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Prevention of Oxidative Stress Caused by Anti-tubercular Drugs Using Aqueous Extracts of *Daucus carota* and *Moringa oleifera*

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ABSTRACT- The antioxidant effect of aqueous extracts of *Daucus carota* root and *Moringa oleifera* leaf were investigated in anti-tubercular drugs (isoniazid, INH and rifampicin, RMP) induced oxidative stress.The test control rats were administered with INH (27mg/kg) and RMP (54mg/kg) for 4 weeks to induce oxidative stress. Rats were pretreated with doses of 100mg/kg and 200mg/kg of aqueous extracts of *Daucus carota* and *Moringa oleifera* respectively for 4 weeks then INH and RMP were administered for another 4 weeks. Rats were also co-administered with 100mg/kg and 200mg/kg of the extracts plus INH and RMP for 4 weeks. The effect of the extracts on oxidative stress was determined by monitoring the serum malondialdehyde (MDA) concentration. Pretreatment and co-administration of *Daucus carota* and *Moringa oleifera* aqueous extracts significantly ($P<0.05$) reduced serum MDA levels compared with the test control. The co-administration of the extracts with the anti-tubercular drugs had a more profound effect in reducing serum MDA concentration compared with pretreatment with the aqueous extracts of *D. carota* root and *M. oleifera* leaf. *Moringa oleifera* leaves and *Daucus carota* rootsmay afford anti-oxidant effect in anti-tubercular drugs- induced oxidative stress.

Index Terms: anti-tubercular drugs, *Daucus carota*, malondialdehyde,*Moringa oleifera*, oxidative stress

1. INTRODUCTION

Anti-tubercular drugs-induced hepatotoxicity is a potential adverse effect of the currently used anti- tuberculosis regimens and occurs in about 9% of patients treated for active tuberculosis (TB)[1,2]. The most effective anti- tuberculosis therapy (standard therapy) is a combination of isoniazid (INH), rifampicin (RMP), and pyrazinamide (PZA) for eight weeks followed by INH and RMP for a further 4-7 months[3]. However, there is a large evidence for toxicity of these standard drugs in humans[4,5,6], with hepatotoxicity being the most serious effect[7]. The rate of hepatotoxicity has been reported to be much higher in developing countries (8 – 30%) compared to advanced countries (2 -3%) with a similar dose schedule[8]. Hepatotoxicity complicates the treatment of 5 – 10% patients treated for active TB[9]. Isoniazid hepatotoxicity is considered idiosyncratic, not as a result of hypersensitivity or allergic reaction, but rather is most probably caused by toxic metabolites[10,11]. The predominant metabolic pathway of INH metabolism is acetylation by the hepatic enzyme N-acetyl transferase 2 (NAT 2)[12]. It is acetylated to acetylisoniazid and then hydrolysed into acetylhydrazine and

isonicotinic acid. Acetylhydrazine is either hydrolysed to hydrazine or acetylated into diacetyhydrazine[11,13]. The major metabolic pathway of rifampicin is desacetylation to desacetylrifampicin and separate hydrolysis produces 3 – formylrifampicin[14,15]. The pathogenesis of INH and RMP – induced damage may involve oxidative stress in the liver mitochondria associated with mitochondrial permeability alterations and increased apoptosis of the hepatocytes, and therefore mitochondrial redox changes have been suggested as crucial events in apoptotic liver cell injury in INH- RMP.

Different parts of the carrot plant were used in Indian traditional medicine for the treatment of a broad spectrum of ailments such as inflammation, leprosy and worm infections[16]. When taken daily, carrots can lower cholesterol and blood pressure[17,18]. Intake of foods rich in carotenoids may be beneficial to blood sugar regulation[19]. Carrots may help slow the ageing process and reduce the risk of many diseases including cancer, heart diseases, cataracts, stroke, high blood pressure, osteoporosis, bronchitis and urinary tract infections[20].

The Moringa tree has great use medicinally both as preventive and treatment, and virtually every part of the tree (bark, roots, fruit, flowers, leaves, seeds, and gum) can be used medicinally. Studies have indicated that the plant possesses antiplasmodial activity[21], radioprotective capacities[22], thyroid hormone regulatory properties[23], hypocholesterolemic action[24], hypotensive[25] and antifungal effects[26,27]. It is also effective as antitrypanosomal[28], antiulcer[29,30], diuretic[31], anti-inflammatory and antispasmodic[32]. Moringa flowers have been shown to possess, antitubercular, antibacterial and depressant property[33]. Aqueous root extracts shows inhibitory effect on central nervous system[34]. The present study was undertaken in an attempt to evaluate the antioxidant effects of *Daucus carota* and *Moringa oleifera* against INH and RMP-induced oxidative stress.

II. MATERIALS AND METHODS

a. Animals

Wistar albino rats weighing 100-130g were obtained from the Animal House, Department of Biological Sciences, Bayero University, Kano. The animals were housed in cages in a room where a 12-hour light/dark cycle was maintained. They were allowed free access to water and feed (a product of Grand Cereals and Oil Mills Ltd) throughout the experimental period.

b. Drugs

Isoniazid tablets BP (300mg) Microlabs Ltd, India and rifampicin capsules BP (300mg) Maxheal Pharmaceuticals, India were used for the research.

c. Preparation of Extracts

Leaves of moringa plant were obtained from the Botanical garden of Biological Sciences Department, Bayero University, Kano Nigeria. They were washed, shade-dried and pulverized into a powder. A known weight of the powder was soaked overnight in

distilled water, filtered and the residue dried. The residue was weighed and concentration of the filtrate was determined from the difference in weight.

