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EVALUATION OF PROTECTIVE AND CURATIVE ROLE OF *MORINGA OLEIFERA* AQUEOUS EXTRACT IN DIMETHYLBENZ(A)ANTHRACENE (DMBA) ACTUATED - NEPHROTOXIC RATS

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ABSTRACT

The point of the present study was to assess the cancer prevention agent and chemopreventive effectiveness of M. oleifera against dimethylbenz(a)anthracene (DMBA) affected nephrotoxicity in rats. Moringa oleifera (MO) is a tropical drumstick tree whose various financial applications are stirring developing global interest. Kidneys vulnerable to damage by poisons, contamination, invulnerable responses and ischaemia. Intense renal disappointment is a continuous complexity of basic disease particularly in the inpatient setting.Neohrotoxicity was induced by DMBA injections , Moringa oleifera extract was administered to rats as protective and curative agents.DMBA induction inspired a huge acceleration in parameters like serum urea, creatinin, protein, electrolytes, cyctatin c and β2 - Microglobulin, add up to and direct bilirubin level and MDA , with a consumption of cancer prevention agent catalysts to be specific SOD, CAT and NO. The helpful adequacy of Moringa oleifera concentrate was seen as far as standardization of changed renal oxidative anxiety parameters and electrolytes and both cyctatin c and β2 - Microglobulin. Explored parameters were restored, about to the ordinary qualities, after MO extract treatment. These outcomes proposed that MO concentrate could act against DMBA-impelled kidney damage in rats by a system identified with its cell reinforcement properties. So also, the oral organization of MO, as therapeutic specialists created comparable changes to those when MO was utilized as a defensive operators, however to a lesser degree.

Key words : Moringa , DMBA , Nephroxicity , Antioxidants, Cyctatin c , β2 - Microglobulin

INTRODUCTION

Therapeutic plants have been utilized by all civic establishments as a wellspring of medications since old times. In the late times, there has been developing enthusiasm for misusing of diverse therapeutic herbs, because of their characteristic cause, cost viability and lesser reactions . Enthusiasm for restorative plants as a re-rising wellbeing help in the support of individual rising so as to wellbeing and prosperity has been fuelled expenses of physician endorsed drugs, and the bioprospecting of new plant-inferred drugs[1]. Kidneys are vulnerable to damage by poisons, disease, insusceptible responses and ischaemia. Intense renal disappointment is a successive difficulty of basic ailment particularly in the inpatient setting . The prognosis of acute renal failure is complicated by secondary injuries induced by free radicals formed during ischaemia/reperfusion injury of the kidney [2]**.** *M. oleifera* is a tropical drumstick tree whose various economic and biological applications are stirring developing global interest. The Moringa tree is developed and utilized as a vegetable (leaves, green cases, blooms, simmered seeds), for flavor (essentially roots), for cooking and restorative oil (seeds) and as a therapeutic plant (all plant organs) [3]. Drumstick tree is exceedingly dry season tolerant, and is developed in semiarid and bone-dry districts of India, Pakistan, Afghanistan, Saudi Arabia, and East Africa accepting a yearly precipitation as low

as 300 mm , albeit such destinations are likely flooded or have a high water table inside of range of the roots. In Arabic it is called "Rawag" [4]. *Moringa oleifera* is a profoundly esteemed plant, dispersed in numerous nations of the tropics and subtropics. It has a noteworthy scope of therapeutic uses with high healthful quality. Distinctive parts of this plant contain a profile of essential minerals, and are a decent wellspring of protein, vitamins, β–carotene, amino acids and different phenolics. The Moringa plant gives a rich and uncommon mix of zeatin, quercetin, kaempferom and numerous different phytochemicals. It is critical for its restorative quality. Different parts of the plant, for example, the leaves, roots, seed, bark, natural product, blooms and youthful cases go about as heart and circulatory stimulants, have antitumor, antipyretic, antiepileptic, mitigating, antiulcer. Other imperative therapeutic properties of the plant incorporate antispasmodic, diuretic, antihypertensive, cholesterol bringing down, cancer prevention agent, antidiabetic, hepatoprotective , antibacterial and antifungal effect. *M. oleifera* parts are being utilized for the treatment of diverse sicknesses in the indigenous arrangement of medication, especially in South Asia.[5] They are well known for their pharmacological activities as well and are utilized for the customary treatment of diabetes mellitus, hepatotoxicity , stiffness, venomous bites furthermore for cardiovascular incitement. Leaves of *M. oleifera* have been utilized as antiulcer, diuretic, anti-inflammatory and for wound mending. Ethanolic concentrate of leaves have demonstrated antifungal movement against various dermatophytes , though methanol extract has a strong CNS depressant activity . The watery concentrate of the leaves has been found to have anti-fertility effect and is exceptionally helpful in controlling the thyroid hormone status in grown-up Swiss rats. Its leaves are likewise utilized as dietary supplement and development promoters because of the critical vicinity of protein, Se, P, Ca, carotene and, tocopherol[6]. In this study, we conjectured that Moringa oleifera leaves might have defensive and therapeutic impact against renal toxicity instigated in rodent's model.

