International Journal of Medicine and Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijmpr

R E S E A R C H A R T I C L E

Evaluation for Hepatoprotective Activity of Methanolic Extracts of *Murraya Koenigii* in Mice

Ramidi Ramadevi[1], Dr. Y. Sridhar[2], Dr. Vijaya Kumar Gampa[3]

[1]*KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.*
[2]*Professor, KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.*
[3]*Professor and Head, Dept. of Pharmacy, KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.*

A B S T R A C T

The present study has been designed to achieve the following aims and objectives. To evaluate the hepatoprotective activity of *Murraya koenigii* on Azathioprine induced oxidative stress in mice. On the basis of our findings, it may be worthy to suggest that *Murraya koenigii* has antioxidant activity against Azathioprine induced oxidative stress in mice by decreasing the oxidative stress biomarkers serum AST, serum ALT in liver *Murraya koenigii* has antioxidant effect, elevated by measuring antioxidant enzymes. There is increase in superoxide dismutase in liver tissue in standard and test extracts treated mice.

Keywords: *Murraya koenigii*, Azathioprine

A R T I C L E I N F O

Corresponding Author
Dr. Gampa Vijay Kumar
Professor and Head, Dept. of Pharmacy,
KGR Institute of Technology and Management, Rampally,
Kesara, Rangareddy, Telangana, India
MS-ID: IJMPR4087

PAPER-QRCODE

A R T I C L E H I S T O R Y: Received 12 June 2019, **Accepted** 27 August2019, **Available Online**10 October 2019

Citation: *Gampa Vijay Kumar, et al.* Evaluation for Hepatoprotective Activity of Methanolic Extracts of *Murraya Koenigii* in Mice. *Int. J. Med. Pharm. Res., 2019, 7(6):202-206.*

CONTENTS

1. Introduction. 202
2. Materials and Method. 203
3. Results and Discussion. 205
4. Conclusion. 206
5. References. 206

1. Introduction

The liver is one of the most important organs of the body. It performs a fundamental role in the regulation of diverse physiological processes, and its activity is related to different vital functions, such as metabolism, secretion, and storage. Its capacity to detoxify endogenous (waste metabolites) and/or exogenous (toxic compounds) substances of organisms, as well as for synthesize useful agents, has been analyzed since the 1970s by many researchers. The liver is also involved in the biochemical processes of growing, providing nutrients, supplying

energy, and reproducing. In addition, it aids in the metabolism of carbohydrates and fats, in the secretion of bile, and in the storage of vitamins.

Because of all of these functions, hepatic diseases continue to among the principal threats to public health, and they are a problem worldwide. Hepatic disease is a term that indicates damage to the cells, tissues, structure, or liver function, and this damage can be induced by biological factors (bacteria, virus, and parasites) and autoimmune diseases (immune hepatitis, primary biliary cirrhosis), as well as by the action of different chemicals, such as some drugs [high doses of paracetamol (PCM) and antitubercular drugs], toxic compounds [carbon tetrachloride (CCl4), thioacetamide, dimethylnitrosamine (DMN), D-galactosamine/lipopolysaccharide (GalN/LPS)], and unquestionably, excessive consumption of alcohol.Despite enormous advances in modern medicine, there are no completely effective drugs that stimulate hepatic function, offer complete protection to the organ, or aid in regenerating hepatic cells. Additionally, some drugs can induce adverse or side effects. Thus, it is necessary to identify alternative pharmaceuticals for the treatment of hepatic diseases, with the aim of these agents being more effective and less toxic.

The use of some plants and the consumption of different fruits have played fundamental roles in human health care. Approximately 80% of the world's population has employed traditional medicine for health care, which is based predominantly on plant materials. Diverse scientific investigations of medicinal plants and the ingestion of fruits have indicated that the properties that are responsible for their beneficial effects could be attributed to the presence of chemical compounds or substances that are biologically active and that are non-essential nutrients for life, called phytochemicals.

In the literature, studies can be found that have examined the impact that different phytochemicals exert on health Among the most frequently cited examples, we find the following: (1) the vinca alkaloids (vincristine, vinblastine, and vindesine); (2) the betalain pigments (betanin and indicaxanthine); (3) the anthocyanins (cranberries); and (4) and resveratrol; all of these have generally been analyzed based on their chemo protective properties against cancer. All of the medicinal plants, as well as the ingestion of certain fruits, have demonstrated different effects on living systems. Although there have been diverse studies directed toward the evaluation of their hepatoprotective potential, the majority of investigations have been directed at analysis of their sedative, analgesic, antipyretic, cardioprotective, antibacterial, antiviral, antiprotozoal, and anticarcinogenic capacities.

