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EFFECTIVE ALLEVIATION OF METHOTREXATE INDUCED HEPATOTOXICITY BY *MURRAYA KOENIGII* LEAF EXTRACTS

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ABSTRACT

Drug induced hepatotoxicity is a distressing cause of liver insult, since the liver is the prime site of metabolism and biotransformation. Hence the present investigation was aimed at evaluating the ameliorative action of potent herbal extracts of *Murraya koenigii* to counteract the drug induced liver injury. These extracts, aqueous and hydro-alcoholic are a rich source of bioactive phytochemicals known for its anti-inflammatory, antioxidant and immunomodulatory activity. Male albino mice weighing 35-40 gm were treated with *Murraya koenigii* (250mg/kg body weight) extracts against Methotrexate induced toxic model for 15 days. The body weight was insignificantly elevated following methotrexate dosing, however this change was reversed by administration of the *Murraya koenigii* extract. The decline in liver weight was evidence of the inhibitory effect of Methotrexate on liver biosynthetic activity. Although the extracts were found to restore the fall in liver weight, complete recovery in this gravimetric parameter was not observed. Biochemical analysis for key liver markers revealed a significant elevation in activity of liver transaminases, indicative of hepatic inflammation. Oral administration of both extracts could effectively curb the rise in ALT and AST, where the hydroalcoholic extract appeared to be more potent. In addition, the significant decline observed in the activity of enzymes involved in oxidative and energy metabolisms, was also efficiently ameliorated *by M. koenigii* extract treatment. Further metabolic insult due to Methotrexate dosing resulted in a significant fall in protein and cholesterol levels, reflecting inhibition of metabolic turnover. Both extracts were able to bring about a significant recovery in these metabolite levels, possibly expediting liver repair. Thus, the extracts being a rich source of antioxidants could lead to recovery, mitigating the induced toxic injury of Methotrexate, possibly by effectively scavenging the free radicals generated. It is also known that *Murraya koenigii* phytochemicals manifest anti- inflammatory effects by suppression of inflammatory inducers. Thus owing to the rampant, unsupervised use of medications, simultaneous administration of Murraya koenigii leaf extracts could provide the much required hepatoprotection to alleviate liver damage.

KEYWORDS: Methotrexate, Liver, Mice, *Murraya koenigii*.

INTRODUCTION

With the large number of environmental changes, increasing dependency on medication and higher consumption of food adulterants the liver being the site of metabolism and biotransformation is primarily at risk of suffering toxic injury.

Drugs are often the main cause of liver injury. More than 900 drugs, toxins and herbs have been reported to cause hepatic injury (Friedman *et al*., 2003). The drug induced injury could be induced through different ways including direct toxic effect; immunological reaction or through active metabolite that is formed by the drug (Kaplowitz, 2001). Certain drugs may cause liver injury when introduced even within the therapeutic ranges. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature. The liver plays a very important role in transforming and clearing chemicals which lead to increase its susceptibility to the toxicity from these agents (Swayeh *et al*., 2014). Therefore it is evident that the liver is a prime target of xenobiotics, drugs and toxins, since it directly carries out interaction with these substances.

In the present study therefore, the effects of drug induced injury on liver was evaluated. The drug selected for this study was Methotrexate, since it is widely used for varied illnesses, at low, moderate and high doses.

Methotrexate is prescribed for cases in Chemotherapy and also as an antimetabolite and immunosuppressant in rheumatoid arthritis. It has also been identified as moderately hepatotoxic. Tripathi *et al*. (2015) have reported that it has been used as a first line drug in many autoimmune diseases pemphigus, myasthenia gravis,

uveitis, chronic active hepatitis. A large number of patients are therefore using this drug, with potential risk to liver damage. The main reason for the selection of methotrexate in the present investigation is due to the significant adverse effects of this drug. The hepatotoxicity warrants repeated check for Liver function in patients on methotrexate. Long term use of methotrexate causes anaemia. Methotrexate seems to be hepatotoxic, nephrotoxic and toxic to respiratory and reproductive system at very low doses for continuous therapy (Patel *et al*., 2014). Ciralik *et al*. (2006) mentioned that methotrexate caused damage to the mucosa of small intestine leading to stomatitis, decreased absorption and gastrointestinal ulceration. Shanbhag *et al*. (2015) reported that methotrexate is contradicted in pregnancy, liver disease and peptic ulcer. Hence, hepatic function should be monitored periodically.

