Protective Effect of *Allium sativum* against Liver Injury Induced by Anti-Tubercular Drugs in Rats

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**Abstract:** *Allium sativum* has been reported to have a lot of therapeutic potentials such as antihypertensive, antimicrobial and hypocholesterolemic. This study has investigated its effect on anti-tubercular drugs hepatotoxicity. Wistar albino rats were pretreated and co-administered orally with aqueous solution of *Allium sativum*. Eighteen rats were divided into six groups of three rats each. Group 1 were normal, while Group 2 were test control administered with anti-tubercular drugs; isoniazid (27 mg/kg bw) and rifampicin (54 mg/kg bw). Groups 3 and 4 were pretreated with 100 and 200 mg/kg of *Allium sativum* for 28 days, then administered with isoniazid and rifampicin at doses 27 and 54 mg/kg respectively for same duration. In groups 5 and 6, same doses of *Allium sativum* and anti-tubercular drugs were administered concurrently for twenty eight days. Serum levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) and unconjugated bilirubin (uBIL) were estimated 24 h after administration of the last dose. The results showed that pretreatment and co-administration of *Allium sativum* solution significantly reduced (p<0.05) serum ALT, AST and ALP activities and uBIL concentration in the rats, compared to the test control administered with anti-tubercular drugs only, to induce liver damage. The results suggest that aqueous extracts of *Allium sativum* can be said to have protective effect on anti-tubercular drugs hepatotoxicity in rats.

**Key words:** *Allium sativum*, anti-tubercular drugs, hepatotoxicity, isoniazid, liver enzymes, rifampicin

**INTRODUCTION**

All drugs have the potential to cause injury and in many cases, such adverse effects can outweigh the benefits (Fingerote, 2008). Drug-induced liver injury is an important and commonly encountered adverse effect with anti-tuberculosis therapy (Sharma, 2004), which comprises of 2 months of Isoniazid (INH), Rifampicin (RMP) and Pyrazinamide (PZA) with or without Ethambutol (ETH), followed by 4 months of treatment with INH and RMP (WHO, 2003; Blumberg et al., 2003). The two main drugs INH and RMP are in the arsenal due to their high bactericidal power (Migliori et al., 2007). During the first 2 months intensive phase of TB treatment, INH kills 90% of viable bacilli, this renders the patient non infectious and reduces the risk of drug resistance (Donald and Schaaf, 2007). During the 4 month continuation phase, RMP kills the last remaining bacilli while INH assists in preventing drug resistance. Anti-Tubercular Drugs-induced Hepatotoxicity (ATDH) is a potential adverse effect of the currently used anti-TB regimens involving isoniazid, rifampicin and pyrazinamide with or without ethambutol and occurs in about 9% of patients treated for active TB (Yee et al., 2003; Tasduq et al., 2005). However, currently available second-line drugs used to treat MDR-TB are four to ten times more likely to fail than the standard therapy for drug susceptible tuberculosis (Nikalje and Mudassar, 2011).

Tuberculosis is a systemic disease caused by *Mycobacterium tuberculosis* (Ouatarra et al., 2011), which most commonly affects the lungs but can also involve almost any organ of the body (George and Stoppler, 2010). With nearly one-third of the global population infected by *Mycobacterium tuberculosis*, TB remains a major cause of death (1.7 million in 2006), (Aristoff et al., 2010). An anti-TB regimen is considered useful when more than 90% of patients are cured and fewer than 5% of patients develop serious intolerance (WHO, 2003).

*Allium sativum* (garlic) has been shown to have a lot of therapeutic potentials such as; antihypertensive (Krishnaraju et al., 2006), anti-rheumatic, stimulant, in treatment of asthma, cold, diabetes, paralysis and chronic fever (Morakinyo et al., 2008; Nadkarni, 1994). It was also found to possess hypocholesterolemic effect (Siligaly and Neil, 1994) and strong antimicrobial activity (Sood...
et al., 2003). Wattenberg (1990) has shown its high potential in the prevention of cancer. Sang et al. (1995) have demonstrated that garlic oil is effective against fat infiltration of the liver. In combination with propranolol, garlic homogenate offered protection from myocardial infarction (Vibha et al., 2011) and nephrotoxicity caused by amikacin (Iman and Mahmoud, 2011).

In recent years, many researchers have examined the effects of plants used traditionally by indigenous healers to support the treatment of liver diseases. Triphala (Mahaboob et al., 2007), Senna occidentalis (Nwaehujor et al., 2011) and Parkinsonia aculeata (Hassan et al., 2008) have all yielded positive results.

This study investigated the protective effect of Allium sativum in hepatotoxicity caused by anti-tubercular drugs in rats.

MATERIALS AND METHODS

Animals: Eighteen wistar albino rats weighing 120-140 g 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 h light/dark cycle was maintained. They were allowed free access to water and feed (a product of Grand Cereals and Oil Mills Ltd, Jos, Nigeria) throughout the experimental period.

Drugs: Isoniazid tablets BP (300 mg) Microlabs Ltd., India. Rifampicin capsules BP (300 mg) Maxheal Pharmaceuticals, India. Garlic once-a-day tablets (300 mg) Kwai® Klosterfrau, Germany.

Preparation of garlic solution: One garlic tablet was dissolved in 100 mL of distilled water to make a 3 mg/mL solution administered to the rats.

Experimental design: The study was conducted at the Animal House, Department of Biological Sciences, Bayero University Kano between September to December, 2010. Rats were divided into 6 groups of three rats each. The drugs and garlic solution were administered orally. Group 1 comprised of the normal rats, while group 2 served as the test control administered with INH (27 mg/kg bw) and RMP (54 mg/kg bw) only for 28 days to induce liver damage. Groups 3 and 4 were pretreated with 100 and 200 mg/kg bw of garlic solution respectively for 28 days then administered with INH (27 mg/kg bw) and RMP (54 mg/kg bw) from 29 to 56th day. Groups 5 and 6 were administered with same doses of the garlic solution and drugs concurrently for 28 days.

