WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 6, 1363-1373. Research Article ISSN 2277– 7105

EFFECTS OF *MORUS INDICA* LEAF EXTRACT ON REPEATEDLY USED OIL (RANCID) FOOD INDUCED TISSUE DAMAGE IN EXPERIMENTAL RATS

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Article Received on
26 March 2016,
Revised on 15 April 2016,
Accepted on 04 May 2016
DOI: 10.20959/wjpr20166-6320

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ABSTRACT

This study deals that the repeatedly using edible oil become rancid and may cause many physiological and biochemical alterations in the body system. Now a day in the fast food stalls mostly such oil is being used and this may changes the food hobbits of the people. This made us to perform this study on (rancid) foods. Experimental animal designing (wistar rats weighed about 150 to 200gm; N=4 & n=6) : Group 1: Served as normal control (NS-10 ml/kg); Group 2: Stress control (Rancid food control) (Repeatedly used oily food was supplied for 14 days); Group 3: Stress animal treated with *Morus indica* leaf extract (MILE)-75 mg/kg *po.);* Group 5: Stress rats were treated with Sylimarin (10 mg/kg *p.o.)* was used as positive standard. Method; The 70% ethanolic extract of dried *Morus indica* leaves were collected and the %yield was Calculated. Phyto Chemical Screenings was performer to find the active ingredients. The parameters analyzed are body weight, fasting blood glucose level, *in-vivo* anti-oxidant activity on Liver tissue such as Catalase (CAT), Super oxide dismutase (SOD), TBARs and vital organ weight of experimental rats. The results are statistically analyzed and tabulated.

1.0 INTRODUCTION

1.1 Rancidity

Deterioration in the fat or oil portion of a food causes unpleasant odors and flavors in foods. Mechanisms of rancidity are oxidative, hydrolytic, and ketonic. The Unsaturated fat

undergoes oxidation to produce Peroxides further decomposition of peroxides produces Oxidative rancidity.

- Oxidative rancidity arises from the decomposition of peroxides.
- The products resulting from the decomposition of peroxides include-Aldehydes, Ketones, Hydrocarbons.
- These help to produce the flavours and odours associated with oxidative rancidity.
- The abnormal characteristics of a product that has undergone oxidative rancidity are paint like or acrid (burning) odour and an abnormal (rancid) taste.
- The colour of a food item is not normally changed due to this deteriorative process.
- The texture of a food product is not affected by the deteriorative condition.

1.2 Health Effects

Rancid oil produces harmful free radicals in the body, which are known to cause cellular damage and have been associated with diabetes, Cancer, Alzheimer's, disease and other conditions.

Rancid oils can also cause digestive distress and deplete the body of vitamins B and E. Dr. Andrew Weil says rancid oil can also cause damage to DNA, accelerate aging, promote tissue degeneration and foster cancer development.

While rancid oil may taste bad, it doesn't normally make you sick, at least not in the short term. Rancid oil does contain free radicals that might increase your risk of developing diseases such as cancer or heart disease down the road. Rancid oils may produce damaging chemicals and substances that may not make you immediately ill, but can cause harm over time. Chemicals such as peroxides and aldehydes can damage cells and contribute to atherosclerosis.

If oxidative rancidity is present in severe quantities, a potential health hazard may exist. High levels of malonaldehyde are found in rancid foods. Malonaldehyde is a decomposition product of polyunsaturated fatty acids. This chemical has been reported to be carcinogenic and a potential health hazard does exist. Eating rancid oil will expose you to accelerated aging, raised cholesterol levels, obesity and weight gain. Daily consumption increases the risk of degenerating diseases such as cancer; diabetes; Alzheimer's disease; and atherosclerosis, a condition in which artery walls thicken due to a buildup of fatty materials (RD. Judy thalheimer, 2015).

The breakdown rate and total formation of toxic compounds depends on the type of oil and temperature. Initially, the oil decomposes into hydroperoxides, then into aldehydes According to a study from the University of Basque Country.

Free radicals are the atoms or molecules with one or more unpaired electrons capable of independent existence. The unpaired electrons that characterized an oxygen free radical confer a high level of instability and thus a high chemical reactivity on the molecule. Free radicals are involved in the pathogenesis diseases like arthritis, cancer, aging, heart attack, atherosclerosis, hyperoxia, asthma, vasospasm, periodontis, sexual disfunction, liver injury, retinal damage, cataractogenisis, stroke, hyperoxia, Trauma.