Hence it is imperative to identify a suitable, safe ameliorative agent to protect users against the associated hepatotoxicity of such drugs.

Medicinal plants have the richest source of bioactive compounds which are used in traditional and modern medicine. People prefer to use herbal drugs because they are considered as safe, inexpensive and have no adverse effects. *Murraya koenigii* is a highly valuable plant for its characteristic aroma and medicinal value and it is widely used in Indian cookery for centuries and have a versatile role to play in traditional medicine. Curry leaf also used in many of the Indian Ayurveda, Siddha, Unani, Amchi, and local health traditions (Gahlawat *et a*l., 2014).

In folk and traditional medicine, it is implicated in the treatment of several ailments including traumatic injury, diabetes, jaundice, stomachache and dysentery. It is reported to possess antibacterial, antidiabetic (Saha *et al*., 2013), antilipidemic (Kesari *et al*., 2007), antiulcer, antiinflammatory, wound healing, enzyme inhibiting, antioxidant (Sathaye *et al*., 2011), antidysenteric, immunomodulatory (Paul *et al*., 2011), anticarcinogenic activity (Darvekar *et al*., 2011; Sasidharan and Menon, 2010). Several study suggested that hepatoprotective effects of plant extracts are associated with their antioxidant plant constituents (Celik *et al*., 2009). *Murraya koenigii* manifests a hypolipidemic activity and plays a vital role in cytokine production as local cytokines production fluctuates during cutaneous wound, diabetes and in hyperlipidemia (Kumar *et al*., 2012). Studies carried out earlier at our laboratory indicated that *Murraya koenigii* manifested potent ameliorative action against Carbon tetrachloride induced gastrotoxicity (Highland *et al*., 2015). Hence, two leaf extract of this plant were selected in the present study to test its ameliorative action against selected drug- induced toxicity.

MATERIALS AND METHODS

The present study was focused on the ameliorative action of *Murraya koenigii* against drug induced toxicity, on certain target tissue (Liver) in male Swiss albino mice. Male albino mice weighing 35-40 gm were treated with *Murraya koenigii* against drug induced toxic model for 15 days.

Healthy, adult, pathogens free, male albino mice (Mus musculus) of Swiss strain, weighing between 35-40 gm were obtained from a recognized supplier. The experimental protocol and number of animals used for experiments were mentioned. The animals were housed in air conditioned animal house with 12h light and 12h dark cycles at a temperature of 26± 2ºC and relative humidity 30 -70%. Animals of different experimental groups were caged separately and maximum of five animals per cage were maintained on a standard chow obtained from Pranav Agro Industry, distilled water was given *ad libitum*. All the animals were acclimatized seven days prior to the commencement of the treatment. The treatment was given daily before feeding so as to avoid interference with food intake. Experiments were conducted in accordance with guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and experimental protocols were approved by Institutional animal ethics committee; CPCSEA Registration no.167/1999/CPCSEA.

Collection of plant material

The selected plant material (*Murraya koenigii* leaves) was collected from Gandhinagar, Gujarat, India, during the month of December-2015. Leaves were carefully selected and washed thoroughly with running tap water, followed by rinsing with distilled water, were shade dried at room temperature and then pulverized into powder and stored in air tight container till further use.

Authentication of the material (*Murraya koenigii* leaves) used in present study was obtained from the Principal Taxonomist, Department of Botany, Gujarat University, Ahmedabad.

Preparation of extract

The crude powder of the plant material (*Murraya koenigii* leaves) was then defatted with petroleum ether for about 24h. After defattation, the extraction was carried out using a soxhlet apparatus. Two extracts were prepared:

1) Hydro-methanolic extracts (methanol: water – 70:30)
2) Aqueous extracts

Animal groups

Adult male albino mice with body weights of 35-40 gm were used in the study. Treatment was administered for fifteen days. The animals were divided into groups as mentioned below:

Group I: control
Control animals were only given standard food pellets and distilled water.

Group II: methotrexate treated
Animals were injected methotrexate (10mg/kg body weight), intraperitoneally with the help of a 26 gauge needle to induce toxicity in the target tissue at every 48 hours for 15 days.

Group III : **methotrexate + aqueous leaf extract (aq) (250 mg/kg body weight).**

Group IV: methotrexate + hydro-methanolic leaf extract (hm) (250 mg/kg body weight).