MATERIALS AND METHODS

Plant readiness

Moringa Oleifera acquired as pure *Moringa oleifera* leaf powder , and purchased from Perfectly Natural Herbs Company, Kalamazoo, MI 49048 , USA. Moringa Oleifera watery concentrate was readied in our research facility according to (REF) [7]. Briefly, 1 gm dried and powdered leaves of Moringa Oleifera was blended with 10 ml bubbling water for 5 mins. The blend was then filtered twice. The watery concentrate stock solution(100mg/ml) was freshly prepared for every set of examinations and put away 4◦c up to 5 days.

Experimental Design

Fifty grown-up female albino rats "Sprague-Dawely strain" measuring 120-130 g were acquired from Animal House Colony of King Fahd Medical Research Center, Jeddah, KSA. Rats were acclimatized to research center conditions for 7 days, kept up at steady 24 C with 12 h light-dull cycle and nourished a standard control diet and water *adlibitum*. After acclimatization, the rats were randomized into five trial groups (n = 10) and sustained on refined eating methodologies readied as pellets. Gatherings were planned as takes after:

Group (1): set as control rats creatures in this gathering got 1 ml oral saline day by day.
Group (2): Renal toxicity quality impelled group. Rats were gotten intraperitoneally dimethylbenz(a)anthracene (DMBA) 15 mg/Kg in a single dose [8].
Group (3) rats got Moringa in a measurements of 200mg/Kg [9]
Group (4): Rats orally directed with Moringa oleifera in a measurements of 200 mg/kg body weight 28 days subsequent to accepting intraperitoneally dosage of dimethylbenz(a)anthracene (DMBA) 15 mg/Kg body weight, (DMBA-MO-C) group.
Group (5): Rats orally directed with Moringa Oleifera in a measurements of 200 mg/kg body weight 14 days before and 14 days subsequent to accepting intraperitoneally dosage of dimethylbenz(a)anthracene (DMBA) 15 mg/Kg body weight , (DMBA-MO-P) group . Toward the end of the exploratory period, the rats were fasted overnight and subjected to diethyl ether anesthesia. The blood tests were quickly gathered from the retro orbital venous plexus and serum tests were gathered for biochemical investigation. At that point, the rats were yielded by cervical separation and the kidney tissue was dismembered and weighed.

Biochemical investigation

Serum electrolytes (Na, K and Cl) ,urea, creatinine and uric acid , malondialdehyde , nitric oxide , superoxide dismutase and catalase compound were

estimated by commercial kits. Cyctatin C and β 2 - Microglobulin were determined according to Christensson et al.,[10] and Naveen et al., [11]