1.3 Antioxidants

Antioxidants are the chemical agents that inhibits the oxidative damage to a target molecule. Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. In a biological system they may protect cells from damage caused by unstable molecules known as free radicals. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols. They are believed to play a role in preventing the development of chronic diseases such as cancer, heart disease, stroke, Alzheimer's disease, Rheumatoid arthritis, cataracts etc., (A. Bendich 1994; WM. Zhang, 2009).

1.4 Functions of anti oxidants

Supports the generation of β cells in pancreas, Supports kidney functions, Protects the liver, Maintain healthy vision, Reduces obesity, Helps in systemic circulation, Maintain good dental health.Improves reproductive functions. Possess good anti-aging effects, Supports the immune system and improves deference power of body, Offers protection against digestive disorders. Supports respiratory system, Improves quality of sleep(A.K. Tiwari, 2001).

1.5 The need of traditional medicines(TM) in the treatment of diseases and disorders.

Presently there is an increasing attention in TMs due to the side effects associated with allopathic chemical agents (therapeutic agent) for the cure of diseases and disorders. So the conventional TMs are mainly used which are obtain from plants, which plays a vital role in the managing of diseases and disorders **(**World Health Organisation, 1993). A number of herbal drugs are described in the scientific text as having medicinal values. a valuable natural

resource and probably safe drugs can play an important Thus the aim of the present study is to evaluate the Anti stress effect of 70% Ethanolic extract of *Morus indica leaf extract* (MILE) in repeatedly used oil food induced stress in rats as per the following strategies(JC. Tilac, 2012; K.R. Kiritikar).

2.0 MATERIALS AND METHODS

2.1 Drugs and chemicals: *In-vivo* anti-oxidant parameters analyzing kits are bought from Agappe diagnosis, Kerala. Animal feed (Hindustan limited) and all other chemicals used in the studies were of analytical grade procure from BROSS (Tirupathi) chemicals through sri Padmavathi school of pharmacy- Tirupathi.

2.2 Instruments: UV-Visible spectrophotometer (Analytical systems, Model no: AUV 2060), electronic balance (Shimadzu, Model no: DS-852 J), homogenizer (Ever shine, Model no: 607) and Auto analyser (Mispa excel, Version: 1.4e), Cooling centrifuge (Remi, Model no: C-24 BL). SD *CHECK (*blood glucose test strip-GOLD) from SD biosensor (Germany),

2.3 Plant materials

a) Collection and Authentification: The leaves of *Morus indica*(03-04-2009) were authenticated by DR.K.Madavachetty, Asst. professor, Dept Of Botany, Sri Venkateswara University, Tirupathi (AP-India) and were collected from Yogimallavaram (Tirupathi-chittoor district A.P).

b) Preparation of the Extract: The fresh leaves of the plants were collected and air dried under shade at room temperature. The leaves were individually powdered mechanically and stored in air tight container for experimental purposes. Maceration technique was followed for extraction of leaves. 70% ethanol was used as solvent. About 600gm of powder was mixed with 1700ml of solvent and kept on mechanical shaker for 4hrs and filtered. The filtered marc obtained was again added with solvent and the procedure was repeated. The contents obtained after shaking was filter through muslin cloth and the filtrate was concentrated under reduced pressure and control temperature to yield a dark gummy solid. The extract was preserved in a refrigerator at 4^{o}C. The percentage yield for all the extracts were calculated and given in the table ie., % yield = {weight of extract produced/weight of leaf powder taken} x 100**.** Phyto Chemical Screening chemical tests were carried out using the extract of *Morus indica* for the presence of phyto chemical constituents(G.E. Trease, 2002).

2.4 Ethical clearance: The study was approved by the Animal Ethics Committee as SPSP:1016/PO/E/S/CPCSEA/2016/006, Sri Padmavathi school Of Pharmacy, Affiliated to JNTUA University. Anantapur- Tirupathi.

2.5 Animal procurement: The adult healthy wistar strain male and female rats were used for this studies, generally female rats are slightly more sensitive (for toxicity studies) these animals are normaly 12-14 weeeks of age and 150-200gm in weight. The required animals were purchased from M/s. Ragavendra enterprises, Bangalore through SPSP, Tirupathi.