The rats were sacrificed 24 h after the last administration. Blood samples were collected and Serum Liver Transaminases (ALT and AST), Alkaline Phosphatase (ALP) activities and Unconjugated Bilirubin (uBIL) concentration were estimated.

Assessment of liver damage:
Biochemical investigations: Serum AST and ALT activities were determined using the method of Reitman and Frankel (1957), uBIL was estimated by the method of Jendrassik and Grof (1938) and ALP estimated using the method of Roy (1970).

Statistical analysis: All results were expressed as mean±SD for each group. Data were analysed with student’s t-test using SPSS. P values of less than 0.05 ($p<0.05$) were considered significant.

RESULTS AND DISCUSSION

The results in Table 1 showed increase in the analysed biochemical parameters in the test control (Group 2), compared to the normal control (Group 1). This increase is in line with the proven hepatotoxic effects of INH and RMP. Administration of garlic both in pretreatment and co-administration significantly ($p<0.05$) reduced serum ALP, AST and ALT activities and uBIL concentration in groups 3-6. Reduction in serum ALP activity was more profound in group 4, while group 5 exhibited the most reduced AST activity compared to the test control. Pretreatment with 100 mg/kg bw of garlic solution (Group 3) lowered serum ALT activity better than groups 4-6. The same treatment favoured serum uBIL by decreasing the concentration, compared to the test control group.

Table 1: Effect of pretreatment and co-administration of Allium sativum and anti-tubercular drugs on serum ALP, AST, ALT activities and uBIL concentration in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose of extracts (mg/kg)</th>
<th>ALP (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>uBIL (μM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>-</td>
<td>29.33±3.60</td>
<td>17.00±9.90</td>
<td>6.00±1.40</td>
<td>2.49±0.49</td>
</tr>
<tr>
<td>Group 2</td>
<td>-</td>
<td>51.00±4.20</td>
<td>52.66±4.60</td>
<td>25.33±12.60</td>
<td>7.46±1.80</td>
</tr>
<tr>
<td>Group 3</td>
<td>Pretreatment 100</td>
<td>47.33±6.20b</td>
<td>37.00±11.70a</td>
<td>8.33±1.20a</td>
<td>4.33±1.60</td>
</tr>
<tr>
<td>Group 4</td>
<td>Pretreatment 200</td>
<td>39.66±3.40b</td>
<td>17.00±1.40b</td>
<td>12.33±2.10a</td>
<td>2.56±0.40</td>
</tr>
<tr>
<td>Group 5</td>
<td>Co-administration 100</td>
<td>40.66±0.40b</td>
<td>14.66±0.80b</td>
<td>12.33±4.20a</td>
<td>2.56±0.40</td>
</tr>
<tr>
<td>Group 6</td>
<td>Co-administration 200</td>
<td>42.66±2.60b</td>
<td>33.33±3.80b</td>
<td>21.66±10.60b</td>
<td>2.89±0.20</td>
</tr>
</tbody>
</table>

n: 3; a: Significant increase ($p<0.05$) as compared with the test control group; b: Significant decrease ($p<0.05$) as compared with the normal control group
Damage to the structural integrity of the liver is reflected by an increase in the level of serum transaminases (Schmidt et al., 1975) because these are cytoplasmic in location and are released into the circulation after cellular damage (Sallie et al., 1991). Anti-tubercular drugs hepatotoxicity is characterised by elevations of serum uBIL concentrations and AST, ALP and ALT activities which has been observed in the course of this study. Oral administration of garlic solution both in the pretreated and co-administered groups significantly decreased levels of the above parameters and thus conferring a protective effect on the liver.

The constituents of garlic are reported to inhibit Phase I drug metabolism through specific inhibition of cytochrome P-450 enzymes, while they enhance the activities of Phase II enzymes that are involved in the conjugation and excretion of drugs and their Phase I metabolites (Guyonnet et al., 2002). This may play an important role in the detoxification of toxic metabolites postulated to be involved in ATDH. Furthermore, many steroidal saponins and sapogenins are present in garlic (Matsuura, 2001) and could play vital roles as anti-inflammatory agents, in the induction of protein synthesis and in tissue regeneration and repair. The interplay of a wide range of phytochemicals (allicin, ajoene, diithin), vitamins (A, B1, C), antioxidants (glutathione) phytochemicals (quercetin, cyanidin), amino acids (Met, His, Cys, Phe, Ile) and minerals (Ca, Se, Mn, Cu, P, Mg, Zn, K, Na, Fe) (Agarwal, 1996) whose synergy can contribute immensely to the restoration of enzyme activities and prevention of cellular degeneration may also account for the hepatoprotective effect of garlic.

Based on the findings from this study which are in agreement with Ezeala et al. (2009) who reported on protective effect of garlic on acetaminophen-induced liver injury, Shaarawy et al. (2009), who reported the protective effects of garlic and silymarin on NDEA-induced rats hepatotoxicity and Obioha et al. (2009), who studied the hepatoprotective potentials of onion and garlic extracts on cadmium-induced oxidative damage in rats, it can be concluded that Allium sativum (garlic) can serve as a potential protective agent against liver injury caused by anti-tubercular drugs.

**CONCLUSION**

Results of this study have shown that both pretreatment and co-administration of aqueous solution of Allium sativum at the administered doses have a preventive effect on anti-tubercular drugs hepatotoxicity in rats. This will go along way to ensure uninterrupted and effective TB therapy, as one of the major drawbacks of the standard TB therapy happens to be liver injury attributed to anti-tubercular drugs.

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