RESULTS

The consequences of the present study unmistakably show that Moringa Oleifera extract has both defensive and therapeutic activity. Table (1) represent the impact of Moringa Oleifera on some biochemical parameters in the control and treated gatherings against DMBA-prompted nephrotoxicity in male rats. Significant elevation ($P < 0.05$) was seen in the levels of creatinine and urea in DMBA challenged rats when contrasted with the corresponding control groups. On the other hand, serum total protein level of DMBA treated rats was essentially diminished ($P < 0.05$) when contrasted with the relating control . However, Pretreatment of rats with Moringa Oleifera before DMBA induction for 14 successive days (DMBA-MO-P) brought about a critical lessening ($P < 0.05$) in the serum levels of urea and creatinine when contrasted with DMBA treated group. Table (2) DMBA intoxication brought about nephrotoxicity which was showed by noteworthy increment ($P < 0.05$) in serum total bilirubin and direct bilirubin when contrasted with other treated groups. Generally, oral organization of MO altogether diminished ($P < 0.05$) the raised levels of both total and direct bilirubin this decrease was more declared in pretreated rats (DMBA-MO-P) than in (DMBA-MO - C) rodent group when contrasted with (DMBA) group. Results from table (3) showed that Intake of aqueous extract of Moringa oleifera 200 mg/kg body weight along with DMBA significantly ($p<0.05$) improved serum electrolyte levels.

Table(4)demonstrated that administration of DMBA evoked sensational noteworthy increment ($p< 0.05$) in the estimations of Cystatine C and β2-Microglobulin. Moringa extract (DMBA-MO-P), diminished the lifted levels of Cystatine C and β2-Microglobulin when contrasted with the comparing DMBA and (DMBA-MO-C) groups. Results acquired from table (5) demonstrated that DMBA organization brought about huge ($p<0.05$) increment in the MDA level and critical ($p<0.05$) diminish in CAT and SOD activity and NO, in examination to control group. Oral administration of fluid concentrate of Moringa oleifera essentially ($p<0.01$) diminished MDA level and fundamentally expanded the CAT, SOD and NO activity either it was utilized as remedial or defensive treatment.

Table 1

Effect of Moringa Oleifera (MO) on kidney functions in all experimental groups (Mean ± SE)

	Urea mg/dl	Creatinine mg/dl	Protein g/L
Healthy control (C)	25.61 ± .0074	0.868 ± 0.0007	1.65± .00223
Renal toxicity (DMBA)	35.166 ± 1.266	1.25 ± .00428	1.15± .0042
Healthy + M.O extract (H-MO)	25.633 ± 0.135	0.86 ± 0.001	1.93 ± 0.0076
Renal toxicity + M.O (curative) (DMBA-MO -C)	31.95 ± 0.406	0.93 ± 0.0077	1.483 ± 0.004
Renal toxicity + M.O (protective) (DMBA-MO-P)	28.8± 0.383	0.94 ± 0.001	1.7± .004

Table 2

Effect of *Moringa Oleifera* (MO) on total and direct billirubin in all experimental groups (Mean ± SE)

	Total Billirubin Umol/L	Direct Billirubin Umol/L
Healthy control (C)	4.566 ± 0.1585	1.233 ± 0.0042
Renal toxicity (DMBA)	8.45 ± 0.0095	3.48 ± 0.006
Healthy + M.O extract (H-MO)	6.45 ± .0067	2.25 ± 0.0042
Renal toxicity + M.O (curative) (DMBA-MO -C)	5.8 ± 0.0025	2.866 ±0.0033
Renal toxicity + M.O (protective) (DMBA-MO-P)	4.88 ±0.003	2.183 ± 0.004

Table 3
Effect of Moringa Oleifera (MO) on serum sodium, potassium and chloride in all experimental groups (Mean ± SE)

	Sodium Mmol/L	Potassium Mmol/L	Chloride Mmol/L
Healthy control (C)	138.16 ± 0.477	4.34 ± 0.009	97.5 ± 0.562
Renal toxicity (DMBA)	124.66 ± 0.666	5.48 ± 0.205	102.5 ±1.384
Healthy + M.O extract (H-MO)	138.33 ± 0.66	4.41 ± 0.0079	97.5 ± 0.763
Renal toxicity + M.O (curative) (DMBA-MO -C)	138.5 ± 1.25	4.66 ± 0.138	95.5 ± 1.11
Renal toxicity + M.O (protective) (DMBA-MO-P)	138.83 ± 0.307	4.25 ± 0.114	94.33 ± 0.714

Table 4
Effect of Moringa Oleifera (MO) on serum cystatin - C and β2 -microglobulin in all experimental groups (Mean ± SE)