2.6 Animal preparation, Housing and feeding: The randomly selected animals were marked for individual identifications. All the animals were individually kept in their cases for 5days before dosing to allow for acclimatization to the laboratory conditions. Animals were housed with $22+3^0$c, Relative humidity approximately 50% was maintained, Artificial lighting and 12hrs light and dark was maintained, and Animal pellets with adequate water was supplied.[2]

2.7 Experimental animal designing: Experimental design for *in-vivo* anti oxidant studies Experimental animals: N=4 and n=6 = 24 Rats. **Group 1:** Served as normal control (NS-10 ml/kg); **Group 2:** Stress control (Repeatedly used oily food was supplied for 14 days); **Group 3:** Stress animal treated with MILE-75 mg/kg *po.);* **Group 5:** stress rats were treated with Sylimarin (10 mg/kg *p.o.)* and used as positive standard.

2.8 Repeatedly using oil food preparation for animals: The freshly prepared rancid edible oil food (chicken, cauliflower pakkodi as prepared in the fast food stalls) was served along with normal rat food for 14 days.

2.9 Collection of blood sample and tissue homogenate: Blood samples were withdrawn from the tip of the tail for FBG,**Tissue processing:** The Liver tissue is removed and washed immediately with ice- cold saline to remove as much as blood possible. Liver tissue was homogenated (5% w/v) in cold potassium phosphate buffer (50 Mm, pH 7.4) using a Remi homogeniser. The unbroken cells and cell debris were removed by centrifugation at 3000 rpm for 10 min, using Remi C-24 refrigerated centrifuge. The obtained supernatant was used for the estimation of catalase (CAT), Superoxide dismutase (SOD), and Thiobarbituric acid reactive substances (TBARS).

2.10 Estimation of blood glucose: SD *CHECK (*blood glucose test strip-GOLD) from SD biosensor (Germany) was used to analyze fasting blood glucose on weekly basis the blood was collected via tail tip of the rats which causes least tissue damage and ideal method. The value difference between strip method and analyzer is ±2 mg/dL.

2.11 *In vivo* Antioxidant parameters

a) ***Estimation of superoxide dismutase (SOD):*** SOD activity was determined by the inhibition of autocatalysed adrenochrome formation in the presence of homogenate at 480nm. The reaction mixture contained 150µl of homogenate, 1.8ml of carbonate buffer (30mM, pH 10.2), and 0.7ml of distilled water and 400µl of epinephrine (45mM). Auto oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate. Activity was expressed as µmol/min/mg protein (Kakkar et al., 1984).

b) ***Estimation of catalase (CAT):*** The catalysis of H_2O_2 to H_2O in an incubation mixture adjusted to pH 7 was recorded at 254nm. The reaction mixture contained 2.6ml of 25mM potassium phosphate buffer pH 7 and 0.1ml of tissue homogenate and was incubated at 37^0C for 15min and the reaction was started with the addition of 0.1ml of 10mM H_2O_2. The time required for the decrease in absorbance from 0.45 to 0.4 representing the linear portion of the curve was used for the calculation of enzyme activity. One unit of catalase activity was defined as the amount of enzyme causing the decomposition of µmol H_2O_2/mg protein /min at pH 7 at 25^0C (Abei,1984).

c) ***Estimation of lipid peroxidation (TBARS***): For TBARS, 0.1ml of tissue homogenate (tris-Hcl buffer, pH 7.4) was mixed with 2ml of TBA-TCA-HCL reagent (thiobarbituric acid 0.37%,0.25N HCL and 15% TCA mixed in 1:1:1 ratio). The resultant solution was placed in water bath for 15min, cooled and centrifuged at 1000rpm at room temperature for 10min. The absorbance of the clear supernatant was measured against reference blank at 535nm. The results were expressed as nM/min/mg tissue protein (Rukkumani et al., 2004).

2.12 Statistical analysis

All the data was expressed as mean ± standard error mean (SEM). statistical significance between more than two groups was tested using statistical package for social sciences (SPSS) version 10 using ANOVA followed by the Dunnet test. Statistical significance was determined at $p<0.001$

3.0 RESULTS AND DISCUSSION

3.1 Results

Table no: 1 Percentage yield of *Morus indica* leaf extract

Extract	solvent	% yield w/w
Morus indica	70% Ethanol	20.24

Table no: 2 Phytochemical screening of MILE

S.NO	CONSTITUENTS	MILE
1.	Tri terpenoids	+
2.	Flavonoids	+
3.	Glycosides	+
4.	Steroids	-
5.	Alkaloids	+
6.	Saponins	-
7.	Carbohydrates	+
8.	Proteins	+
9.	Tannins	+

