indicated by elevated levels of hepato specific enzymes like Serum SGOT, SGPT, ALP and Serum BILIRUBIN.

Table 7. Effect of Extract of *Moringa oleifera* Leaf Extract on SGOT, Alkaline Phosphatase and Serum Bilurubin.

Sr. No.	Groups	SGOT	ALKALINE PHOSPHATASE	Sr. Bilirubin
1	Control	265 ± 4.75ns	328.00±12.06 ns	5.20 ± 1.65ns
2	standard	231.66±14.33**	211.5±12.01**	2.51 ±0.92**
3	MO 100 mg	242.66 ± 11.63*	258.83 ± 6.11*	3.18 ± 1.07*
4	MO 200 mg	237.66 ± 4.16*	243.16 ± 1.15*	2.75 ±1.02**
5	MO 400 mg	202 ± 13.63**	221.33±11.01**	2.6 ±0.92**

Values are * p<0.01 – significant, **P<0.001 -- Highly Significant ns – non-significant

Effect on serum Glutamate Oxaloacetate Transaminase (SGOT) levels-

In group 1[control] Rats had serum SGOT level of (265 ± 4.75 IU/L) when measured on day 21. In group 2[standard] Rats had serum SGOT level of (231.66±14.33 IU/L). In group 3[MO 100] Rats had serum SGOT level of (242.66 ± 11.63 IU/L). In group 4 [MO 200] Rats had serum SGOT level of (242.66 ± 11.63 IU/L). In group 5[MO 400] Rats had serum SGOT level of (242.66 ± 11.63 IU/L). There was significantly higher difference in SGOT Levels in standard group (P<0.05) when compared to serum SGOT levels in control rats (265 ± 4.75 IU/dl).

Effect on serum Alkaline Phosphatase (ALP) levels-

In group 1[control] Rats had serum ALP level of (328.00 ±12.06 IU/L) when measured on day 21. In group 2[standard] Rats had serum ALP level of (211.5 ±12.01 IU/L). In group 3[MO 100] Rats had serum ALP level of (258.83 ± 6.11 IU/L). In group 4 [MO 200] Rats had serum ALP level of (243.16 ±1.15 IU/L). In group 5[MO 400] Rats had serum ALP level of (221.33 ± 11.01 IU/L).

There was significantly higher difference in ALP Levels in standard group (P<0.05) when compared to serum SGOT levels in control rats (328.00 ±12.06 IU/dl).

Effect on serum Bilirubin total levels-

In group 1[control] Rats had serum Bilirubin level of (5.20 ±1.65 IU/L) when measured on day 21. In group 2[standard] Rats had serum Bilirubin level of (2.51 ± 0.92 IU/L). In group 3[MO 100] Rats had serum Bilirubin level of (3.18 ± 1.07 IU/L). In group 4 [MO 200] Rats had serum Bilirubin level of (2.75 ±1.02 IU/L). In group 5[MO 400] Rats had serum Bilirubin level of (2.60 ± 0.92 IU/L).

There was significantly higher difference in serum Bilirubin Levels in standard group (P<0.05) when compared to serum Bilirubin levels in control rats (5.20 ± 1.65 IU/dl).

HISTOPATHOLOGICAL EXAMINATION

Group 1:

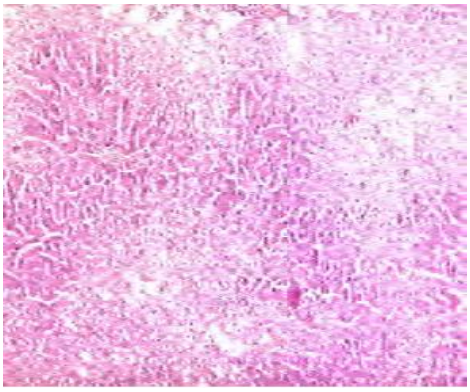

Fig 1. Histopathology of Liver in Group 1

The histology of the liver rat (**group I**) shows wide spread inflammation, vascular congestion, dilated sinusoidal spaces and focal necrosis.

Group 2:

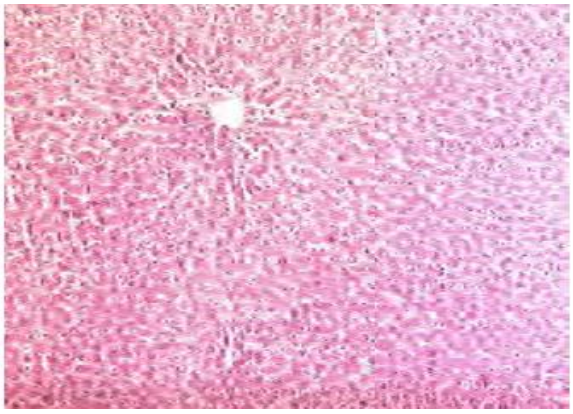

Fig 2. Histopathology of liver in group 2

The histological examination of the liver tissues in rats treated with liv 52 (**group II**) showed significant improvement in the liver tissue. Only minor distortion in architecture and vacuolation was observed.