Animals were injected intraperitoneally with methotrexate (10mg/kg body weight) along with aqueous and hydro-methanolic extracts.

GRAVIMETRIC ANALYSIS
Body and organ weights
The body weight of control and all the treated mice were recorded to the nearest milligram on a digital balance (Repetach). The animals were weighed before and at the end of each week prior to necropsy. Similarly, weights of organs were recorded to the nearest milligram on digital balance after necropsy (Citizen, Japan).

Data collection
At the end of each treatment, the animals were first weighed on digital balance (Repetac) and necropsied according to CPCSEA specification. Vital tissue liver was dissected out carefully and blood was collected for the serum to further analysis.

BIOCHEMICAL PARAMETERS
Alanine aminotransferase (ALT) (EC.2.6.1.2)
ALT was estimated from the serum by Modified UV (IFCC) method using Autospan Liquid Gold kit reagents in IFCC kit and results obtained on automated Biochemical Analyser.

Aspartate transaminases (AST) (EC.2.6.1.1)
AST was estimated from the serum by IFCC method (using Reckon Diagnostics Pvt. Ltd. IFCC kit and the results obtained on automated Biochemical Analyser. (Ladue *et al.*, 1954).

Phosphorylase (E.C. 2.4.1.1)
Phosphorylase activity in liver of control and all treated groups of animals was assayed by the method of Cori *et al.* (1943).

Alkaline phosphtase (ALKpase) (E.C.3.6.1.3)
Alkaline phosphatase activity was estimated in liver by the method of Bessy *et al.* (1946).

The enzymes alkaline phosphatase hydrolyses the substrate p-nitrophenyl phosphate into inorganic phosphate and p-nitrophenol. The quantity of p-nitrophenol released under standardized condition is measured at 410 nm.

Succinate dehydrogenase (E.C. 1.3.5.1)
The activity of SDH was estimated in liver of control and all treated groups of animals according to the method of Beatty *et al.* (1966) using 2(4- lodophenyl) 3-(4-Nitrophenyl) 5 – phenyl tetrazolium chloride (INT) as an electron acceptor.

Adenosine triphosphatse (ATPase) (E.C.3.6.1.3)
The ATPase activity in liver of control and all treated groups of animals was assayed by the method of Quinn and White (1968); while inorganic phosphate liberated was estimated using the method of Fiske and Subbarow (1925).

Total protein
Protein levels in liver of control and all treated groups of animals were estimated by the method of Lowry *et al.* (1951).

CHOLESTEROL
The levels of the cholesterol in liver of control and all treated groups of control and all treated groups of mice were estimated by the method of Zlatkis *et al*. (1953).

STATISTICAL ANALYSIS OF THE DATA
For each biochemical parameter, a minimum of 4-6 replicates were done. Values are expressed as Mean ±S.E. The Student's't' test was used to verify levels of significance at the $p<0.05$ value.

RESULTS
Body weight of Control and Methotrexate treated animals were monitored before and after during the Methotrexate treatment regimen:

As shown in Table 1, there was no significant alteration in body weight before and after the entire experiment in the control animals. Methotrexate administration did not cause significant increase in body weight, post treatment, however the reversal of body weight increase could be observed in the animals fed Methotrexate along with the *Murraya koenigii* leaf extract. The hydromethanolic extract appeared to have a more potent effect in mitigating this increase (Table 1).

Table 1: Showing body weight in control and Methotrexate treated mice and amelioration with aqueous and hydro-methanolic extracts of *Murraya koenigii* (leaf).

No.	GROUPS	BODY WEIGHT (B.T.) (gm)	BODY WEIGHT (A.T.) (gm)
1.	CONTROL	40 ± 2.2	42±3.6
2.	MTX (10 mg/kg)	44 ± 3.1$^{N.S}$	46±2.9^{NS}
3.	MTX +AQ (250 mg/kg)	35 ± 1.8$^{N.S}$	38±2.1^{NS}
4.	MTX +HM (250 mg/kg)	41 ± 4.7$^{N.S}$	42 ± 3.3 $^{N.S}$

N= 4 /group
Values are Mean±S.E
B.T. =Before treatment
A.T. =After treatment
N.S.= Not significant

Organ weight

Liver

Methotrexate caused a significant decrease in net liver weight. The extract administration did not manifest an increase as compared to the treatment Group. However, as compared to control complete recovery could not be observed (Table 2).

