PROTECTIVE EFFECT OF RUTIN ON ACETAMINOPHEN-INDUCED ACUTE HEPATIC DAMAGE IN RATS

Awah Francis M1*, Chukwumezie Princess U2, Ezema Ogechukwu C3, Emiliarita Iloakasy4, Ubokudom Queen I5

1, 2, 3, 4, 5 Department of Biochemistry, Madonna University, Elele Campus, Rivers State, Nigeria
*Corresponding author: E-mail: awambuh@yahoo.com; Tel: (+234) 8057431113

Received: 18/03/2012; Revised: 16/04/2012; Accepted: 25/04/2012;

ABSTRACT

Acetaminophen is a widely used analgesic and antipyretic drug; overdose however, can cause acute hepatic and renal damage. In this study, rutin a natural antioxidant belonging to the class of bioflavonoids was investigated for its hepato- and nephro-protective capabilities in acetaminophen-induced damage. Male albino rats were divided into five groups. Group A (control) was given normal saline only, group B was given acetaminophen only (8 g/kg body weight) for seven days, while groups C, D and E were co-administered acetaminophen (8 g/kg body weight) and 100, 200 and 500 mg/kg body weight of rutin respectively for seven days. On the eighth day the rats were killed. Liver and kidney function tests were performed using Randox diagnostic reagent kits. Oxidative stress status was assessed by assaying for catalase, superoxide dismutase, ascorbate and malondialdehyde using standard methods. Oral administration of acetaminophen produced liver damage as rats in group B had significant elevations \((p < 0.05)\) in serum aspartate aminotransferase \((\text{AST})\) and alanine aminotransferase \((\text{ALT})\) compared to group A, C, D and E. Creatinine levels were significantly \((p < 0.05)\) maintained to normal levels in group C, D and E rats as compared with group B. Significantly low activity \((p < 0.05)\) of superoxide dismutase \((\text{SOD})\) were observed in group B, relative to groups A, D and E. Co-treatment with 200 and 500 mg/kg body weight rutin also significantly lowered the level of lipid peroxidation while ascorbate was elevated compared to group B. These results suggest that in-vivo, rutin could counteract the deleterious effects caused by acetaminophen metabolic intermediates and could therefore be used as an antidote in combination with acetaminophen to protect the liver in case of an overdose.

Keywords: Rutin, acetaminophen, hepato-protective effect
INTRODUCTION

Acetaminophen (paracetamol) is a widely used analgesic and antipyretic (Cranswick and Coghlan, 2000; Moller et al., 2005; Bertolini et al 2006). Its mechanism of action is considered to be via the inhibition of cyclooxygenase (COX-2) (Hinz et al., 2008). While generally safe for use at recommended doses, acute overdoses of acetaminophen can cause potentially fatal multiple-organ damages, particularly liver damage and acute kidney failure (Jaeschke et al., 2002; Mahadevan et al., 2006; Ryder and Beckingham, 2001). Acetaminophen toxicity is not from the drug itself but from the alkylation electrophilic metabolites, N-acetyl-p-benzoquinoneimine (NAPQI) (Mitchell et al., 1973; Cohen et al., 1997). Acetaminophen is metabolized primarily via phase II metabolism in the liver, into non-toxic products before excretion in the kidney (Muldrew et al., 2002). A small, yet significant amount is metabolized via the hepatic cytochrome P$_{450}$ enzyme system, which is responsible for the formation of NAPQI. At normal doses, NAPQI is quickly detoxified by conjugation with glutathione. Following overdose, this detoxification pathway becomes saturated, and, as a consequence, NAPQI depletes hepatic glutathione (Mitchell et al., 1973). NAPQI is then free to react with cellular membrane molecules, resulting in acute hepatic damage. Animal studies have shown that hepatic glutathione is depleted to less than 70% of normal levels for hepatotoxicity to occur (Richardson, 2000). The increasing liver damage alters biochemical markers of liver function (hepatic transaminases), leading to abnormal rise in serum levels. In some cases, acute kidney failure may be the primary clinical manifestation of toxicity. In these cases, it has been suggested that the toxic metabolite is produced more in the kidneys than in the liver (Boutis and Shannon, 2001).

There is evidence pointing to the fact that oxidative stress is involved in acetaminophen toxicity. Free radicals such as superoxide anion may be formed via a number of mechanisms including formation from cytochrome P$_{450}$ (Puntarulo and Cederbaum, 1996). Superoxide anion rapidly reacts with nitric oxide forming peroxynitrite which is another very toxic radical (Hinson et al., 2002). In addition, during formation of NAPQI by cytochrome P$_{450}$, the superoxide anion formed, undergoes dismutation leading to the formation of another reactive oxygen species (ROS) hydrogen peroxide (Dai and Cederbaum, 1995). Also, peroxidation of acetaminophen to the semiquinone free radical could lead to increased superoxide anion generation via the redox cycling between the acetaminophen and the semiquinone (de Vries, 1981).