Group 3:

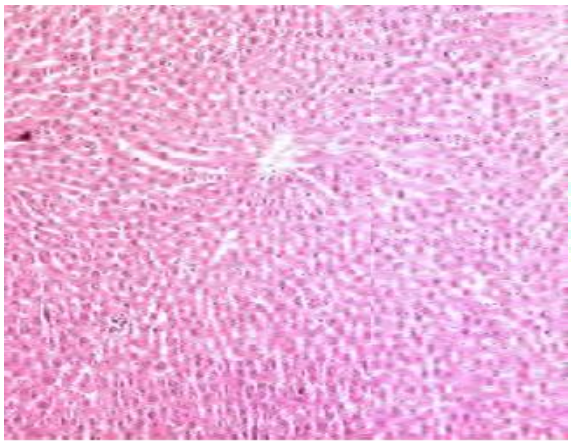

Fig 3. Histopathology of liver in group 3

The histological examination of the liver tissues in rats treated with *ethanolic leaf* extract 100 mg /kg (**group III**) showed improvement in the liver tissue. Minor vacoulation in the cytoplasm of the hepatocytes and minor focal hepatocellular necrosis was observed.

Group 4:

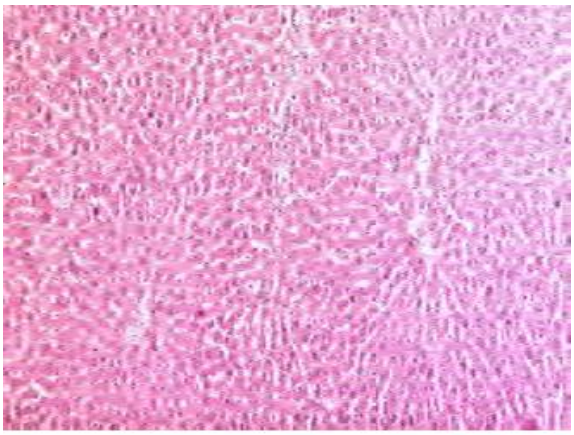

Fig 4. Histopathology of liver in group 4

The histological examination of the liver tissues in rats treated with ethanolic leaf extract Moringa 200 mg /kg (**groups IV**) showed very minor change in cytoplasm of hepatocytes; Sinusoids begin to appear; Normal architecture of the liver in some areas.

Group 5:

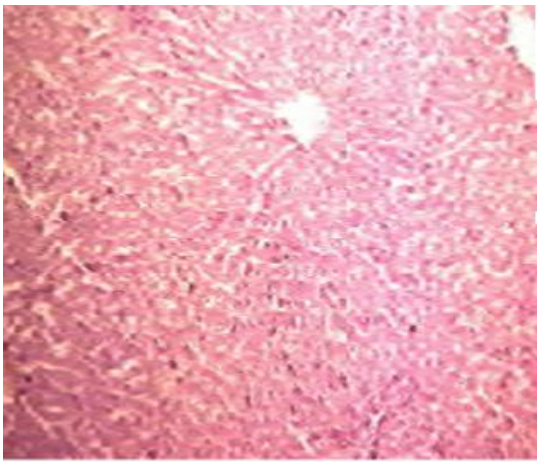

Fig 5. Histopathology of liver in group 5

The histological examination of the liver tissues in rats treated with ethanolic leaf extract of *Moringa oleifera* 400 mg / kg (**groups V**) showed significant improvement in the liver tissue. Only minor distortion in architecture and vacuolation is observed

DISCUSSION:

The aim of research is to find out new herbal origins from plants, which are potent and non-toxic agents. These plants are traditional medicinal plants. Generally herbal drugs are free from side effects /adverse effects and are low cost medicines which will be beneficial for the people of our country. The improvement in quantity control and standardization of herbal drugs and their products have led to the development of effective quality medicines from plants. In present investigation, we have not only authenticated the leaf of *Moringa oleifera* but also standardized the leaf extract with various physical parameters like total ash, acid insoluble ash, loss on drying, water and ethanol soluble extractive values.

The study shows that plant contains phytochemicals like phenolic, flavonoids and tannins, glycosides; saponins could be responsible for the potential activity of the plant.

