Safety Evaluation of *Mangifera Indica* Bark for Raw Water Purification

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A B S T R A C T

Background:

Synthetic coagulants commonly used for water treatment are associated with undesirable properties, such as inefficacy and toxicity in addition to being expensive. Natural coagulants are considered safe and economical alternatives for developing countries where the plants are abundantly available.

Methods:

The phytochemical composition and safety of water samples treated with Mangifera indica (M. indica) were evaluated in 13 groups of albino rats (N=36) for four weeks and compared with water samples treated with alum and calcium hypochlorite, using biochemical and hematological parameters.

Results:

Mangifera indica bark contained alkaloids, flavonoids, saponins, phenols, tannins, terpenes, steroids and cardiac glycoside. Both the raw water samples (rivers, ponds & streams) and treated waters (alum, calcium hypochlorite and plant material) did not caused any significant ($p>0.05$) changes to the activities or levels of transaminases (AST and ALT), alkaline phosphatase, total serum proteins, urea, creatinine, sodium, potassium, platelet and mean corpuscular hemoglobin concentration compared with those in normal control rats. Water samples treated with M. indica caused significant increases ($p<0.05$) in packed cell volume, hemoglobin, and red blood cells of the animals while the untreated water samples significantly increased the white blood cell. However, alum treated water significantly increased ($p<0.05$) the concentrations of serum urea, sodium and potassium while calcium hypochlorite treated water significantly ($p<0.05$) increased the creatinine and potassium concentrations.

Conclusion:

The use of M. indica bark in water purification confers hematopoietic properties to the water and reduces adverse effects on the biochemical parameters, thus could be considered as an effective and safe agent for water purification.

Keywords:

Mangifera Indica; Hematology and Biochemicals; Phytochemicals; Purification; Rivers, Streams and Ponds.

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INTRODUCTION

The purity of water consumed by humans is very crucial since it directly impacts our health. More than half of all illnesses and deaths among children are caused by germs, which enter the body via water and food (1). The World Health Organization has estimated that up to 80% of all diseases in the world are caused by inadequate purification and sanitation of water or unavailability of healthy water (2). Every day, two million tons of industrial sewage and agricultural wastes are discharged into the water worldwide (3). According to the UN estimates, the amount of wastewater produced annually is about 1500 km^3, six times more than the capacity of all of the rivers in the world (3).

Historically, the use of natural materials of plant origin has been practiced for many years to purify polluted

surface waters. Egyptian inscriptions afforded the earliest recorded knowledge of plant materials used for water treatment, dating back perhaps to 2000 BC in addition to boiling and filtration of water in modern times (4). However, the safety evaluation should be a major criterion in the selection of plant materials for water purification.

Of the large number of plant materials used over the years, the seeds from *Mangifera indica (M. indica)* have been shown to be one of the most effective primary coagulants for water treatment especially in rural communities (5). Mango trees (*Mangifera indica* Linn Anacardiaceae) are naturally abundant in West Africa. The bark and leaves of this tree have astringent prosperities and are used in Nigeria as lotion to relieve toothache, sore gums and throat or infused in the treatment of malaria, diarrhea and dysentery (5). All parts of this plant are rich in tannins and flavonoids, which are useful in health promotion, disease prevention and drug production (6). This study, investigated the safety of this plant in an attempt to recommend or refute its usage for the purification of surface waters.

MATERIALS AND METHODS

Sample Collection and Preparation: Fresh bark of *M. indica* was obtained from Minna Niger State, Nigeria, and was identified by a botanist at the Department of Biological Science at Federal University of Technology, Minna (FUTMINNA), Nigeria.

Sample Preparation and Phytochemical Analysis: The plant materials were washed and dried for 2 weeks at 37°C, and finely powdered, using a grinder mill. Qualitative phytochemical analyses of the plant were carried out, using the standard procedures as described previously (7,8).

Experimental Animals: Healthy albino rats (N=39) were procured from animals holding unit of FUTMINNA. They were allowed unrestricted access to rat food pellets and water. The ethical principles governing the use of laboratory animals as set by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research and also the internationally accepted principles for laboratory animal use and care, as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed.