Rutin is an antioxidant that belongs to a class of plant secondary metabolites called bioflavonoid that are also known as rutoside, sophorin and quercetin-3-rutinoside (Yang et al., 2008). It is sometimes referred to as Vitamin P, although not strictly a vitamin. Rutin is gotten from natural sources like buckwheat, tomato, orange, carrot, sweet potato, black tea and apple peels (Kreft et al., 1999; Fabjan et al., 2003, Wang et al., 2003). Ingestion of rutin is said to have abundant health benefits. Rutin enhances the effectiveness of vitamin C, lowers blood cholesterol levels as well as works as a very potent antioxidant (Guo et al., 2007; Caillet et al., 2007; Jiang et al., 2007). Rutin is also helpful in treating glaucoma, high blood pressure, heart disease and allergies (Rosane et al., 2006; Sheu et al., 2004). It is reported to possess anti-inflammatory, anticancer, antibacterial, antiviral and antiprotozoal properties (Webster et al., 1996; Guardia et al., 2001; Calabro et al., 2005; Kwon et al., 2005; Martínez et al., 2005; Luo et al., 2008).

Oxidative stress, hepatotoxicity and nephrotoxicity have been reported to be hallmarks in the toxicity of acetaminophen. Rutin is known to have a potent in vitro antioxidant activity; however, little data is available regarding the in vivo antioxidant potentials. This study was aimed at investigating the in vivo antioxidant potential, hepatoprotective and nephroprotective effects...
of rutin in albino rats administrered with high
doses of acetaminophen.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used were of analytical grade. Acetaminophen, acetic acid, L-ascorbic acid, sulfuric acid, potassium dihydrogen phosphate (KH₂PO₄), potassium hydroxide (KOH), ferric chloride (FeCl₃), ethylenediaminetetraacetic acid (EDTA), sodium carbonate (Na₂CO₃), acetaminophen, methanol, ferrous sulfate (FeSO₄·7H₂O), hydrogen peroxide (H₂O₂), thiobarbituric acid (TBA), Folin–Ciocalteu’s reagent (FCR) and trichloroacetic acid (TCA) were all purchased from Sigma Chemical Co. (St. Louis, MO).

Animals

All animals were cared for in accordance with the principles and guidelines of the ethical committee for conduction of animal studies in Madonna University, Elele, Nigeria. Eight-week old healthy male albino rats of the Wistar strain with an average weight of 200–250 g were used in the study. The experimental animals were housed in aluminum cages in an animal house properly ventilated with good sanitary conditions.

Experimental design

The acetaminophen-induced hepatotoxicity model experiment was employed in this study. A total of 25 rats received water ad libitum and vital feed grower pelletized mash (Grand Cereals and Oil Mills Ltd, Nigeria) and were randomly divided into five groups (n = 5): Group A (control), was given normal saline only; Group B, acetaminophen-only (8 g/kg body weight); Group C, rutin (100 mg/kg body weight) + acetaminophen; Group D, rutin (200 mg/kg body weight) + acetaminophen; and Group E, rutin (500 mg/kg body weight) + acetaminophen. Groups B, C, D and E were intragastrically co-administered 8 g/kg acetaminophen for seven days. All animals were anaesthetized with chloroform and killed on the eighth day. Blood was collected by cardiac puncture in plain tubes. This was then centrifuged at 5000 rpm for 10 min to obtain the serum for biochemical assays. The liver was removed, weighed and individually homogenized in ice cold phosphate buffer solution (0.1 M, pH 7.4) to give a 10 % (w/v) liver homogenate. Tissue homogenates were prepared and the homogenate was centrifuged at 5000 rpm for 20 min. The supernatant was used for biochemical assays.

Assessment of liver function and kidney function

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, and urea levels were assayed in fresh serum using the commercial kits supplied by Randox (UK). These analyses were carried out according to the manufacturer’s protocols.

Assessment of Liver homogenate antioxidants and oxidative stress markers

The liver homogenate total protein concentration was measured by the method of Lowry et al. (1951). Catalase (E.C.1.11.1.6.) activity was determined according to Aebi (1984) with phosphate buffer pH 7.0, at 240 nm. Total superoxide dismutase (mitochondrial Mn-containing and cytosolic Cu- and Zn-containing forms E.C. 1.15.1.1) activity was determined by the method of Beauchamp and Fridovich (1971) at room temperature. Measurement of the extent of lipid peroxidation in the liver homogenates was determined based on the formation of thiobarbituric acid reactive substance as described by Buege and Aust (1978). Ascorbate levels in homogenates were determined following the method of Tietz (1986). The spectrophotometric readings were performed in a Jenway UV/visible spectrophotometer (Camlab, UK).

