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EFFECT OF PURE MANGIFERIN VIS-A-VIS CRUDE ETHANOLIC EXTRACT FROM MANGIFERA INDICA BARK ON HAEMATOLOGICAL PARAMETERS IN MALE ALBINO RATS

Arnab Das[1], Chaitali Bose[2], Subhasish Ghosal[2], Indrani Chakraborty[3], Nirmal Pradhan[1]*

[1]Post Graduate Department of Physiology, Hooghly Mohsin College, Chinsurah, Hooghly, West Bengal, India.

[2]Dept. of Physiology, Presidency University, Kolkata, West Bengal, India.

[3]Dept. of Physiology, Krishnanagar Govt. College, Nadia, West Bengal, India.

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***Correspondence for Author**
Dr. Nirmal Pradhan
Post Graduate Department of Physiology, Hooghly Mohsin College, Chinsurah, Hooghly, West Bengal, India.

ABSTRACT

Different parts of mango tree have great medicinal value in different clinical fields. But the bark extract is very important in the field of pharmaceutical research and clinical applications. The present study was conducted to evaluate the effect of crude ethanolic extract (mother) of *Mangifera indica* bark in comparison to pure mangiferin on haematological indices in adult male albino rats. In this study, eighteen animals were divided at random into three groups i.e. vehicle control, *M.indica* bark extract and pure mangiferin, which received intraperitoneally 0.1ml 70% methanol, 0.1ml bark extract and 1mg pure mangiferin in 0.1ml 70% methanol/100gm b.wt./day accordingly for consecutive 14 days. Results showed no change in body growth rate, weights of heart and lung, where weights of adrenal and spleen were significantly increased in extract treated group than control. But the weights of kidney and spleen were reduced in pure mangiferin group in respect to extract treatment. Significant reduction of liver weight was observed in pure mangiferin group than control. The SGOT, SGPT and serum ALP showed no significant change. Haematological indices like Hb, TC of RBC and PCV were significantly increased in pure mangiferin group when compared to control. Notably, TC of RBC was also significantly high in pure mangiferin group when compared to extract treatment. But MCV and MCH showed significant reduction in pure mangiferin group than control and extract treatment. The MCHC showed no change. TC of

WBC was significantly higher in treated groups than control. From the results, it may be concluded that mangiferin of *M.indica* bark extract may be an active haemopoietic principle, which can be therapeutically used for haemopoiesis.

KEY WORDS: Mangiferin, *Mangifera indica*, Bark extract, RBC, WBC, Haemoglobin.

INTRODUCTION

Recently the World Health Organization (WHO) has reported about 119 plants-derived pharmaceutical drugs, of which 74% are used in modern medicine that are correlated directly with traditional uses as Herbal plant medicine. In the present scenario, about 80% of the world population presently uses herbal medicine for primary health care. The remaining 20 %, who mainly reside in developed countries, consuming costly medicines. [1] *Mangifera indica* L. is a medicinal plant traditionally used in tropical regions. *Mangifera indica* L. belongs to the family Anacardiaceae. The tree is a native of tropical Asia and is indigenous to the Indian subcontinent.[2] The natural C-glucoside xanthone mangiferin (Fig. 1,2) [2-C-β-$_D$gluco-pyranosyl-1,3,6,7-tetrahydroxyxanthone; $C_{19}H_{18}O_{11}$; MW- 422.35; melting point-anhydrous 271°C][3] has been reported to found in various parts of *M.indica*, i.e. leaves,[4] fruits,[5] stem bark,[6,7] heartwood[8] and roots,[9] but which is predominant in stem bark.[6,7]

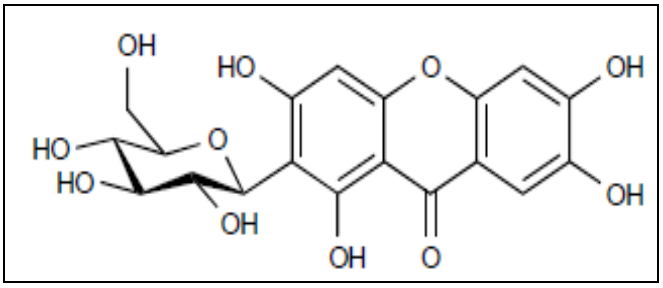

Fig. 1: Chemical structure of mangiferin.