Safety Profile Evaluation: The powdered plant material was suspended in the water samples from the local rivers, streams and pond waters at 0.5g/l concentration and was left to stand for 30 minutes to allow the coagulated particles to precipitate (9). Following centrifugation, the supernatants were filtered, using filter paper and the resultant water samples were used for the toxicological analyses. The rats were grouped into 13 (A-M) of 3 animals each and were administered the water samples for 28 days as described below:

Group A = Untreated stream water (raw stream water)

Group B = Alum treated stream water

Group C = Calcium Hypochlorite treated stream water

Group D = *M. indica* bark treated stream water

Group E = Untreated pond water (raw pond water)

Group F = Alum Treated pond water

Group G = Calcium Hypochlorite treated pond water

Group H = *M. indica* bark treated pond water

Group I = Untreated river water (raw river water)

Group J = Alum Treated river water

Group K = Calcium Hypochlorite treated river water

Group L = *M. indica* bark treated river water

Group M = Control: normal filtered water

At the completion of the study, blood samples were collected from the animals, centrifuged and the serum samples were collected for the biochemical analyses as described previously (10).

Biochemical Parameters: The activities or concentrations of serum AST, ALT, ALP, total proteins, albumins, bilirubins, urea, creatinine and chloride in the serum samples of rats were determined spectrophotometrically, using standard laboratory procedures (11-15).

Haematological Parameters: The hematological components, such as haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), platelets (PLT) and differential counts (granulocyte, lymphocytes, eosinophils, monocytes and neutrophils) were determined, using the automated hematologic analyzer SYSMEX KX21 (SYSMEX Corporation, Japan) employing the methods described by Dacie and Lewis (16).

Data Analysis: The data were analyzed using statistical package for social science (SPSS, v18). Differences between groups were compared using analysis of variance, ANOVA ($P<0.05$) followed by Duncan's multiple range test.

RESULTS

Phytochemical Composition: The *M. indica* samples contained alkaloids, flavonoids, saponins, phenols, tannins, terpenes, steroids, cardiac glycosides as shown in Table 1.

Table 1. Qualitative phytochemical contents of *M. indica.*

Phytochemicals	Inference
Flavonoids	+
Phenols	+
Tannins	+
Saponins	+
Alkaloids	+
Cardiac glycosides	+
Anthraquinones	+
Terpenes	+
Steroid	+

Effects on Serum Biochemical Parameters Reflecting Liver Integrity: The effect of raw and treated water samples on the biochemical parameters reflecting liver integrity in rats are shown in Table 2. The daily administration of either raw or water samples treated with alum, calcium hypochlorite & plant materials to the rats for 28 days did not caused any significant changes ($p>0.05$) to the activities of aspartate amino transaminase (AST), alkaline phosphatase, alanine amino transaminase (ALT) activities and total proteins concentrations compared with those in the control rats (Table 2).

Effects on Serum Biochemical Parameters Reflecting Kidney Integrity: The effect of raw and treated water on the biochemical parameters, reflecting kidney integrity of the rats are shown in (Table 3). The daily administration of either raw or plant-treated water samples to the rats for 28 days did not cause any significant changes ($p>0.05$) to the concentrations of serum urea, creatinine, sodium and potassium, compared to those in the control rats. However, rats administered with alum treated water experienced significant increases ($p<0.05$) in the serum urea, sodium and potassium concentrations, compared to those in the control rats. Similarly, calcium hypochlorite significantly ($p<0.05$) increased the serum creatinine and potassium concentrations, compared to those in the control rats (Table 3).

Effects on Hematological Parameters: The effect of raw and treated water on hematological parameters in rats are shown in Table 4. The daily administration of either raw or treated water (alum, Calcium hypochlorite and plant) to albino rats for 28 days did not cause any significant changes ($p>0.05$) to the concentrations of platelet and MCHC, compared to those in the control rats. However, rats administered with plant treated water showed a significant increase ($p<0.05$) in the concentrations of PCV, Hb and RBC, compared to those in the control rats (Table 4). Also the rats administered the raw water showed a significant increase in WBC count, compared to those in the control rats (Table 4).