Statistical analysis

The data were analysed using the Statistical Package for Social Sciences (SPSS) version
10.0 for Windows. Analysis of variance (ANOVA) was used to compare means, and values were considered significant at \( p < 0.05 \). All the results are expressed as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

Acetaminophen overdose is the most frequent cause of drug-induced liver injury in many parts of the world. In the present study, we investigated the protective potential of rutin when co-administered with high doses of acetaminophen to induced hepatotoxicity. Considering the fact that oxidative stress is a major hallmark in hepatotoxicity, an antioxidant like rutin with potent \textit{in vitro} radical scavenging capabilities could be effectively used to prevent, manage or treat liver damage.

Effect of rutin on liver function in acetaminophen-intoxicated rats

Acetaminophen-induced hepatic damage is a commonly used model for hepatoprotective drug screening and the extent of hepatic damage is assessed by the serum level of AST, ALT and ALP (Sallie et al., 1991). In this study the hepatotoxic effect of acetaminophen overdose was confirmed in accordance with previous reports (Jaeschke et al., 2003; Mahadevan et al., 2006). The acetaminophen metabolite NAPQI caused damage in the hepatocytes leading to a leakage of ALT and AST into the serum. Co-administration of rutin at the doses of 100, 200 and 500 mg/kg with toxic doses of acetaminophen significantly \( (p < 0.05) \) protected the liver from damage as shown by the serum transaminases (AST and ALT) compared to the control \( (96.2 \pm 7.8 \text{ U/L and } 46.5 \pm 9.8 \text{ U/L respectively}) \) and the acetaminophen-only groups \( (534.8 \pm 44.2 \text{ U/L and } 236.9 \pm 20.6 \text{ U/L respectively}) \) (Table 1). AST is found in cardiac, hepatic, muscle and kidney tissues while ALT is produced principally in the liver where it catalyses transamination reactions. ALT is therefore more specific for hepatocellular damage than AST and remains elevated in the serum for longer periods, due to its longer half-life. AST is found in the cell cytoplasm and mitochondria while ALT is found solely in the cytoplasm, hence in an inflammatory condition, there is simply leakage of cytoplasmic enzymes into circulation and ALT will rise more than AST (Bramstedt, 2006). In this study, the level of AST rose above the ALT, suggesting gross cellular necrosis in acetaminophen poisoning, resulting in both cytosolic and mitochondrial AST. However, ALP levels were not significantly different \( (p > 0.05) \) between the control, acetaminophen-only and acetaminophen + rutin co-treated rats. As observed in this study, rutin significantly attenuated the hepatotoxic effects of acetaminophen and assisted in maintaining the normal integrity of the hepatocytes. Since oxidative damage and inflammation play central roles during drug-induced damage, the observed protective effect of rutin could possible be due to its inherent anti-inflammatory activity (Guardia et al., 2001) and free radical scavenging and anti-lipid peroxidation capabilities (Gao and Zhou, 2005). Alternatively, inhibition of cytochrome \textit{P}_{450} \textit{isoenzymes (CYPs) could also have reduced the toxicity of acetaminophen since formation to the toxic metabolite NAPQI will be minimized (Bear and Teel, 2000). These observations suggest that rutin may find clinical application in a variety of conditions where oxidative stress causes cellular damage.
Table 1: Serum hepatic enzymes levels of control and acetaminophen-intoxicated rats

<table>
<thead>
<tr>
<th></th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>96.2 ± 7.8*</td>
<td>46.5 ± 9.8*</td>
<td>296.2 ± 26.5</td>
</tr>
<tr>
<td>Acetaminophen Only</td>
<td>534.8 ± 44.2</td>
<td>236.9 ± 20.6</td>
<td>314.8 ± 24.2</td>
</tr>
<tr>
<td>Acetaminophen + 100 mg/kg Rutin</td>
<td>280.2 ± 25.4*</td>
<td>166.8 ± 14.4*</td>
<td>290.2 ± 35.4</td>
</tr>
<tr>
<td>Acetaminophen + 200 mg/kg Rutin</td>
<td>218.6 ± 23.3*</td>
<td>146.6 ± 21.3*</td>
<td>308.6 ± 23.3</td>
</tr>
<tr>
<td>Acetaminophen + 500 mg/kg Rutin</td>
<td>182.4 ± 14.4*</td>
<td>151.3 ± 22.7*</td>
<td>282.4 ± 14.4</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM; * p < 0.05 vis-à-vis the Acetaminophen-only group