Table 2: Showing organ weight of liver in control and Methotrexate treated mice and amelioration with aqueous and hydro-methanolic extracts of *Murraya koenigii* (leaf).

No.	GROUPS	ORGAN WEIGHT (LIVER) (mg)
I	CONTROL	2502.9±0.98
II	MTX (10 mg/kg)	2033.8 ± 0.92$^{\#\#}$
III	MTX +AQ (250 mg/kg)	1136.0 ± 1.96**
IV	MTX +HM (250 mg/kg)	1354.4 ±1.33**

N=4 /group
Values are Mean±S.E
**P<0.001
= compared to control
* = compared to Mtx treated

The results obtained after assay of the activity of ALT, AST, Phosphorylase, ALKpase, SDH, ATPase and protein, cholesterol levels in liver of control (Group I) and Methotrexate treated (Group II) and extract treated mice (Groups III and IV) are mentioned below:

Alanine aminotransferase (ALT) activity

Serum transaminases were affected by administration of Methotrexate. As shown in Table 3, the results indicated that ALT level increased significantly ($p < 0.001$) after Methotrexate treatment, as compared to the control (Group I). Oral feeding of the aqueous extract of *Murraya koenigii* leaves brought about a significant recovery ($p<0.001$) in the liver ALT activity in Methotrexate treated animals. Similarly, the hydromethanolic extracts of *Murraya koenigii*, had a significant ameliorative action ($p<0.001$) and could bring the liver ALT levels back to normal values. However, no complete mitigation was seen after the administration of HM extract (Table 3).

Aspartate transaminase (AST) levels

Serum AST activity was also affected by Methotrexate dosing. As shown in Table 3, the results indicated that AST level increased significantly ($p<0.001$) after Methotrexate treatment, as compared to the control (Group I). Oral feeding of aqueous extract was able to significantly ($P<0.001$) ameliorate the toxic effect thus bringing the liver AST activity similar to control values. Similarly, the hydromethanolic extracts of *Murraya koenigii*, had a significant ameliorative action ($p<0.001$), and could lower the liver AST levels back to normal values. But complete mitigation was not observed after administration of extracts (Table 3).

Phosphorylase activity

Phosphorylase activity was significantly elevated ($p< 0.001$) in the Methotrexate treated animals (Group II) as compared to animals of the control Group I. However, administration of the extracts (aqueous and hydromethanolic) appeared to bring about a significant recovery in the Phosphorylase activity in the Group III and Group IV. The result however indicated that complete mitigation of the Methotrexate induced toxicity was not observed after both the extracts administration (Table 3).

Table 3: Showing activities of AST, ALT and Phosphorylase in liver of control and Methotrexate treated mice, with aqueous and hydro-methanolic extracts of *Murraya koenigii* (leaf).

No.	GROUPS	ALT (IU/L)	AST (IU/L)	PHOSPHORYLASE[a]
I	CONTROL	65.6 ±8.6	157.3 ±11.2	75.23±9.6
II	MTX (10 mg/kg)	280.9±12.5##	332.7±16.9##	90.66±11.2##
III	MTX +AQ (250 mg/kg)	61.5±9.2**	228.2±7.4**	84.23±9.4*
IV	MTX +HM (250 mg/kg)	87.7±10.1**	179.1±7.5**	81.66±8.3**

N= 4 /group a=µg of inorganic phosphate released / mg protein/15'
Values are Mean±S.E IU/L =International unit/liter
*P<0.01 **P<0.001
= compared to control
* = compared to Mtx treated

Alkaline phosphatase (ALKpase) activity

Methotrexate treatment for 15 days brought about a significant decline in the ALKpase activity in the liver of Group II animals (p<0.01). The result suggested that animals of Group III (p<0.01) and IV (p<0.001) showed significant recovery in the liver ALKpase activity, which was comparable to the control values. The hydromethanolic extract was seen to be more effective than the aqueous extract in bringing about complete reversal of the Methotrexate induced toxicity. (Table 4).

Succinate dehydrogenase (SDH) activity

SDH activity in the liver of animals of Methotrexate treated Group II showed highly significant decline (p<0.001) after 15 days Methotrexate treatment as compared to the control (Group I). Group III and IV registered a highly significant increase (p<0.001) after 15 days as compared to the MTX Group II (p<0.001). Thus extract treatment hence had effective mitigative action, reversing the hepatotoxic effects of Methotrexate however complete reversal was not seen (Table 4).