(+)Presence of constituents (-) Absence of constituents

Table no: 3 Effect of MILE on BODY WEIGHT of experimental rats

S.No	Drug Treatment	Animal weight in gm			% Change in wt gain
		0 day	7th day	14th day	
1	Normal Control (NS-10 ml/kg)	164.16 ± 0.25 [a]	168.83 ± 1.05 [a]	172,16 ± 1.35 [a]	+ 4.87
2	Stress control	165.0O ± 1.27 [a]	166.83 ± 1.09 [a]	169.66 ± 178 [a]	+ 2.83
3	stressed + Test	157.00 ± 0.09 [a]	174.33 ± 1.97 [a]	187.83 ± 1.02 [a]	+ 19.63
4	stressed + STD	154.33 ± 0.90 [a]	170.83 ± 1.45 [a]	180.00 ± 1.57 [a]	+ 16.56

Data are expressed as mean ± S.E.M; (n=6). Values are statistically significant at P< (0.01^a-0.05^b) the 3 and 4 groups are compared with control (1) and stress control (2).

Table no: 4 Effect of MILE on FASTING BLOOD GLUCOSE level of experimental rats.

S.No	Drug Treatment	Fasting blood glucose(mg/dL)			% changes in FBG
		0th day	7th` day	14th day	
1	Normal Control (NS-10 ml/kg)	103.00 ± 1.98[a]	111.50 ± 0.93 [a]	115.50 ± 0.93 [a]	12.13
2	Stress control	101.00 ± 0.09 [a]	104.27 ± 1.37 [a]	105.83 ± 1.93 [a]	04.78
3	stressed + Test	102.00 ± 0.33 [a]	83.20 ±0.87 [a]	66.00 ± 0.79 [a]	- 35.29
4	stressed + STD	107.26 ± 0.91 [a]	93.29 ±1.28 [a]	80.00 ± 1.35 [a]	- 25.41

Data are expressed as mean ± S.E.M; (n=6). Values are statistically significant at P< (0.01^a-0.05^b) the 3, 4 and 5th groups are compared with control (1) and stress control (2).

Table no: 5 Effect of MILE on ANTI-OXIDANT activity on Liver tissue of experimental rats

S.No	Drug Treatment	CAT (µM/min/mg protien)	SOD (µM/min/mg protien)	TBAR (µM/min/mg protien)
1	Normal Control (NS-10 ml/kg)	90.23±1.90[a]	3.46±1.54 [a]	11.96±1.14
2	Stress control	68.45±1.64 [a]	3.08±1.09 [a]	25.56±2.01
3	stressed + Test	76.50±2.09 [a]	4.94±0.98 [a]	11.01±1.09
4	stressed + STD	72.39±0.98 [a]	4.93±0.87 [a]	12.12±1.12

Data are expressed as mean ± S.E.M; (n=6). Values are statistically significant at P< (0.01[a]-0.05[b]) the 3, 4 and 5[th] groups are compared with control (1) and stress control (2)

Table no: 6 Effect of MILE on vital organ weight of experimental rats

S.No	Drug Treatment	BRAIN (gm)	HEART (gm)	LIVER (gm)	KIDNEY (gm)
1	Normal Control (NS-10 ml/kg)	1.59 ± 1.35 [a]	0.52 ± 1.35 [a]	5.00 ± 1.45 [a]	1.20 ± 1.23 [a]
2	Stress control	1.29 ± 2.01 [a]	0.52 ± 2.06 [a]	4.69 ± 1.25 [a]	1.23 ±1.37 [a]
3	stressed + Test	1.66 ± 2.31 [a]	0.78 ± 1.03 [a]	5.18 ± 1.54 [a]	1.48 ± 1.22 [a]
4	stressed + STD	1.46 ± 1.11 [a]	0.73 ± 1.56 [a]	5.44 ± 1.45 [a]	1.36 ±0.84 [a]

Data are expressed as mean ± S.E.M; (n=6). Values are statistically significant at P< (0.01[a]-0.05[b]) the 3, 4 and 5[th] groups are compared with control (1) and stress control (2).

3.2 DISCUSSION

Table no. 1 states that 70% hydro ethanolic extract has better solvent efficacy by providing maximal percentage yield (20.24%w/w) of *Morus indica* life extract as gummy product. 70% of ethanol provides anti bacterial activity.