	Cystatine C	β 2 -Microglobulin
Healthy control (C)	1.536 ± 0.00135	3.25± 0.114
Renal toxicity (DMBA)	3.7 ± 0.00632	5.45 ± 0.0067
Healthy + M.O extract (H-MO)	2.416 ± 0.00477	4.523 ± 0.0084
Renal toxicity + M.O (curative) (DMBA-MO -C)	2.6 ± 0.0073	3.8 ± 0.0036
Renal toxicity + M.O (protective) (DMBA-MO-P)	2.25 ± 0.00718	3.26 ± 0.0042

Table 5
Effect of Moringa Oleifera (MO) on serum antioxidants against DMBA-induced nephrotoxicity in rats. (Mean ± SE)

	SOD U/g	MDA nmol/g	Catalase U/g	Nitric Oxide µmol/L
Healthy control (C)	11.28± .535	9.86 ± .408	1.95 ±.0035	127.36 ± 1.96
Renal toxicity (DMBA)	6.96 ± .174[a]	18.37±1.804[a]	1.125±.0083[a]	70.5 ± 3.24[a]
Healthy + M.O extract (H-MO)	10.083±.212[b]	10.39 ±.221[b]	1.82 ± .135[b]	126.17 ±3.23[b]
Renal toxicity +M.O(curative) (DMBA-MO -C)	11.15 ± .563[b]	14.41±.423[bc]	1.86 ± .003[b]	102.33±1.33[bc]
Renal toxicity +M.O(protective) (DMBA-MO-P)	11.43±.364[bc]	14.24 ± .63[bc]	1.93±0042[b]	129.25± 1.09[b]

DISCUSSION

The *M. oleifera* has shown remarkable potential which could be harnessed for medicinal and nutritional purposes. In recent times, several studies have been able to demonstrate the medicinal significance of *M. oleifera* [12]**.** Moringa is an important plant that used as human food and in medicine . Leaves of this plant are reported to have biological importance, including thyroid hormone regulation, hypocholesterolemic, gastric ulcers, antitumor agent antihyperglycemic, hypotensive agent and antidiabetic properties. Moringa leaves as well as the, roots, gums, flowers, seeds. It is used for treating of inflammations [13]**,** liver disease and hematological, renal and hepatic function[9]**.** In the evidence of medicinal and nutritional benefits of *M. oleifera*, *M. oleifera* was administered for healthy rats (H-MO), for another group of rats 14 days pretreated with aqueous extract of *M. oleifera* before DMBA induction of nephrotoxicity and 14 days after the induction (protective group) (DMBA-MO-P), and for a group of rats treated with aqueous extract of *M. oleifera* 28 days after the induction of nephrotoxicity (DMBA-MO-C) (curative group), in order to study the potential role Moringa role in attenuating DMBA -induced nephrotoxicity in rats [14]**.** Results of the study showed that DMBA