Adenosine triphosphatase (ATPase) activity

A significant decline in ATPase activity was observed following Methotrexate treatment for 15 days in the liver of animals of Group II (p<0.01) as compared to the control Group I. ATPase activity in liver however manifested a significant recovery in extract treated ameliorative Groups III and IV with resultant values comparable to control. A remarkable recovery was evident with the administration of both the extracts. (Table 4).

Table 4: Showing activities of ALKpase, SDH and ATPase in liver of control and Methotrexate treated mice, and amelioration with aqueous and hydro-methanolic extracts of *Murraya koenigii* (leaf).

No.	GROUPS	ALKpase[a]	SDH[b]	ATPase[c]
I	CONTROL	11.45±2.9	394.9±16.3	25.18±1.3
II	MTX (10 mg/kg)	8.58±0.7#	209.6±10.9##	18.02±0.9#
III	MTX+AQ (250 mg/kg)	10.23±1.2*	303.9±10.1**	26.38±2.2*
IV	MTX+HM (250 mg/kg)	11.04±`1.5**	379.7±13.9**	25.27±1.7*

N= 4/group a=µ moles of p-nitro Phenol released /100 mg tissue weight
*p< 0.01 **p<0.001 b= µg formazan formed /15min /100 mg t.w.
Values are Mean±S.E c=µ moles of inorganic phosphate released/ mg protein/30'
= compared to control
* = compared to Mtx treated

Protein content

Protein content was decreased significantly (p<0.01) in liver of mice following Methotrexate intoxication for 15 days as compared to the control Group I. *Murraya koenigii* extract appeared to be effective in expediting liver repair and subsequently an increase could be seen in the level of protein In group III and IV (p<0.01). The hydromethanolic extract appeared to be more potent since a complete mitigation was observed after its administration (Group IV) (Table 5).

Cholesterol content

Toxicity induced due to Methotrexate treatment for 15 days reduced the cholesterol content of liver significantly in Group II (p<0.001) as compared to control Group I. After the aqueous and hydromethanolic extract treatment for 15 days, both extracts could effectively alleviate the hepatotoxic changes and restore the cholesterol levels in liver of animals of Group III and IV (p<0.001) bringing the values close to normal (Table 5).

Table 5: Showing Protein and Cholesterol levels in liver of control and Methotrexate treated mice and amelioration with aqueous and hydro-methanolic extracts of *Murraya koenigii* (leaf).

No.	GROUPS	PROTEIN[a]	CHOLESTEROL[a]
I	CONTROL	9.95±0.4	2.64±0.12
II	MTX (10 mg/kg)	5.79±0.25#	1.62±0.09##
III	MTX +AQ (250 mg/kg)	8.64±0.7*	2.05±0.17**
IV	MTX +HM (250 mg/kg)	9.08±0.6*	2.04±0.11**

N=4 /group a=mg / 100 mg tissue weight
*P<0.01 **P<0.001
Values are Mean±S.E
= compared to control
* = compared to Mtx treated

DISCUSSION

The liver is one of the major organs in our body responsible for metabolism of toxic chemicals and drugs. Thus it is a target organ for all toxic chemicals. Liver disease is increasing globally due to the rampant use of medication, which causes significant morbidity and mortality.

Methotrexate is most widely vilified for its Hepatotoxicity. This was first noted in leukemia patients given high daily doses of the drug (Colsky *et al*., 1955). In the 1960s it was reported that psoriatic patients developed cirrhosis, despite being maintained on low-dose methotrexate (Epstein *et al*., 1969). Methotrexate is well known to cause serum aminotransferase elevations and long term therapy has been linked to development of fatty liver disease, fibrosis and even cirrhosis. The literature on methotrexate is extensive, but with great variability in rates of liver test and biopsy abnormalities at different doses, dose regimens and durations of therapy. It is clear therefore, that any patient subjected to such drugs is at high risk for hepatotoxicity and/or nephrotoxicity. The liver function is subsequently monitored putting the patient into much stress. A good ameliorative agent if administered along with a drug could provide an important protective action for this vital organ. Hence the present study was focused at evaluating the hepatotoxic effect of Methotrexate and identifying a suitable mitigative herbal product which could minimize toxic side effects on the liver.