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Today, we are witnessing a great deal of public interest in the use of herbal remedies. Furthermore many western drugs had their origin in plant extract. There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. Given their potential to produce significant therapeutic effect, they can be useful as drug or supplement in the treatment / management of various diseases. Herbal drugs or medicinal plants, their extracts and their isolated compound(s) have demonstrated spectrum of biological activities. Such have been used and continued to be used as medicine in folklore or food supplement for various disorders. Ethno pharmacological studies on such herbs/medicinally important plants continue to interest investigators throughout the world.

The leaves, the bark and the roots of *Murraya koenigii* (L.) Spreng. can be used as a tonic and a stomachic. The bark and the roots are used as a stimulant by the physicians. They are also used externally to cure eruptions and the bites of poisonous animals. The green leaves are stated to be eaten raw for curing dysentery, and the infusion of the washed leaves stops vomiting.A strong odiferous oil occurs in the leaves and the seeds of *Murraya koenigii* (L.) Spreng. The chemical examination of this oil has been made by Nigam and Purobit (1961). Gautam and Purobit (1974) reported that this essential oil exhibited a strong antibacterial and antifungal activity.An alkaloid, murrayacinine, is also found in this plant.

Materials

Trichloroacetic acid (TCA), nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH), phenazinemethosulfate (PMS), ferrozine, glutathione reduced, bathophenanthrolinesulfonate disodium salt, Thiobarbituric acid (TBA), and 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Hydrogen peroxide, ammonium iron (II) sulfatehexahydrate [$(NH_4)_2Fe(SO_4)_26H_2O$], 1-chloro-2,4-dinitrobenzene (CDNB), chloramine-T, hydroxylamine hydrochloride, Dimethyl-4-aminobenzaldehyde, and 2,4-dinitro phenylhydrazine (DNPH) were obtained from Merck, Mumbai, India. Ferritin was purchased from MP Biomedicals, USA. Streptomycin sulphatewas obtained from HiMedia Laboratories Pvt. Ltd, Mumbai, India. The standard oral iron-chelatingdrug, desirox, was obtained from Cipla Ltd., Kolkata,India.

2. Materials and Methods

Collection and Authentification of Plant Material

The leaves of *murraya koenigii* is collected and authenticated by Dr K Madhava Chetty, Department Of Botany, Sri Venkateswara University, Tirupati.

Extraction of Plant Material

The leaves are grinded in to a coarse powder with the help of suitable grinder.

Cold Extraction (Methanol Extraction)[40,41]

In this work the cold extraction process was done with the help of methanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of methanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent(drying procedure)

The filtrates (methanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum desiccator for 7 days[42].

Preliminaryphytochemicalscreening[43,44]

Freshly prepared leaf extract of plant were tested for the presence of phytochemical constituents by using reported methods.

Acute toxicity studies[45]

The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organization for Economic Co-operation and Development) guidelines no 423. Female Albino Swiss mice(25-35 g) were taken for the study and dosed once with 2000 mg/kg. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing the 2000 mg/kg dose to be safe. Thus, 1/10 and 1/20 doses of 2000 mg/kg i.e. 200 mg/kg and 100 mg/kg were chosen for subsequent experimentation.

Induction procedure[46,47,48]**:-**

Induction of oxidative stress:-

50mg/kg. of Azathioprine solution was given through i.p route to all the group of animals and the samples were collected from the animals through retro-orbital plexus root and the liver, kidney bio marker parameters were estimated like SGOT, SGPT.

Experimental design:-

The animals were assigned to five groups, each group containing six mice (weight-20-25gm):

Group I: mice were orally administered with normal saline (1.2ml/day) for 21days as the normal control.

Group II: mice were administered with Azathioprine (50mg/kg. I.P)(single dose)

Group III: mice treated with *Murraya koenigii* (100mg/kg) by oral for 21days.administered with Azathioprine (50mg/kg. I.P)(single dose)

Group IV: mice treated with *Murraya koenigii* (200mg/kg) by oral for 21days.administered with Azathioprine (50mg/kg. I.P)(single dose)

Group V: group-IImice treated with ascorbic acid (10mg/kg) by oral for 21days.administered with Azathioprine (50mg/kg. I.P)(single dose)

Collection of blood samples and liver[16-18]:

Blood samples were collected from all the groups of animals 24hours after the 21st day of treatment through puncture of retro orbital plexus and were centrifuged at 3000 revolutions per minute (RPM) for 15 minutes. Serum was separated and stored at-20 c and used for estimating SGOT, SGPT, levels. mice were killed by over anesthesia. A midline abdominal incision is made to open up the abdominal cavity and access the liver. The liver are removed rapidly and washed with saline. Then fixed quickly in formaldehyde. The liver was homogenized in 0.25 M cold sucrose solution and centrifuged at 5000 rpm for five minutes. The supernatant which is store at -20^0C used for the quantitative estimation of superoxide dismutase within 48hours by using UV spectrophotometry.