administration causes nephrotoxicity as manifested by dramatic increased values of urea, creatinine, blood electrolytes, Cystatine C and β2-Microglobulin, together with a reduction in serum protein as compared to all treated groups. Results showed the major target organ of toxicity of DMBA is kidney, then comes the mammary glands, skin and liver .Bowman's capsule also seem to be more sensitive to DMBA induced renal toxicity. Many studies provided experimental techniques that DMBA exposure produced ROS thus causing cell damage or cell death . DMBA is known to be cytotoxic, mutagenic, immunosuppressive and carcinogenic agent [15]**.** It induces potentially malignant tumors in vital organs such as liver, kidney, brain and bladder. In addition, there are high evidences that DMBA induces the ROS production which result in DNA damage, depletion of cell antioxidant defense system and lipid peroxidation [16]**.** DMBA is polycyclic hydrocarbon, is environmental pollutants which has multiple toxic and carcinogenic effects. DMBA-considered an ideal model for studing the chemopreventive importance of medicinal plants and studying plants active ingredients**.** Nephrotoxicity may be resulting to guide cytotoxic harm to kidney structures by toxicants, to immunologic procedures, to aberrant harmfulness because of changes in renal hemodynamics, or to the creation of endogenous nephrotoxic substances renal cortical mitochondria are the source of reactive oxygen metabolites**.** Nephrotoxic effects developed in tubular and glomerular epithelial cells due to mechanisms that disturb normal cellular functions of mitochondria and membrane integrity, which induces kidney toxicity [17]**.S**erum urea (BUN) and creatinnine (CR) are biochemical indicators of kidney damage. BUN and CR not only reflect the nitrogenous compounds metabolism in organism, but also the glomerular filtration function damage. In the present study, we found that BUN and CR were significantly increased in the DMPA-treated group, significant increase was noticed in CR after the administration of DMBA may compromise the capacity of renal function. CR increase DMBA might interfere with creatinine metabolism leading to increase its synthesis. Combined administration of *M. oleifera* plus DMBA to rats resulted in reduced levels of serum BUN and CR. Results was in agreement with previous study **[18]** *M. oleifera* can modulate the serum BUN and CR levels to ameliorate the toxic effect of DMBA even used as curative or protective treatment. Plasma levels of creatinine and urea are among the major biochemical indices commonly used to evaluate renal functions. Creatinine is a by-product of muscle metabolism and under normal physiological condition the amount excreted per day is constant and correlates with body mass. Conversely, when creatinine level is elevated due to retention in the blood, it could be used to evaluate glomerular filtration rate [19]**.** Urea, on the other hand, is formed as means to rid body of nitrogenous waste from protein degradation. It is formed in the liver and excreted by the kidney in urine. Elevated plasma urea level has been linked to reduced renal function. In the present study, levels of plasma creatinine and urea were determined [20]**.** However, the feeding of diets containing different concentrations of M. oleifera to rats prevented the elevation of the indices for renal dysfunction. These findings are in support of previous studies which showed that M. oleifera offered nephro- and hepatoprotection [8]**.** Results showed a significant increase in serum protein content after administration of *M. oleifera* as curative or protective agent. Earlier studies have revealed M. *oleifera* as being rich in protein content [21]**.** DMBA intoxication resulted in significant increase in serum total bilirubin and direct bilirubin as compared to other treated groups. Oral administration of MO significantly reduced the elevated levels of both total and direct bilirubin this reduction was more announced in pretreated rats protective group (DMBA-MO-P) rather than in curative group (DMBA-MO-C) when compared to G2. Oral treatment with *M. oleifera* extract (200 mg/kg b.w), significantly improved serum electrolyte levels. Our results revealed significant alteration to levels of rat plasma electrolytes caused by oral exposure to DMBA. On the contrary, the significant reductions in levels of plasma sodium and potassium caused by DMBA were averted by M. oleifera supplemented diets. The maintenance of normal levels of plasma electrolytes is crucial to homeostatic balance [22]**.** Perturbation of plasma electrolyte balance may affect the pH, osmolality, and blood volume with adverse impact on the kidney and other related body organs. M. oleifera has been shown to possess diuretic effect and this may have contributed to protecting against homeostatic imbalance imposed by DMBA exposure [23]**.** Moreover, the maintenance of normal level of plasma sodium and potassium by M. oleifera diet is consistent with reported therapeutic potential including the improvement of the health