The ethno-medicinal plant, *Murraya koenigii*, popularly known as curry leaves (family Rutaceae) is native to India, Sri Lanka and other South Asian countries (Rastogi and Mehrotra, 1998). *Murraya koenigii* is an extremely common plant and its leaves are regularly used as a condiment, on a daily basis. They are extensively used in Indian food preparations to add characteristic flavor and aroma to the conventional Indian cuisines (Birari *et al*., 2010).

Murraya koenigii leaves find mention in Ayurveda for their anti-diabetic properties (Satyavati, 1987) while, traditionally they are used for curing ailments like stomach ache, constipation, diarrhea, nausea, vomiting etc. In the past few years as well, *Murraya koenigii* has been the centre of research focus and several researches have scientifically demonstrated that this potent herb proves effective having hepatoprotective activity and free radical scavenging potential (Sathaye *et al*., 2012), hypolipidemic action (Kumar *et al*., 2012). It also manifests good antioxidative, anti-lipid per-oxidative actions (Mitra *et al*., 2012), anti-inflammatory (Muthumani *et al*., 2009), hepatoprotective (Pande *et al*., 2009), anti-hypercholesterolemic (Iyer *et al*., 1990; Khan *et al*., 1996). Thus, the leaves of the curry plant have the potential to provide protection against oxidative stress. The *Murraya koenigii* leaves can be considered to be a mocktail of potent phyto-constituents which have pronounced ROS scavenging and antioxidant activities. The aqueous *Murraya koenigii* leaf extract also seemed to have lead chelating potential (Bandyopadhyay *et al*., 2013) and could be effective in scavenging toxic metal ions.

In the present study, the effect of two extracts, aqueous (aq) and hydromethanolic (hm) extracts of *Murraya koenigii* were investigated for possible ameliorative action of against the toxicity induced by Methotrexate on the liver of Swiss albino mice.

The administration of Methotrexate caused an insignificant Increase in body weight, possibly due to inhibition of metabolic processes from the toxic impact of Methotrexate. This could also be correlated with our biochemical analysis showing decrease in protein and cholesterol. Treatment of the Methotrexate intoxicated mice with aqueous and hydromethanolic extracts of leaves of *Murraya koenigii* resulted in recovery of body weight, suggesting an ameliorative effect. Similarly, the tested extracts also proved effective in bringing about a recovery in weight of target organ Liver. The decrease in liver weight on Methotrexate administration possibly reflects inhibition of tissue synthesis within the tissue which is validated by decline obtained in protein levels in Liver. It was reported by Bennett and Brown (2003) that Methotrexate inhibits protein synthesizing transcription enzymes in the liver. Hence due to Methotrexate induced toxicity in the liver, lower levels of metabolites could have resulted in decreased tissue weight. However, this decrease was counteracted by the *Murraya koenigii* leaf extracts, to near normal values.

Methotrexate administration in this study caused a significant increase in the activity of ALT and AST in liver. Elevated levels of ALT and AST are sensitive indicators of liver injury. These results are in agreement with Tunali-Akbay *et al.* (2010) who demonstrated that rats treated with Methotrexate exhibited a toxic effect in liver where it provoked a notable elevation in serum activities of ALT and AST. ALT is a cytosolic enzyme of the hepatocyte and its increased serum activity reflects a leakage in plasma membrane permeability, which in turn, is associated with cell death. ALT is considered to be one of the best indicators of liver necrosis (Rosen *et al*., 2000). Methotrexate is also known to cause elevation of hepatic transaminases (Bennett and Brown, 2003). Al-motabagani *et al.*(2006) reported that MTX can bind to the enzyme hydrofolate reductase, which bans conversion of folic acid to folinic acid causing block in amino and nucleic acid synthesis. This might lead to damage of organelles and plasma membranes of hepatic parenchymal cells interfering with their function and allowing leakage of enzymes depending upon the degree of hepatic damage. A similar result was obtained by Diwan et al. (2013).

The results of our study indicate that there was a significant elevation in the levels of serum ALT in Methotrexate treated group, a finding which is in agreement with several researchers (Fu *et al.* 2008; Vaghasiya *et al.* 2009). Vardi *et al.* (2010) evaluated the effect of a polyherbal formulation on methotrexate induced hepatotoxicity in rats, but did not report full amelioration. In our study treatment with *Murraya koenigii* leaf extracts in combination with MTX brought about a significant reduction in ALT and AST activities, from their elevated state, to almost normal levels, possibly due to its active phytochemical constituents which are known to alleviate such inflammatory effects.