Estimation of biochemical parameters:-

The following are the biochemical parameters estimated to evaluate the effect of the test materials against the experimentally induced oxidative stress in mice. They are SOD, ALT (SGPT), AST (SGOT).

Estimation of Superoxide Dismutase (SOD)[41,42]

Superoxide dismutases are the enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen.

Extraction procedure: 3ml of packed blood cells were lysed by the addition of equal volume of cold deionized water. Hemoglobin was then precipitated by the addition of chloroform: methanol (1.5:1). This was diluted with 500µl of water and centrifuged for 15 minutes at 3000 rpm. The supernatant containing SOD was taken for the measurement of its activity.

Assay procedure: 0.88ml of riboflavin solution (1.3×10^{-5} M in 0.01M potassium phosphate buffer, pH 7.5) was added to 60µl of O-dianisidine solution (10^{-2} M in methanol) and to this 100µl of clear separated SOD was added and optical density was measured at 460nm. Then the cuvette containing reaction mixture was transferred to the illuminating box, illuminated for 4min., and optical density was remeasured against blank containing methanol in place of enzyme. The change in the optical density was determined. The SOD content was determined from the standard graph prepared using pure bovine SOD.

Serum Glutamate Pyruvate Transaminase (SGPT)[43,44]

Alanine transaminase present large amounts in liver, kidney, heart and skeletal tissues. It is also present in spleen, lungs, pancreas, brain and erythrocytes at lower concentration. Primary to liver damages and secondary to other causes result in elevated levels of GPT.SGPT converts L- Alanine and α- ketoglutarate to pyruvate and Glutamate. The pyruvate formed reacts with 2,4, Dinitrophenyl hydrazine to procedure a hydrazone derivative, which in an alkaline medium produces a brown coloured complex whose intensity is measured. The reaction does not obey Beer's law and hence a calibration curve is plotted using a pyruvate standard. The activity of SGPT (ALAT) is read off this calibration curve

Serum Glutamate Oxaloacetic Transaminase (SGOT)

Aspartate transaminase is present in all human tissues of the body. It also presents large amounts in liver, kidneys, heart and skeletal muscles. Elevated levels are associated with liver disease or damage, myocardial infraction, muscular dystrophy. In myocardial infraction GOT levels increase after 3-8 hours of onset of attack and returns to normal in 4-6 weeks. The duration and extent of increase in levels is

proportional to the severity of attack.SGOT converts L-Aspartate and α- ketoglutarate and Glutamate. The oxaloacetate formed reacts with 2,4, Dinitrophenyl hydrazine to procedure a hydrazone derivative, which in an alkaline medium produces a brown coloured complex whose intensity is measured. The reaction does not obey Beer's law and hence a calibration curve is plotted using a pyruvate standard. The activity SGOT (AST) is read off this calibration curve *(Excel diagnostics Pvt, Ltd,Hyd, India).*

Estimation of serum bilirubin

About 0.2 ml of serum was taken in a tube and to this 1.0 ml of water was added. A 0.5 ml of HCl was added to the blank tube. To the sample tube, 2.5 ml of mmethanol and 0.5 ml of diazo reagent were added. The tubes were incubated at room temperature for 30 minutes. The development of blue color in the reaction mixture was read at 540 nm in a Shimadzu spectrophotometer, along with bilirubin calibrator solutions. The values were expressed as mg/dl.

Statistical analysis:

All the values were expressed as mean ±standard deviation (S.D). Statistical comparisons between different groups will be done by using one way analysis of variance (ANOVA) followed by dunnett's test. P <0.05 will be considered as statistically significant[36-38].

3. Results and Discussion

Percentage of Yield

% yield of ethanol extract = (weight of extract)/(powder taken for extraction) × 100

= 20 /200 ×100 = 10%.

% Yield of the *Murraya koenigii* is found to be **10.0**

Table 1: Results of Phytochemical Analysis Ethanolic Extract of *murraya koenigii*

Name of the Phytochemical Constituents	Ethanol extract
Saponins	-
Alkaloid	+
Glycoside	_
Reducing sugar	+
Tannin	+
Flavonoid	++
Steroid	_
Anthocyanin	_
Phenol	+
Amino acid	_
Protein	++

+: Indicates the presence and -: Indicates the absence of phytoconstituents

***In-Vivo* Studies**

Superoxide dismutase:

Superoxide dismutase is class of enzyme that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. It is an important antioxidant defence in nearly all cells exposed to oxygen. Superoxide dismutase activity was estimated in tissue homogenate with help of pure bovine superoxide dismutase standard.