Table 2. Effect raw and treated water on liver function indices in rats.

	Experiment	AST (U/L)	ALT (U/L)	ALP (U/L)	PROTEIN (mg/dL)
Stream water	Untreated	16.00±4.62 [a]	16.45±3.46 [a]	132.98±2.74 [a]	25.43±3.73 [a]
	Alum Treated	15.40±3.57 [a]	16.56±1.02 [a]	126.54±3.22 [a]	23.56±2.78 [a]
	Calcium Hypochlorite	15.34±1.24 [a]	15.43±0.89 [a]	122.34±3.94 [a]	24.34±2.34 [a]
	M. indica bark	16.40±2.51 [a]	15.78±1.78 [a]	128.93±1.90 [a]	25.34±1.98 [a]
Pond Water	Untreated	15.50±2.78 [a]	16.23±1.89 [a]	146.78±2.67 [a]	31.78±2.89 [b]
	Alum Treated	16.40±1.67 [a]	17.78±0.21 [a]	143,56±4.09 [a]	25.67±3.21 [a]
	Calcium Hypochlorite	16.52±2.35 [a]	16.72±1.35 [a]	13215±4.56 [a]	23.45±3.45 [a]
	M. indica bark	16.20±1.52 [a]	16.76±0.78 [a]	145.03±3.78 [a]	26.56±2.93 [a]
River Water	Untreated	15.00±3.56 [a]	16.67±2.78 [a]	134.56±3.97 [a]	23.45±3.23 [a]
	Alum Treated	15.04±1.42 [a]	18.21±1.82 [a]	143.98±4.32 [a]	26.93±1.09 [a]
	Calcium Hypochlorite	16.42±0.73 [a]	16.98±0.21 [a]	131.56±2.45 [a]	24.82±2.17 [a]
	M. indica bark	16.40±1.38 [a]	16.78±0.32 [a]	154.34±3.67 [a]	26.56±1.92 [a]
Control	Normal rats	15.89±0.94 [a]	16.72±0.21 [a]	141.67±3.89 [a]	26.34±2.87 [a]

Data are expressed as Mean ± SEM of triplicate determination. Values followed by different superscript alphabet were significantly different at $p<0.05$.

Table 3. Effect of raw and treated water on kidney function indices in rats.

	Treatments (mg/kg)	Potassium (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Sodium (Mmol/l)
Stream	Untreated	4.35±0.54 [a]	72.73±3.42 [a]	3.21±0.67 [a]	124.32±2.33 [a]
	Alum Treated	9.83±0.12 [b]	142.93±5.43 [b]	3.34±0.82 [a]	163.93±4.32 [b]
	Calcium Hypochlorite	5.35±0.45 [a]	81.34±3.45 [a]	4.19±0.36 [b]	185.45±3.34 [b]
	M. indica bark	5.32±0.21 [a]	69.23±4.54 [a]	2.89±0.32 [a]	122.45±3.45 [a]
Pond	Untreated	4.23±0.76 [a]	65.34±4.32 [a]	3.21±0.61 [a]	102.93±3.23 [a]
	Alum Treated	7.46±0.67 [b]	68.64±3.45 [b]	3.01±0.42 [a]	151.23±4.34 [b]
	Calcium Hypochlorite	5.67±0.13 [a]	69.34±3.67 [a]	5.10±0.92 [b]	138.45±2.34 [a]
	M. indica bark	4.61±0.77 [a]	74.82±2.44 [a]	3.11±0.83 [a]	119.23±3.23 [a]
River	Untreated	4.23±0.21 [a]	71.45±0.32 [a]	2.93±0.21 [a]	125.43±4.34 [a]
	Alum Treated	7.87±0.45 [b]	114.34±4.53 [b]	2.97±0.72 [a]	166.67±5.34 [b]
	Calcium Hypochlorite	5.54±0.32 [a]	79.34±2.78 [a]	4.78±0.31 [b]	188.93±2.34 [b]
	M. indica bark	5.42±0.21 [a]	59.34±0.43 [a]	2.93±0.31 [a]	128.34±4.34 [a]
Control		5.10±0.43 [a]	72.63±3.45 [a]	3.01±0.32 [a]	126.34±3.45 [a]

Data are express as Mean ± SEM of triplicate determination. Values followed by different superscript alphabet were significantly different ($p<0.05$)

Table 4. Effect of raw and treated water on haematological parameters of rats.