of renal tissues and general well-being. Although, supplementing of M. oleifera as curative or protective, caused a decreases in the level of sodium and potassium, the alterations were found not to be significant when compared to the control group given normal saline daily [24]. Administration of DMBA elicited significant increase in values of Cystatine C and β 2 -Microglobulin . Administration of moringa as protective agent before toxicity induction for 14 days (DMBA-MO-P), reduced the elevated levels of Cystatine C and β 2 -Microglobulin as compared to the corresponding DMBA and (DMBA-MO -C) groups. Administration of moringa as curative (DMBA-MO-C) was found to cause a significant decrease in Cystatine C and β 2 -Microglobulin values as compared to DMBA group. β2-Microglobulin is a free filterable protein under regular condition, and is reabsorbed totally in the proximal tubules. However, nephron damage leads to an increase in β 2-Microglobulin concentration in the urine. In the present study, elevated excretion of cystatin c and β2-Microglobulin indicate that DMBA exposure can induce renal tubule dysfunction [25]. Cystatin C is an endogenous cysteine proteinase inhibitor, produced by nucleated cells at a steady rate [26]. It is freely filtered by the glomerulus and reabsorbed, then catabolized, although not secreted by tubules. Studies approved the superiority of serum cystatin C when compared to creatinine, in detecting minor reduction of GFR. Previous studies on cystatin C showed that serum cystatin C considered to be better than serum CR in detecting acute changes of GFR [27]. Results indicate that serum cystatin C performs well as a marker to detect ARF. In addition, cystatin C may be used to detect the development of ARF one to two days earlier than serum CR, the current standard marker for ARF, this is important because early detection of ARF will provide extra time in preventing ARF progression. In the present study, malondyaldehyde (MDA) , catalase (CAT) activities ,superoxide dismutase (SOD) and nitric oxide (NO) were determined as they considered important components of cellular defense system against any oxidative stress. DMBA administration increased the MDA with parallel reduction in CAT and SOD and NO levels, in comparison to control group. DMBA induces substantive nephrotoxicity which characterized by kidney tubular necrosis, proteinuria with upregulation of specific signals like tumor necrosis factor-α, chemokines and cytokines . It also induced different histological alterations, include renal tubule dilutions; sloughing of epithelium which indicates tubules disintegration [8]. Normal kidney contains several antioxidant enzymes, which are directly and indirectly involved in the prevention of kidney injury which occurs due to excessive production of free oxygen radicals. These ROS promote MDA formation, a lipid peroxidation end product in kidney [28]. The content of MDA is a good index of increased oxidative stress in renal tissue, causing raised peroxidation processes. Lipid peroxides are unstable and can be decomposed to forming complex compounds, including reactive carbonyl compound, which is the most abundant malondialdehyde [29]. This study revealed that, MDA concentration increase significantly in both pre-treated and post-treated groups with DMBA as compared with control groups. These results are in agreement with [8]. This increase in MDA concentration related to the damage occurs in kidney cells due to production of reactive oxygen species by DMBA [30]. On the other hand, MDA concentration decreases significantly in both pre-treated and post-treated groups with MO as compared with DMBA groups. This decrease revealed the ability of the extract to resistant the oxidative damage occurred by DMBA. Reactive oxygen species ROS distort the normal functioning of antioxidant enzyme cascade such as SOD and CAT. SOD act to convert superoxide (O^{2-}) to hydrogen peroxide (H_2O_2) it considered a major defence system for aerobic cells in reducing toxic effects of SOD radical [30].Catalase decomposes hydrogen peroxide (H_2O_2) into H_2O and O_2 and protects the tissue from highly reactive hydroxyl radicals. Results of the study reveals that, free radical production as an outcome of oxidative stress due to DMPA raises the activities of SOD and CAT in rat kidney. Increased activities may be the adaptive response of the body to ROS attack. ROS are known to promote endothelial dysfunction through several mechanisms, depleting the cellular levels of nitric oxide (NO), an endogenous antioxidant, and promoting formation of lipid inflammatory mediators, direct action on the vascular endothelium, [31]. On the other hand, oral administration of aqueous extract of *Moringa oleifera d*ecreased increased the CAT, SOD and NO activities even it was used as curative or protective treatment. The antioxidant property of *Moringa m*ay is due to phenolic compounds. *Moringa p*ods contain important bioactive compounds including isothiocyanates, thiocarbamates, flavonoids and glucosinolates,

which quench ROS, regenerate membrane-bound antioxidants by chelating metal ions. Many previous studies, demonstrated the antioxidant activity of *Moringa e*xtract[32] **.**

CONCLUSION

The data obtained from this study indicated that *Moringa* extract has chemopreventive properties by antioxidant status enhancement and ROS quenching at least partially, alleviates DMBA-induced nephrotoxicity by preventing lipid peroxidation enzyme system and increasing antioxidant enzyme activities. Moreover, the use of *Moringa* extract prior to DMBA treatment (as protection) is more effective than its curative effect in preventing DMBA-induced nephrotoxicity. Therefore, administration of *Moringa* extract is a great agent in preventing kidney injury and dysfunction thus protecting kidney tissue against oxidative damage.

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