It was observed from the experiments carried out that administration of both extracts aqueous and hydromethanolic extracts resulted in significant recovery in the activity of these enzymes bringing the levels back to normal, specifically the hydromethanolic extracts were found more effective than aqueous extracts. In support of our finding Ghosh *et al.* (2012) also observed a dose dependant protection of the activities of SGOT and SGPT when the rats were pre-treated with *Murraya koenigii* extracts.

In the course of treatment with Methotrexate, the phosphorylase activity in the liver was significantly increased. Phosphorylases are enzymes that catalyze the addition of a phosphate group from an inorganic phosphate (phosphate + hydrogen) to an acceptor. *Murraya koenigii* extracts also brought about a reversal in the activity of phosphorylase. Methotrexate administration caused a significant decrease in the activities of Adenosine triphosphate (ATPase) and Succinate dehydrogenase (SDH) in liver, which suggested impaired oxidative metabolism and energy generation in the cells, since ATPase and SDH both are related to these functions. The potent free-radical scavenging ability of Murraya leaf extracts had a positive impact in restoring metabolic balance in the hepatocytes. Succinate dehydrogenase (SDH) catalyses the oxidation of Succinate to fumarate in TCA cycle associated with oxidative metabolism. ATPase is an integral membrane protein, which catalyses the hydrolysis of ATP into ADP and free phosphate ion. ATP also imports metabolites necessary for cell metabolism and export toxin waste materials and solids. MTX also diminished the alkaline phosphatase activity. Alkaline phosphatase (ALKpase) is a hydrolase enzyme, which hydrolyses phosphates at alkaline pH and serves as a marker enzyme of Endoplasmic reticulum as well as plasma membrane and helps in evaluating the integrity of plasma membrane (Akanji and Niumanse, 1987). Alkaline phosphatase levels are indicative of hepatobiliary inflammation and stress. Hence methotrexate induced elevation of ALKpase clearly reflects altered hepatocyte activity.

In the present study it was observed that administration of both aqueous and hydromethanolic extracts resulted in a significant recovery in the activity of these enzymes restoring the levels to near normal values. Specifically the hydromethanolic extracts were found more effective than aqueous extracts. The significant inhibition of these metabolically active enzymes by MTX is an indication of the generation of free radicals and ROS which further affect the cell pathways. Amelioration by *Murraya koenigii* was therefore possible due to its potent antioxidant phytochemicals which can scavenge the toxic free radicals. Antioxidant phytochemicals such as mahanimbine, Murray and mahanine have been shown to be potent scavengers of free radicals in *Murraya koenigii*. The carbazole mahanimbine from curry leaf exhibits anti-oxidant activity (Parthasarthy, 2008).

Biochemical analysis in the liver revealed that toxic effects of Methotrexate caused a decline in protein and cholesterol content. Protein is an integral part of metabolism necessary for all cells of the body and help in building tissue and cells. The liver is the site for biosynthesis of a large number of proteins and a fall in protein levels reflects the toxic effect of MTX on the liver biosynthetic activity. Moreover, MTX has been reported to inhibit enzymes for transcription, suppressing protein synthesis. Cholesterol is the substrate for steroid synthesis, particularly the non-esterified cholesterol. Diminished levels of cholesterol also point to impaired biosynthetic activity. This reduction in two chief metabolites could be correlated with decline in tissue weight. *Murraya koenigii* extracts manifested an effective ameliorative action, increasing the protein and cholesterol content to optimum levels, through its anti-inflammatory action. *Murraya koenigii* extract is therefore the compound of choice in most endemic areas because of its efficacy, ease of administration and its availability (Orisakwe *et al*., 2003).

The mechanism of liver injury with methotrexate is believed to be direct toxicity, through inhibition of RNA and DNA synthesis in the liver and producing cellular arrest. (Katzung *et al.*, 2010) Methotrexate therapy has been shown to increase hepatic stellate cell numbers, but the mechanism by which fibrosis is induced has not been clearly elucidated. Concurrent therapy with folate has been shown to reduce the rate of serum enzyme elevations during low dose methotrexate therapy.

CONCLUSION

Therefore, Methotrexate causes toxic inhibition to the biochemical and metabolic pathways in liver. Oral administration of *Murraya koenigii leaf* extracts thus proved effective in having a curative action on the altered liver function, bringing about remediation and repair of the injury in liver tissue.

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CONFLICTS OF INTEREST

The authors hereby wish to state that there is no Conflict of Interest in the study carried out.

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