Table no 2 shows that the phytochemical constitutions of *Morus indica* leaf extract by performing, triterpinoids ,flavonoids, glycosides, steroids ,Alkaloids, saponins, carbohydrates, proteins ,tannins ,tests as per the standard procedure. *Morus indica* leaf extract shows the presence of triterpinoids, flavonoids, glycosides, steroids ,Alkaloids, saponins, carbohydrates, proteins, tannins.

Table no 3 indicates the percentage change in body weight body weight of normal control (+4.87%), stress control (+2.83%) ,test treated (+19.63%),standard treated (+16.56%) animal

groups, when the test group gained maximum percentage of weight may be due to nutritional effect of drug and which minimized increased oxygen level than the stress control .

Table no. 4 states that the fasting blood glucose level of experimental rats (percentage changes). The test treated group (-35.29%) showed the maximal reduction in blood glucose level. This is may be because of hypoglycemic effect of *Morus indica* leaf extract when compare to stress control(4.78%) and normal control (12.13%).

Table no. 5 shows that the effect of *morus indica* leaf extract on anti-oxidant activity on liver tissue of experimental rats.

i. The quantity of Catalase in stress control (68.45±1.64 µm/min/mg) is decreased than the normal (90.23±1.90µm/min/mg) but in the test treated(76.50±2.09µm/min/mg)and the standard treated (72.39±0.9809µm/min/mg) shows the incremental of catalase level than the stress control but it does not matches the normal control level(90.23±1.90µm/min/mg)this may be due to higher level of free radical generation ofrancid oil

ii. The quantity of SOD in stress control (3.08 ± 1.09 µm/min/mg) is decreased than the normal (3.46 ± 1.54µm/min/mg) but in the test treated(4.94±0.98µm/min/mg)and the standard treated (4.93±0.87µm/min/mg) shows the incremental of SOD level than the stress control but it does not matches the normal control level(3.46±1.54µm/min/mg)this may be due to higher level of free radical generation ofrancid oil

iii. The quantity of TBAR in stress control (3.08 ± 1.09 µm/min/mg) is decreased than the normal (3.46 ± 1.54µm/min/mg) but in the test treated(4.94±0.98µm/min/mg)and the standard treated (4.93 ± 0.87µm/min/mg) shows the incremental of TBAR level than the stress control but it does not matches the normal control level (3.46 ± 1.54µm/min/mg)this may be due to higher level of free radical generation of rancid oil

Table no. 6 indicates the weight of the vital organs of experimental rats.

i. **Brain:** The test treated group shows (1.66 ± 2.31g) more than the stress control(1.29±2.01g) animal .similarly the standard treated(1.46±1.11) group showed more weight than the stress control.Both the test and standard are near to normal control(1.59±1.35).since stress control brain weight showed lesser brain weight than the normal control even the inter group body weight are significant.

ii. **Heart:** The test treated group shows (0.78 ± 1.03g) more than the stress control(0.52±2.06g) animal .similarly the standard treated(0.73±1.56g) group showed

more weight than the stress control.Both the test and standard are near to normal control(0.52±1.35g).since stress control heart weight showed lesser heart weight than the normal control even the inter group body weight are significant.

iii. **Liver:** The test treated group shows (5.18 ± 1.54g) more than the stress control(4.69±1.25g) animal .similarly the standard treated(5.44±1.45g) group showed more weight than the stress control.Both the test and standard are near to normal control(5.00±1.45g).since stress control liver weight showed lesser liver weight than the normal control even the inter group body weight are significant.

iv. **Kidney:** The test treated group shows (1.48±1.22g) more than the stress control(1.23±1.37g) animal .similarly the standard treated(1.36±0.84g) group showed more weight than the stress control.Both the test and standard are near to normal control(1.20±1.23g).since stress control kidney weight showed lesserkidney weight than the normal control even the inter group body weight are significant.

4.0 CONCLUSION

The *Morus indica* leaf extract has better nutritional effect due to flavones, hypoglycemic action and anti –oxidant activity this may be due to various phytochemical ingredients such as , triterpinoids ,flavonoids, glycosides, steroids ,Alkaloids, saponins, carbohydrates, proteins ,tannins. so the repeatedly used oil might have been rancid and might have been caused the free radical generation which shows change in organ weight .the organ weights are decreased mainly in brain and liver.This requires further more studies on varies parameters such as haematological and metabolic activity with the help of specific active ingredient of *Morus indica* leaf extract.

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