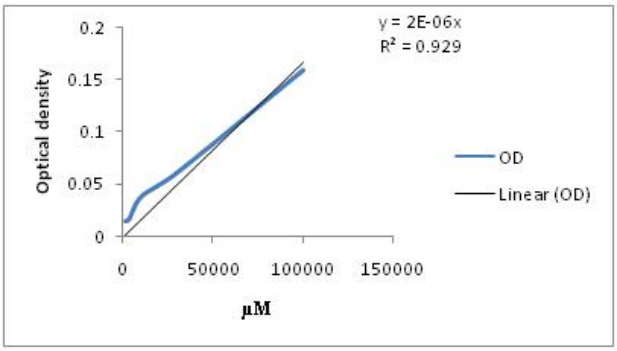

Figure 1: Standard graph of superoxide dismutase

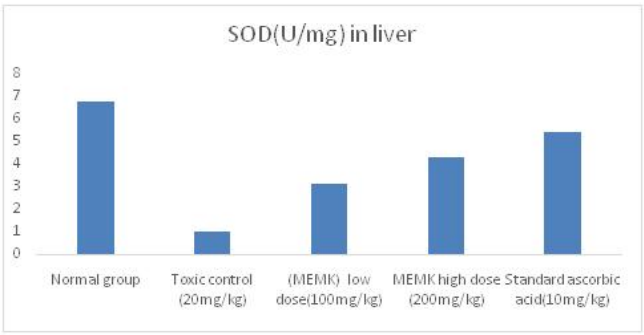

Figure 2: Effect of MEMK on superoxide dismutase levels in liver and kidney tissue homogenate in mice treated with AZP.

In this study, we found that 50mg/kg dose of azathioprine causes significant (p<0.001) decrease in superoxide dismutase levels. This reduction indicates that oxidative stress and toxicity is produced with azathioprine. Post treatment with MEMK at the dose of 100mg/kg and 200mg/kg after a 50mg/kg dose of azathioprine administration, shown a significant (p<0.001, p<0.0001) dose dependent increase in levels compared to toxic control group.

Serum Alanine Aminotransferase (ALT):

Azathioprine and test compound effects on ALT in mice from various groups shown in following figure. Measurements of ALT levels in AZP intoxicated female albino mice, and treated mice with MEMK indicate the effect of treatment. The normal control group ALT level show 132.65±1.28IU/L. After AZP treatment, the ALT level is 201.3± 22.5IU/L. This AZP treated group ALT level was increased compared to the normal control group in 21days. After 21days treatment, the *Murraya koenigii* low dose ALT level was (174.9± 9.71IU/L) decreased compared to the toxic control group has shown significance (**p<0.001) and at high dose ALT level was (150.2± 7.5IU/L) decreased compared to the toxic control group has shown significance (***p<0.0001). On treatment standard ascorbic acid serum ALT level 141.1 ± 8.8, has shown significant (***p<0.0001).

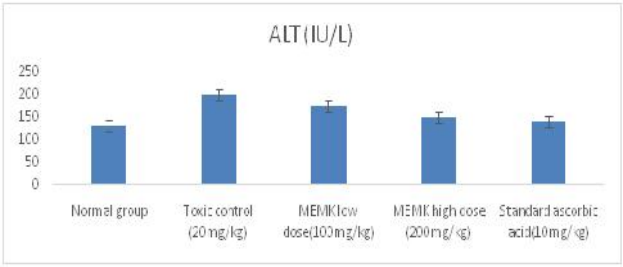

Figure 3: Effects of MEMK on serum ALT levels in mice treated with azathioprine

Serum Aspartate Aminotransferase (AST):

The above table shows the effect of test compound on serum AST levels in mice intoxicated with AZP. After 21days, the normal control group shows the AST level is 140.7±10.43IU/L. In AZP control group level is 210.5± 11.5IU/L, increased compared to the normal group. Treatment with MEMK at low dose AST level was (160.0± 10.3IU/L) decreased compared to the toxic control group has shown significance (***p<0.0001) and at high dose AST level was (150.3± 9.92IU/L) decreased compared to the toxic control group has shown significance (***p<0.0001). On treatment standard ascorbic acid serum AST level 141.0± 8.06, has shown significant (***p<0.0001).