The ability of the extract to increase tail flick latency period confirms the analgesic activity of the extract. The data collectively indicates the ethanolic extract of *Moringa oleifera* possesses analgesic properties, which are probably mediated by both central and peripheral inhibitory mechanisms as well as via inhibition of prostaglandin synthesis. The *Moringa oleifera* plant can therefore be proposed to have a potential benefit in the management of pain.

In conclusion animal models have demonstrated the analgesic effects of *Moringa oleifera* leaves. Many phytochemicals may be involved in the anti-inflammatory process. Among them, quercetin and many other bioactive compounds such as other like flavonoids and phenolic acids naturally present in *Moringa oleifera* leaves, may be involved in to analgesic. [16] Further studies should be devoted to investigate the potential analgesic and anti-inflammatory action and the mechanism of action of other bioactive compound naturally present in *Moringa oleifera* leaves. Finally, human studies are needed to evaluate the anti-inflammatory and analgesic properties of *Moringa oleifera* leaves also in human beings.

The Alloxan induced Diabetes Mellitus causes necrosis of islets of Langerhans which leads to β cell destruction. The herbal drug ethanolic extract of *Moringa oleifera* in different doses were given to alloxan induced rats for 21 days treatment. The study shows that the Ethanolic extract of *Moringa oleifera* possess antidiabetic activity.

From the result, the present study indicates that oral administration of ethanolic extract of *Moringa oleifera* dose dependently improved the blood glucose profile in three weeks treatment in diabetic rats. Hence the ethanolic extract of *Moringa oleifera* plant leaves have a hypoglycemic potential.

Scientific evidences suggest a potential use of *Moringa oleifera* leaves in the treatment of diabetes. Phytochemicals which are isolated from *Moringa oleifera* leaves may be involved in the glucose homeostasis. [17] Isothiocyanates one of the phytochemical is known to reduce insulin resistance and hepatic gluconeogenesis from previous phytochemical data. Some polyphenol compounds are abundantly found in *Moringa oleifera* leaves, like phenolic acids and flavonoids, which may have led to its effects on glucose homeostasis. [18]

These compounds exert anti-diabetic effects targeting various cellular signaling pathways in pancreas, liver, skeletal muscle and white adipose tissue. The phytochemical may exert their influence on β-cell mass and function, as well as insulin sensitivity in peripheral tissues. [19] Their effects may be due to antioxidant, enzyme inhibition, receptor agonist or antagonist activity or through novel mechanisms yet to be elucidated. Phenolic compounds, flavonoids and tannins may be also involved in the ability of *Moringa oleifera* leaves extract to inhibit the intestinal sucrose and modulating the pancreatic α-amylase actions. [20]

Our studies have shown the hypoglycemic effects of *Moringa oleifera* leaves, but further larger randomized studies controlled for large population with respect to factors, such as sex, age, race, nutritional status and dietary habits in human are required before using the leaves as herbal drug for the treatment of diabetes.

Liver is an organ with multiple functions of the body which are concerned with regulation of internal chemical environment. [21] Therefore, damage to the liver can be fatal in long term. There is an ever-increasing need for an agent which could facilitates regeneration by the proliferation of liver parenchymal cells and arrests growth of fibrous tissue.

The hepatoprotective activity was screened in carbon tetrachloride (CCl4) induced Albino rats of Wistar strain models. The various biochemical parameters like serum glutamic oxaloacetic transaminase, serum alkaline phosphatase and total bilirubin were estimated in our study. The hepatoprotective activity was further strongly strengthened by the histopathological studies of the liver.

Our study we found the hepatoprotective activity of the *Moringa oleifera* extract. The results of evaluation of hepatoprotective activity indicated that the ethanolic extract possesses significant hepatoprotective activity at the dose levels of 200 mg/kg and 400 mg/kg body weight. However, it was less as compared to the standard drug liv 52 which was used as a standard in our study.

From the above results, it may be concluded that the ethanolic leaf extract of *Moringa oleifera* is non-toxic and safe. As the results indicated that the extract possesses significant hepatoprotective activity the plant can be considered as a low cost, potent, herbal medicine for treatment of liver disorders.

CONCLUSION:

It can be concluded that the Ethanolic Leaf Extract of *Moringa Oleifera* possess Analgesic, Hypoglycemic and Hepatoprotective activities.