	Treatment (mg/kg)	PCV	HB	RBC	WBC	Platelet	MCHC
Stream	Untreated	45.43±2.45 [a]	13.44±0.34 [a]	6.45±0.21 [a]	9.34±0.34 [b]	321.34±4.32 [a]	33.45±0.55 [a]
	Alum Treated	46.27±1.23 [a]	13.52±0.51 [a]	6.43±0.23 [a]	5.21±0.19 [a]	321.45±4.32 [a]	33.45±0.21 [a]
	Calcium Hypochlorite	45.67±2.89 [a]	13.46±0.56 [a]	6.43±0.32 [a]	5.32±0.32 [a]	321.43±6.52 [a]	33.67±0.32 [a]
	*M. indica*bark	53.45±4.87 [b]	16.78±1.56 [b]	9.78±0.56 [b]	5.13±0.45 [a]	325.56±5.32 [a]	33.56±0.32 [a]
Pond	Untreated	42.58±0.45 [a]	13.02±0.62 [a]	6.23±0.23 [a]	7.98±0.23 [b]	327.62±8.64 [a]	33.21±0.42 [a]
	Alum Treated	46.32±1.32 [a]	13.67±0.51 [a]	6.56±0.13 [a]	5.78±0.34 [a]	316.45±9.89 [a]	33.68±0.23 [a]
	Calcium Hypochlorite	45.67±2.22[a]	13.89±0.28 [a]	6.32±0.89 [a]	5.17±0.25[a]	321.28±4.32 [a]	33.32±0.78 [a]
	*M. indica*bark	52.13±250 [b]	15.97±0.31 [b]	9.56±0.35 [b]	5.27±0.32 [a]	316.54±5.43[a]	33.39±0.23 [a]
River	Untreated	47.67±2.67 [a]	14.52±0.23 [a]	6.78±0.45 [a]	6.01±0.21[a, b]	321.23±6.34 [a]	33.92±0.17 [a]
	Alum Treated	46.32±2.98 [a]	14.52±1.34 [a]	6.28±0.56 [a]	5.83±0.45 [a]	332.23±2.34 [a]	33.56±0.25 [a]
	Calcium Hypochlorite	45.53±2.56 [a]	13.32±0.82 [a]	6.96±0.21 [a]	5.28±0.54[a]	335.67±3.56 [a]	33.67±0.32 [a]
	*M. indica*bark	53.34±1.43 [b]	17.21±0.12 [b]	7.98±0.67[a, b]	5.89±0.28 [a]	321.23±11.82 [a]	33.35±0.21 [a]
Control		43.48±2.78 [a]	14.67±1.21 [a]	6.23±0.23 [a]	5.68±0.34 [a]	341.24±8.95 [a]	33.83±0.21 [a]

Values followed by different superscript alphabet were significantly different ($p<0.05$)
PCV: Packed Cell Volume, HB: Haemoglobin, RBC: Red Blood Cell, WBC: White Blood Cell, MCHC: Mean corpuscular Haemoglobin Concentration.

DISCUSSION

The evaluation of hematological parameters (RBC, WBC & PLT) provides valuable information regarding the adverse effects of foreign components on the blood and explains blood-related functions of chemical compounds (17). It has been established that the consumption of medicinal plant-based substances can alter the normal values of hematological indices (18).

Among the erythrocyte indices evaluated in this study, rats administered with water treated with *M. indica* bark had a significant increase ($p<0.05$) in the concentrations of PCV, Hb and RBC, compared to those in the control rats (Table 1). The results suggest that *M. indica* bark exhibited hematopoietic properties by stimulating the erythropoietin release from the kidneys, which is the humoral regulator of RBC production (19). These findings are consistent with those reported by a previous study (20). That study observed that *M. indica* extract administered to animals demonstrated hematopoietic effect, as manifested by increases in the levels of PCV, erythrocyte, leukocyte, platelet and lymphocyte counts. Hemoglobin and RBC are essential for the respiratory gas exchange. The findings of this study also indicated that the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues of the animals were improved (21).