Carrots roots were purchased from a local market, washed and grated into smaller pieces. They were dried and pulverized into powder. The powder (20g) was soaked in 200cm^3 of distilled water overnight and filtered. The residue was allowed to dry, weighed and subtracted from the initial weight of the powder to determine the concentration of the filtrate. The filtrates were stored in the refrigerator and used for the experimental work.

d. Experimental Design

Thirty six (36) wistar albino rats were divided into 12 groups of three rats each. Group 1 were the normal rats while group 2 served as the test control administered with INH (27mg/kg) and RMP (54mg/kg) only for 4 weeks to induce liver damage. Groups 3 and 4 were pretreated with 100mg/kg and 200mg/kg of extracts respectively for 4 weeks, then administered with INH (27mg/kg) and RMP (54mg/kg) for another 4 weeks. For groups 5 and 6, same doses of the extracts and drugs were administered concurrently, thirty minutes apart for 4 weeks. The remaining six groups were used for a similar set of experiment using *Daucus carota* aqueous extract. Blood samples were collected 24 hours after the last administration and concentration of serum malondialdehyde (MDA) was estimated.

e. Estimation of Serum MDA

This was done using the method of Hunter *et al*. (1963)[35] as modified by Gutteridge and Wilkins (1982)[36].

f. Statistical Analysis

All results were expressed as mean$\pm$SD for each group. Data were analysed with student's t – test. P values of less than 0.05 ($P<0.05$) were considered significant.

III. RESULTS

The effect of pretreatment and co-administration of *Moringa oleifera* and *Daucus carota* extracts on the level of serum MDA in rats treated with anti-tubercular drugs is presented in Table 1. Anti-tubercular drugs exerted a significant ($P<0.05$) increase in serum MDA concentration in group 2, whereas both pretreatment and co-administration with varied doses of aqueous extracts of *Moringa* and *Daucus carota* caused significant ($P<0.05$) decreases in serum MDA levels. The co-administration of the extracts showed more profound effect in reducing serum MDA concentration compared to pretreatment.

Earlier investigations have shown that oxidative stress is the major mechanism of INH-RMP induced hepatotoxicity in experimental rats[37,38]. RMP is a potent inducer of cytochrome P-450 system which mediates generation of toxic metabolites of drugs and their

covalent binding to hepatic macromolecules[39], while INH is believed to mediate hepatotoxicity through the production of toxic metabolites[11,40]. In the present study, free radicals formed either by the reaction of metabolites with oxygen or by the interaction of superoxide radicals with H_2O_2, seem to initiate peroxidative degradation of membrane lipids rich in polyunsaturated fatty acids. This leads to formation of lipid peroxides which in turn give products like MDA that cause loss of integrity of cell membrane and damage to hepatic tissue. In rats treated with INH and RMP alone, the increase in MDA indicates enhanced peroxidation leading to a failure of the antioxidant defence mechanism to prevent formation of excess free radicals. Moringa extract combined with anti-tubercular drugs significantly prevented lipid peroxidation.

Numerous studies showed the elevation of a variety of detoxification and antioxidant enzymes and biomarkers as a result of treatment with moringa or with phytochemicals isolated from moringa[41,42,43].

Being an excellent source of nutrients, minerals, vitamins, etc moringa has been described as a nutritional dynamite. The combination of antioxidant enzymes and phytochemicals found in moringa coupled with the plant's outstanding nutritive value may have been responsible for the protective effect exerted in the prevention of oxidative stress attributed to antitubercular drugs. Antioxidant and free radical scavenging activities of moringa leaf extract has been reported[44], a fact which has been substantiated in this study. Thus it can be concluded *Moringa oleifera* leaves afford antioxidant properties in oxidative stress caused by antitubercular drugs.

Carrot is an excellent source of vitamin A and a very good source of vitamin C; therefore it may be useful in reducing oxidative stress on the body cells. Being an excellent source of several antioxidant compounds such as phenolic compounds that play an important role in antioxidant properties of carrots and the other hydroxycinnamic derivatives such as dicaffeoylquinic acids in the extract may exert some strong antioxidant activities along with chlorogenic acid[45].

Carotenes in the carrot extract include b-carotene, a-carotene, g-carotene, lycopene, cryptoxanthin, leutin and many partly degraded carotenoids such as abscisic acid, trisporic acid, -apocarotenoids, e.g. violaxanthin[46]. Some of the above active principles have the potential to minimize the deleterious effects of free radicals including the peroxy radicals [47]. This confirms that carrot extract could effectively protect tissues against the free radical mediated oxidative stress as evidenced by significantly decreased MDA levels in serum.

Table 1: Effect of pretreatment and co-administration of aqueous extracts of *Moringa oleifera/Daucus carota* and antitubercular drugs on serum MDA concentration.

Groups	**Dose of extract (mg/kg)**	**Serum MDA (µmol/L)** ***Moringa oleifera***	***Daucus carota***
Group 1 (Normal)	-	0.13±0.04	0.13±0.04
Group 2	-	0.66±0.16[a]	0.67±0.16

(Test control)			
Group 3	Pretreatment 100mg/kg	0.33 ± 0.12^{b}	0.23 ± 0.04^{a}
Group 4	Pretreatment 200mg/kg	0.13 ± 0.04^{b}	0.27 ± 0.16^{b}
Group 5	Co-administration 100mg/kg	0.13 ± 0.04^{b}	0.17 ± 0.09^{b}
Group 6	Co-administration 200mg/kg	0.23 ± 0.05^{b}	0.17 ± 0.09^{b}

a = significant increase ($P<0.05$) as compared with the normal control group.

b = significant decrease ($P<0.05$) as compared with the test control group.

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