This pharmacologically active compound, mangiferin also found in different angiosperm families and ferns. [9-11]

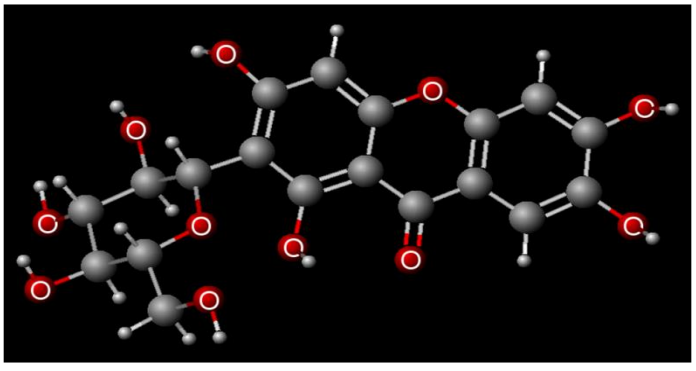

Fig. 2: Structure of mangiferin in 3D (by using Marvin Beans 5.3 software).

The bark extract of mango tree has been reported to have many medicinal uses in treatment of different diseases. Beside this, pure mangiferin also have been reported to have antioxidant,[3] [10-12] radioprotective,[13-14] antitumor,[15-16] immunomodulatory,[11,15,17-23] anti-allergic,[24] anti-inflammatory,[25-26] antidiabetic,[27-31] lipolytic,[32] antibone resorption,[33] monoamine oxidase inhibiting,[34] antiviral,[15,35] antifungal,[12] antibacterial[12,36] and antiparasitic properties.[37]
But no report yet is available regarding comparative haemopoietic activity of mango bark extract and mangiferin pure. So, in the present study, we desired to find the comparative efficacy of the same.

MATERIALS AND METHODS

Plant Material

Pure (98.2%) mangiferin from *Mangifera indica* (bark) was purchased from Sigma-Aldrich (USA) and crude mother of *Mangifera indica* bark from Nityananda Homeo Hall, Kolkata, West Bengal, India.

Animal Selection and Maintenance

18 adult male albino rats (*Rattus norvegicus* L. of Wistar strain), weighing 140g ±10 were selected and used for the experiment. The rats were maintained under standard laboratory condition (temperature 25 ± 2˚C, 12/12hr dark and light, relative humidity 40-60%) with free access to standard normal diet, prescribed by ICMR, NIN, Hyderabad, India[38] and water *ad libitum*. The animals were acclimatized to the laboratory condition for a period of one week before starting the experiment. All the animals were fed twice a day. Animals in each group were housed in clean and large polypropylene cages. Animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) guided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. Ref.no. PU 796/03/ac/CPSEA. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Experimental Design and Drug Administration

The rats were randomly allocated into three groups of 6 animals each. Treatment scheduled for 14days and drugs administered intraperitoneally (i.p.). Body weights of the animals were monitored regularly during entire tenure of the experiment by top pan weighing machine and doses were adjusted according to the body weight changes. As -

a) Control group - given 0.1ml 70% alcohol /100gm b.wt./day.

b) Extract treated group - treated with crude ethanolic extract of *M.indica* bark mother at a dose of 0.1ml/100gm b.wt./day.
c) Pure mangiferin group - treated with pure mangiferin at a dose of 1 mg in 0.1 ml 70% methanol/100gm b.wt./day.

Blood and Tissue Collection

At the end of treatment, rats were anaesthetized with anesthetic ether and blood was collected after sacrifice from hepatic vein. About 1ml of blood was dispensed into specimen vials containing anti-coagulant EDTA (sodium ethylene diamine tetra-acetic acid), while another 2 ml was dispensed into clean clotting vials without anticoagulant and left to clot for serum, to be used for biochemical studies. Different organs like heart, lungs, liver, kidney, spleen and adrenal glands were dissected out, trimmed, blotted and weighed immediately after sacrifice. Tissues and blood samples were kept in 4°C for further experiments.