White blood cells and the differential values are known for the body's defense role against foreign body and infectious agents as reflected by the production, transportation and distribution of antibodies after immune responses (10). The significant increase in WBC counts in rats given raw water samples possibly suggests that the immune system of the animals responded by augmenting the production of WBC in order to overcome the stresses induced by the contaminated and untreated waters.

The evaluation of serum biochemical indices in animals has become the most reliable tools for assessing the integrity and functionality of organs, the risk assessment of pathological conditions and general health status (22). Liver enzymes, such as AST and ALT, are biomarkers of hepatic integrity and to a certain extent can be used to assess hepatic cells damage (21). The ALT activities; however, provide more valuable information regarding the integrity of liver cells than does AST. Alkaline phosphatase is often used to assess the integrity of plasma membranes and endoplasmic reticula (10). In the present study, the daily administration of either raw or water samples treated with alum, calcium hypochlorite and *M. indica* bark to albino rats for 28 days did not caused any significant changes to the activities of AST, ALT, alkaline phosphatase, and the total proteins concentrations, compared to those in the control rats (Table 4). This observation suggests that the integrity and functionality of the endoplasmic reticula and plasma membranes in the liver cells were not compromised (21). It also indicates that the extract did not inhibit or stimulate the activities of the enzymes. These findings are consistent with those observed by Ukpo *et al*. (23) who evaluated the immunostimulatory and biochemical effects of the extract of *M. indica* in male rats.

The levels of total serum proteins, electrolytes, creatinine and urea reflect the synthetic and excretory roles of both kidneys and the liver (24). The observed non-significant differences in serum protein concentrations, as shown in rats administered the untreated or treated water samples (alum, calcium hypochlorite and *M. indica* bark) suggest that the treated water did not interfere with the equilibrium in the rate of either synthesis or inhibition of total serum proteins and direct bilirubin in the animals.

The kidneys regulate the excretion of urea and reabsorption of electrolytes into the blood stream.

When the normal glomerular function is compromised, substances normally cleared by the kidneys, such as urea and creatinine, accumulate in the blood (10). Calcium hypochlorite significantly increased the creatinine and potassium concentrations in the serum of animals, compared to those in the control rats (Table 3). The significant increase in the serum urea, sodium and potassium concentrations following the administration of the alum treated water to rats may suggest that normal functioning of the liver and kidney tubules with respect to these electrolytes were compromised (25). However, the preserved functions of the rat organs administered the treated water samples is an indication of its safety for water purifications as opposed to the inherent adverse effect of chemicals, such as alum and calcium hypochlorite that are regularly used in water purification processes. Therefore, it is unlikely that the use of *M. indica* bark for water purification may cause adverse effects on the hematological and other serum biochemical parameters.

The results of phytochemical screening obtained in this study demonstrate similarities to those reported by other studies that determined the phytochemical constituents of different parts of *M. indica* (22-24). Our results were consistent with those of Doughari, and Manzara (26) on the antibacterial activity of crude leaf materials from *M. indica.* This preliminary analysis revealed the presence of tannins, glycosides, saponins and phenols in the *M. indica* leaves. Another study (27) determined the phytochemical constituents in *M. indica* and reported the presence of alkaloid, flavonoids, tannins, saponins, glycosides and anthraquinones in the leaves. Findings from the present study also supported the results of the previous studies (28, 29). These components are known to be biologically active because they protect the plant against infections and predations by animals. In addition to the phytochemical properties and safety of the *M. indica* bark, a previous study has also reported that *M. indica* has therapeutic effects against ulcers and gastritis (30). Therefore, the medicinal benefits of this plant plus the low costs make it a better alternative means for water purification.

CONCLUSION

The use of *M. indica* bark for water purification confers hematopoietic properties to the water and does not have adverse effects on the biochemical parameters in rats, thus provides for a better and safer alternative to the existing coagulating chemicals used for water purification purposes.

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CONFLICT OF INTEREST

The authors declared no conflict of interests in conducting this study.

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