REFERENCES:

1. V. Subhose, P. Srinivas, and A. Narayana, "Basic principles of pharmaceutical science in Ayurvĕda," Bulletin of the Indian Institute of History of Medicine, vol. 35, no. 2, pp. 83–92, 2005
2. World Health Organization (WHO). National Policy on Traditional Medicine and Regulation of Herbal Medicines. Geneva: 2005. Report of WHO global survey.
3. Anwar F., Latif S., Ashraf M., Gilani A. H. (2007). *Moringa oleifera*: a food plant with multiple medicinal uses. Phytother. Res. 21, 17–25
4. Senthilkumar A, Karuvantevida N, Rastrelli L, Kurup SS, Cheruth AJ. Traditional Uses, Pharmacological Efficacy, and Phytochemistry of *Moringa peregrina* (Forssk.) Fiori. -A Review. *Front Pharmacol*. 2018; 9:465. Published 2018 May 11. doi:10.3389/fphar.2018.00465
5. El-Awady M. A., Hassan M. M., El-Sayed S. A. H., Gaber A. (2016). Comparison of the antioxidant activities, phenolic and flavonoids contents of the leaves-crud extracts of *Moringa peregrine* and *Moringa oleifera*. Int. J. Biosci. 8, 55–62.
6. Jahn S. A. A. (1996). On the introduction of a tropical multipurpose tree to China traditional and potential utilisation of *Moringa oleifera*Lamark. Senckenb. Biol. 75, 243–254.
7. Manzoor M., Anwar F., Iqbal T., Bhnager M. I. (2007). Physico-chemical characterization of *Moringa concanensis* seeds and seed oil. J. Am. Oil Chem. Soc. 84, 413–419.
8. Ullah M. F., Bhat S. H., Abuduhier F. M. (2015). Antidiabetic potential of hydro-alcoholic extract of *Mringa peregrina* leaves: implication as functional food for prophylactic intervention in prediabetic stage. J. Food Biochem. 39, 360–367.
9. Koheil M. A., Hussein M. A., Othman S. M., El-Haddad A. (2013). *In-vivo* antioxidant activity of *Moringa peregrina* against STZ – induced oxidative stress in type 2 diabetic rats. Mol. Clin. Pharmacol. 4, 65–75.
10. Elbatran S. A., Abdel-Salam O. M., Abdelshfeek K. A., Nazif N. M., Ismail S. I., Hammouda F. M. (2005). Phytochemical and pharmacological investigations on *Moringa Peregrina* (Forssk) Fiori. Nat. Prod. Sci. 11, 199–206.
11. Elabd E. M. Y., Zahran H. A., Abdalla A. M. (2017). A comparative study of the effects of three *Moringa* species on obesity- induced oxidative stress state in liver tissue. Int. J. Pharma. Bio Sci. 8, 572–584.
12. Azim S. A. A., Abdelrahem M. T., Said M. M., Khattab A. (2017). Protective effect of *Moringa peregrina* leaves extract on acetaminophen -induced liver toxicity in albino rats. Afr. J. Trad. Complement. Altern. Med. 14, 206–216.
13. Deraedt R., Joughney S., Delevakee F. and Falhour M. Release of prostaglandin E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol.1980; 51: 17-24.
14. Ahmed M. F., Kazim S. M., Ghori S. S., Mehjabeen S. S., Ahmed S. R., Ali S. M., et al. . (2010). Antidiabetic activity of *Vinca rosea* extracts in alloxan-induced diabetic rats. Int. J. Endocrinol. 2010:841090. 1
15. Sharma A., Chakraborti K. K., Handa S. S. (1991). Anti-hepatotoxic activity of some Indian herbal formulations as compared to silymarin. Fitoterapia 62, 229–235.
16. Medhi B., Khanikor H.N., Lahon L.C., Mohan P. and et al., Analgesic, antiinflammatory and local anaesthetic activity of Moringa in laboratory animals. Int. J. Pharmacogn. 1996; 34: 3: 207-212.
17. Tende J.A., Ezekiel I., Dikko A.A.U. and Goji A.D.T. Effect of Ethanolic Leaves Extract of Moringa oleifera on Blood Glucose Levels of Streptozocin-Induced Diabetics and Normoglycemic Wistar Rats. Br. J. Pharm. Toxicol. 2011; 2:1: 1-4.
18. Jaiswal D, Rai PK, Mehta S, et al. Role of Moringa oleifera in regulation of diabetes-induced oxidative stress. Asian Pac J Trop Med 2013; 6: 426-32.
19. Kumari DJ. Hypoglycaemic effect of Moringa oleifera and Azadirachta indica in type 2 diabees mellitus. Bioscan 2010; 5:211-4.
20. Omodanisi EI, Aboua YG, Chegou NN, Oguntibeju OO. Hepatoprotective, Antihyperlipidemic, and Anti-inflammatory Activity of Moringa oleifera in Diabetic-induced Damage in Male Wistar Rats. Pharmacognosy Res 2017; 9:182-7.
21. Almazroo OA, Miah MK, Venkataramanan R. Drug Metabolism in the Liver. Clin Liver Dis. 2017 Feb; 21(1):1-20.