Biochemical Assay

Blood samples in the clotting vials without anticoagulant were allowed to clot at room temperature and then spinned with centrifuge at 3000 rpm for 15 minutes. The sera were aspirated with clean and dry pipettes. The biochemical parameters were determined with the help of standard assay kits (Span Diagnostics Ltd., Surat, India). The parameters are serum glutamic oxaloacetic transaminase [SGOT, also known as aspartate transaminase (AST)], serum glutamic pyruvic transaminase [SGPT, also known as alanine aminotransferase (ALT)] and serum alkaline phosphatase (ALP) were measured by following methods –

a) SGOT and SGPT levels – by Reitman and Frankel Method[39]
b) Serum alkaline phosphatase – by King and King Method[40]

Haematological Assay

The whole blood with anticoagulant was used for assay of the haematological parameters, i.e. total count of red blood cell (TC of RBC), total count of white blood cell (TC of WBC), haemoglobin (Hb) concentration, Packed Cell Volume(PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) by following methods –

a) Hb(gm%) – haemoglobin concentration was determined by the cyanomethaemoglobin method. [41]
b) Total count of RBC and WBC – were estimated using improved Neubauer counting chamber. [42]

c) PCV (Packed Cell Volume) – with Wintrobe haematocrit tubes.

d) MCV, MCH and MCHC were calculated as -

i. MCV (in cubic microns) = (PCV x 10)/RBC(in million/mm^3)

ii. MCH (in picograms) = (Hb in gm/dl x 10)/RBC(in million/mm^3)

iii. MCHC (in g/dl) = (Hb in gm/dl x 100)/PCV

Statistical Analysis

The recorded values are expressed in mean ± SEM. The control group, extract treated group and pure mangiferin treated group were compared to each other by using one way ANOVA with post hoc Tukey's multiple comparison test. The tests were performed using Graph Pad InStat version 3 software. The value of $P<0.05$ is considered to be statistically significant.

RESULTS

Treatment of *Mangifera indica* bark extract (mother) and pure mangiferin to adult male rats at a dose of 0.1ml and 1mg/100gm b.wt./day accordingly for consecutive 14 days caused no significant changes in body growth rate (Table-1, Fig. 3), relative weights of heart and lung when compared among the groups (Table-2, Fig. 4). Weights of kidney and spleen were significantly ($P<0.05$) reduced in pure mangiferin group in compared to extract treatment (Table-2, Fig. 4). Though, the splenic weight in extract group was significantly ($P<0.01$) increased than control. The weight of liver in pure group was significantly ($P<0.01$) reduced than control. Again, the adrenal weight was significantly ($P<0.05$) increased in extract group when compared to control (Table-2, Fig. 4). The serum level of SGOT, SGPT and ALP showed no change when compared among the groups (Table-3, Fig. 5). The haematological studies of Hb (gm%) was significantly ($P<0.05$) high in pure mangiferin group in comparison to extract treatment. Similar result was found in TC of RBC ($P<0.05$) and PCV ($P<0.01$) which were also significantly high in pure mangiferin group than control. The TC of RBC was also significantly ($P<0.05$) increased in pure mangiferin group when compared with extract treated group. But, MCV and MCH showed significant ($P<0.05$) reduction in pure group when compared to control and extract treated group, whereas MCHC was unaltered. The TC of WBC was significantly ($P<0.05$) high in both drug treated group in comparison to control (Table- 4, Fig. 6 and 7).

Table 1: Percentage Change in Body Growth Rate.

Groups	Body growth rate (gm%)
Control	12.918 ± 0.906
Extract	11.938 ± 0.393
Pure	10.568 ± 0.693

Values are expressed as mean ± SEM, n=6. P<0.05 is considered as significant.