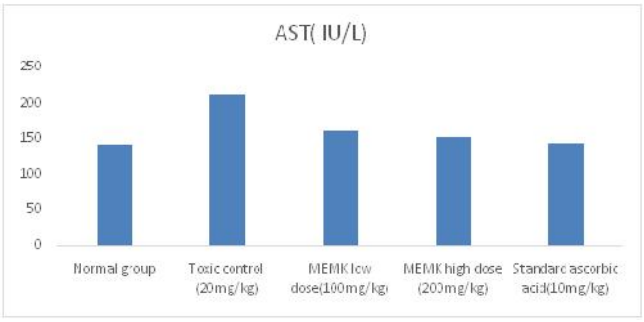

Figure 4: Effects of MEMK on serum AST levels in mice treated with azathioprine

4. Conclusion

On the basis of our findings, it may be worthy to suggest that *Murraya koenigii* has antioxidant activity against Azathioprine induced oxidative stress in mice by decreasing the oxidative stress biomarkers serum AST, serum ALT in liver. *Murraya koenigii*has antioxidant effect, elevated by measuring antioxidant enzymes. There is increase in superoxide dismutase in liver tissue in Azathioprine induced oxidative stress in mice.*Murraya koenigii*has hepatoprotective effect against Azathioprine induced toxicity in liver by observing the histopathological changes in mice liver tissue.

5. References

[1] Kartik J. Salwe, Mirunalini R., Jervin Mano, K. Manimekalai, Hepatoprotective and antioxidant activity of Murraya Koenigii leaves extract against paracetamol induced hepatotoxicity in Rats, http://www.ijbcp.com/index.php/ijbcp/article/view/1692.

[2] Debosreeghosh, syedbenazirfirdaus, elinamitra, hepatoprotective activity of aqueous leaf extract of murraya koenigii against lead-induced hepatotoxicity in male wistar rat, int j pharm pharmsci, vol 5, suppl 1, 285-295.

[3] SathayeSadhana, Amin Purnima, Mehta Vinam, Kaur Harpreet, Hepatoprotective Activity Of Murraya Koenigii Against Ethanolinduced Liver Toxicity Model In Experimental Animals, International Journal of Pharma and Bio Sciences,2012/02/01,430-438.

[4] Sathaye S, Bagul Y, Gupta S, Kaur H, Redkar R, Hepatoprotective effects of aqueous leaf extract and crude isolates of Murraya koenigii against in vitro ethanol-induced hepatotoxicity model, ExpToxicolPathol. 2011 Sep;63(6):587-91.

[5] Sherien Kamal Hassan, Nermin Mohammed El-Sammad1, AmriaMamdouh Mousa1,Maha Hashim Mohammed1, Abd el Razik Hussein Farrag2, Amani Nassir Eldin Hashim34, Victoria Werner,4Ulrike Lindequist, Mahmoud Abd El-Moein Nawwar3, Hypoglycemic and antioxidant activities of Caesalpinia ferreaMartius leaf extract in streptozotocin-induced diabetic rats, Asian Pac J Trop Biomed 2015, 5(6): 462–471

[6] Sambasivam Manikandaselvi, Vellingiri Vadivel and Pemaiah Brindha2, Murraya koenigii L.: A nutraceutical plant, Journal of Chemical and Pharmaceutical Research, 2015, 7(12):137-142

[7] Komal Moon, S. S. Khadabadi, U.A. Deokate, S.L. Deore, Murraya koenigii F-An Overview, Report and Opinion 2010;2(3)

[8] Karasawa, H., Takaishi, K. and Kumagae, Y. (2011), Obesity-Induced Diabetes in Mouse Strains Treated With Gold Thioglucose: A Novel Animal Model for Studying β-Cell Dysfunction. Obesity, 19: 514–521. doi:10.1038/oby.2010.171

[9] Kannur, Dayanand & Hukkeri, V.I. & Akki, K.S.. (2007). Antidiabetic activity of Murraya koenigii seed extracts in rats. Fitoterapia. 77. 546-9. 10.1016/j.fitote.2006.06.013.

[10] Chakrabarti S1, Biswas TK, Seal T, Rokeya B, Ali L, Azad Khan AK, Nahar N, Mosihuzzaman M, Mukherjee B, Antidiabetic activity of Murraya koenigii F. in chronic type 2 diabetic model in Long-Evans rats and evaluation of insulin secretagogue property of its fractions on isolated islets, J Ethnopharmacol. 2005, 10; 97(1):117-22.

[11] Sudeep Parameshwar, K.K. Srinivasan & C. Mallikarjuna Rao, Oral Antidiabetic Activities of Different Extracts of Murraya koenigii Seed Kernels, Pharmaceutical Biology Vol. 40 , Iss. 8,2002.