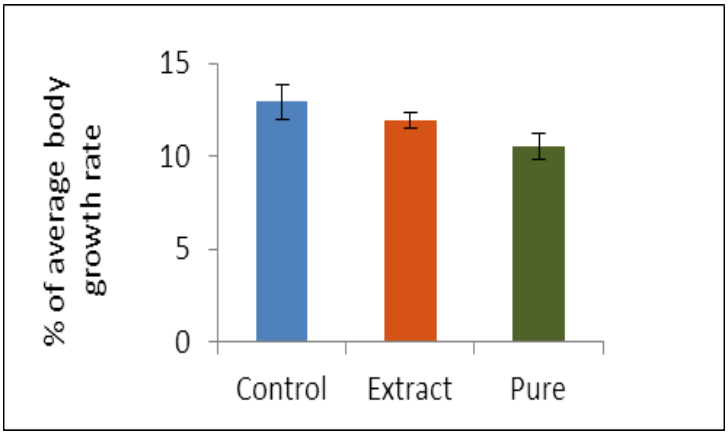

Fig. 3: Percent (%) changes in average body growth rate.

No significant (P> 0.05) change has been found.

Table 2: Change in Different Organ Weights (gm) In Control and Experimental Groups of Rats.

Organs	Control	Extract	Pure
Liver weight	2.937 ± 0.131	2.700 ± 0.059	2.503 ± 0.052#
Kidney weight	0.625 ± 0.029	0.687 ± 0.018	0.550 ± 0.049*
Spleen weight	0.223 ± 0.004	0.262 ± 0.006#	0.223 ± 0.009*
Heart weight	0.312 ± 0.012	0.320 ± 0.007	0.292 ± 0.011
Lungs weight	0.512 ± 0.027	0.535 ± 0.056	0.632 ± 0.117
Adrenal Gland weight	0.112 ± 0.002	0.118 ± 0.002*	0.113 ± 0.001

*Values are expressed as mean ± SEM, n=6. P<0.05 is considered as significant. * P<0.05, # P<0.01*

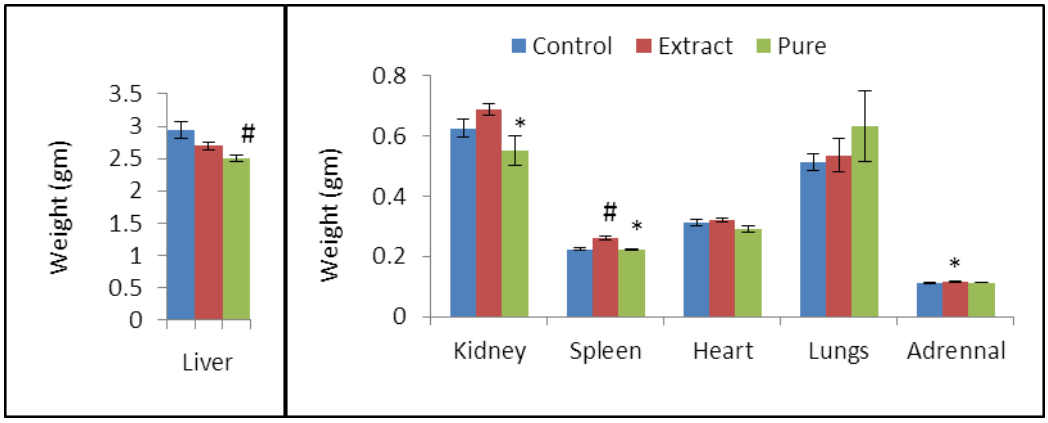

Fig. 4: Change in different organ weights in different groups. (* P<0.05, # P<0.01).

Table 3: Change of Biochemical Parameters in Control and Experimental Groups of Rats.

Parameters	Control	Extract	Pure
SGOT (IU/L)	27.031 ±0.555	24.661 ±1.772	26.192 ±1.302
SGPT (IU/L)	18.032 ±1.197	18.332 ±1.432	17.425 ±1.552
Serum ALP (KA unit)	23.350 ±1.492	22.680 ±1.139	20.490 ±1.892

Values are expressed as mean ± SEM, n=6. P<0.05 is considered as significant.

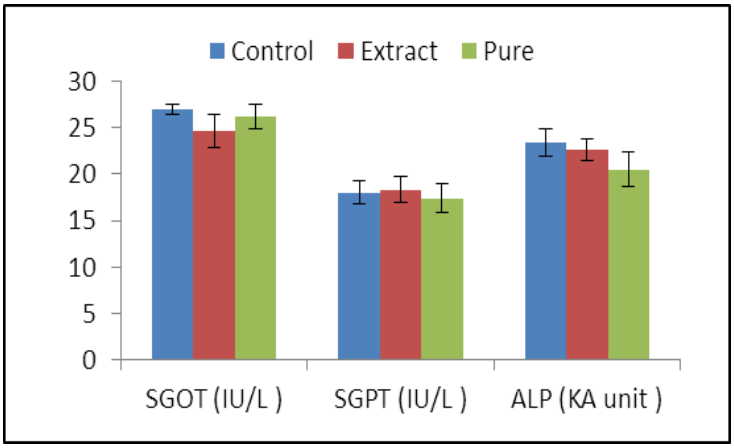

Fig. 5: SGOT, SGPT and serum ALP levels in different groups.

No significant (P>0.05) changes have been found.

Table 4: Change in Haematological Parameters in Control and Experimental Group of Rats.

Parameters	Control	Extract	Pure
Total count of RBC (million/ mm^3)	7.679 ±0.698	7.342 ±0.354	10.459 ±1.020*
Total count of WBC ($x10^3$/ mm^3)	3.630 ±0.293	6.071 ±1.044*	4.689 ±0.258*
Hb (gm%)	13.050 ±1.603	12.730 ±0.827	15.220 ±0.422*
PCV (%)	43.150 ±1.072	42.870 ±0.561	46.100 ±0.813#
MCV (fl)	58.076 ±4.189	58.889 ±2.110	45.606 ±3.168*
MCH (pico gm)	16.779 ±0.768	17.282 ±0.339	14.993 ±0.911*
MCHC (g/dl)	29.899 ±3.035	29.608 ±1.541	33.065 ±1.085

Values represent the mean ± SEM, n = 6. P<0.05 is considered as significant. * P<0.05, # P<0.01.

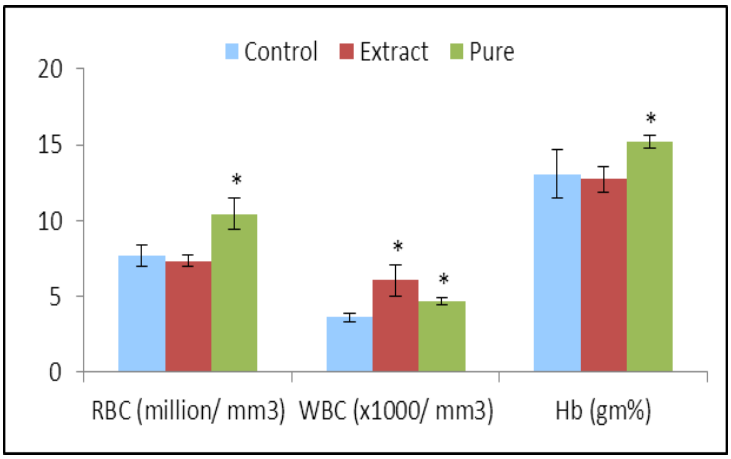

Fig. 6: Total count of RBC, WBC and haemoglobin (gm%) in different groups. (*P<0.05, # P<0.01).

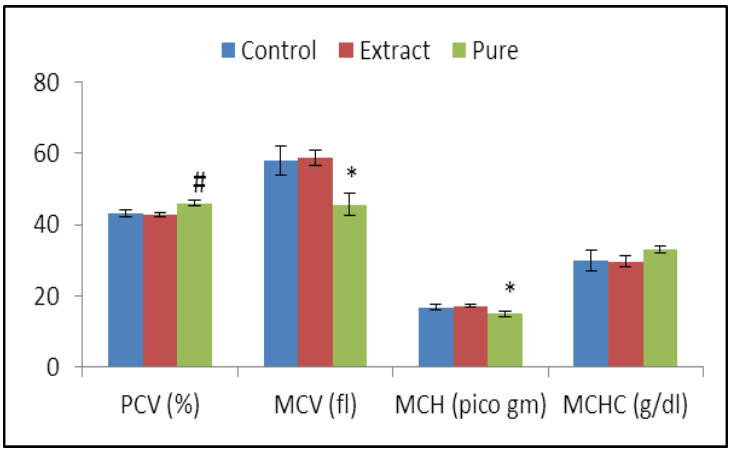

Fig. 7: PCV, MCV, MCH and MCHC in different groups. (*P<0.05, # P<0.01).

DISCUSSION

In the present study, intraperitoneal administration of crude extract(mother) and pure mangiferin at the dose of 0.1ml and 1mg/100gm b.wt/day for 14 days consecutively to mature male albino rats caused no significant change in body growth rate, relative weights of heart, lung, SGOT, SGPT and serum ALP level, showing the possibility of non-toxicity of drugs in a dose and duration dependent manner.[43-44] The weight of kidney and spleen were reduced in pure mangiferin treated group, which show that pure mangiferin is comparatively non-harmful than crude extract of *Mangifera indica* bark, possibly due to antioxidant property of mangiferin[45] and may be due to absence of other phytochemicals with mangiferin[46] as present in crude extract of *Mangifera indica* bark. Again the weights of spleen and adrenal glands were significantly increased in extract treated group, which may be due to drug induced stress. This observation is also supported by other studies of Adebayo et al. [47] and

Nidhi Mishra, [48] where the increase of body weight and organ weight is an indication of cell constriction. Again the significant reduction in organ weights i.e. kidney and spleen in pure mangiferin treated group in compare to extract treatment is mostly due to anti- inflammatory nature of mangiferin. [25-26] So, the comparison in between control and pure mangiferin showed no significant change. The increase in splenic weight in extract treated group indicates the immune-modulation by drug treatment,[49] which can be supported by the significant rise in WBC count in treated groups indicating the immune stimulatory property of mangiferin, which is also supported by other studies.[50] But the cause of reduction in liver weight in pure group in respect to control is unknown, which should be studied further. The rise in haemoglobin level, total count of RBC and PCV in treated groups which is predominant in pure mangiferin treatment, explain the stimulatory effect of mangiferin on haemopoiesis.[50] As mangiferin acts as antioxidant,[3,10-12] which possibly protects the blood cell injury by preventing lipid peroxidation,[50] where red blood cells are extremely susceptible to lipid peroxidation, since they are rich in unsaturated membrane lipids, have rich supply of oxygen and transition metal catalyst. Lipid peroxidation occurs as a result of the reaction between free radicals and membrane lipids and is considered as an important feature of cellular injury. [51] The rise of RBC and haemoglobin by pure mangiferin treatment may be due to erythropoietin released from kidney which acts as humoral regulation for RBC production.[52-54] Similar fashion also have been found in rise of haematocrit value may be due to rise of haemoconcentration.[48,55]

CONCLUSION

From the above study it can be concluded that

1. Pure mangiferin is comparatively nontoxic than crude extract of *M.indica* bark in a dose-dependent manner.
2. Pure mangiferin may slow down the natural process of oxidative breakdown of RBCs than crude extract.
3. Pure mangiferin may increase the rate of erythropoiesis better than crude form.
4. Crude extract of *M.indica* bark may promote the immune stimulatory activities by increasing WBC production than pure mangiferin. Finally, it can be interpreted that this two drugs appear to be very promising alternative for haemopoiesis. But the pure mangiferin is better potent than crude extract in erythropoiesis. So, mangiferin may be potent as antianaemic drug. Further studies are required for critical explanation.

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