AST-Aspartate Aminotransferase; ALT-Alanine Aminotransferase; ALP-Alkaline Phosphatase

Table 2: Serum urea and creatinine levels of control and acetaminophen-intoxicated rats

<table>
<thead>
<tr>
<th></th>
<th>Urea (mmol/L)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.32 ± 1.65</td>
<td>0.43 ± 0.06*</td>
</tr>
<tr>
<td>Acetaminophen Only</td>
<td>7.82 ± 1.37</td>
<td>1.62 ± 0.07</td>
</tr>
<tr>
<td>Acetaminophen + 100 mg/kg Rutin</td>
<td>6.69 ± 1.59</td>
<td>0.97 ± 0.02*</td>
</tr>
<tr>
<td>Acetaminophen + 200 mg/kg Rutin</td>
<td>6.63 ± 0.58</td>
<td>0.84 ± 0.04*</td>
</tr>
<tr>
<td>Acetaminophen + 500 mg/kg Rutin</td>
<td>6.34 ± 0.50</td>
<td>0.65 ± 0.03*</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM; * p < 0.05 compared to the Acetaminophen-only group

Effect of rutin on kidney function in acetaminophen-intoxicated rats

In addition to liver damage, the acetaminophen metabolite N-acetyl-p-benzoquinoneimine (NAPQI) also induces kidney damage. Increase in serum concentrations of urea and creatinine is prominent in acute nephrotoxicity (Erdem et al., 2000). The results of the present study showed that the acetaminophen-only rats had higher urea level (7.82 ± 1.37 mmol/L) compared to the control (6.32 ± 1.65 mmol/L) and those co-treated with rutin, though the mean difference was not statistically significant (p < 0.05) (Table 2). Serum creatinine levels were significantly higher (p < 0.05) in acetaminophen-only rats (1.62 ± 0.07 mg/dL) compared to rats treated with rutin at 100 mg/kg (0.97 ± 0.02 mg/dL), 200 mg/kg (0.84 ± 0.04 mg/dL) and 500 mg/kg (0.65 ± 0.03 mg/dL) and the control (0.43 ±
0.06 mg/dL) (Table 2). The level of creatinine in the acetaminophen-only rats was very high which could be as a result of the inflammation of the kidney caused by the free radicals generated by the acetaminophen overdose that led to the decreased filtration rate of the nephron. This suggests that rutin possesses dose-dependent protective effects against acetaminophen-induced kidney damage. As a potent antioxidant rutin possibly scavenged the free radicals generated during acetaminophen-intoxication thereby preventing renal damage by oxidants and stabilizing the renal function. The observed nephroprotective potential of rutin is in accordance with previous reports by Alsaif (2009).

Effect of rutin on hepatic antioxidant enzymes activity in acetaminophen-intoxicated rats

According to the present data, the extent of reactive oxygen species production by the administered of acetaminophen is significantly quenched by rutin in a dose-dependent manner; thereby reducing the extent of liver damages among the rats co-treated with rutin and acetaminophen, relative to normal and acetaminophen-only rats. Superoxide dismutase (SOD) and catalase (CAT) are endogenous antioxidant enzymes responsible for the detoxification of deleterious oxygen radicals. The protective effect(s) of rutin was evident through significantly higher levels ($p < 0.05$) of total SOD activities among the rutin co-treated rats (10.63 ± 1.58 and 10.34 ± 1.50 U/mg protein for 200 and 500 mg/kg rutin respectively) relative to acetaminophen-only rats (5.82 ± 0.37 U/mg protein) (Table 3). Acetaminophen decreased the liver total SOD activity by about 50% relative to the normal healthy control rats indicating that the high dose of acetaminophen administered to the rats, constituted a stressor agent that lead to depletion of the liver tissue antioxidant enzymes. Table 3 clearly indicates that rutin co-treatment has increased the SOD, but not CAT, activity among the acetaminophen-treated rats relative to control rats. This suggests that the antioxidant enzyme CAT is not very much affected probably because the major free radicals involved in acetaminophen toxicity are superoxide anion and peroxynitrite. The decreased activity in total SOD could be due to exhaustion of the enzyme because of increased generation of free radicals such as superoxide anion during NAPQI metabolism and peroxidation. Rutin co-treatment significant increased ($p < 0.05$) the hepatic SOD activity (Table 3) by possibly scavenging the free radical generated thereby preventing radical-induced hepatic damage. The increase in total SOD activity in rutin co-treated rats is a definite indication of hepatoprotective action of the drug (Curtis and Mortiz, 1972). Previous studies have revealed another possible mechanism of action of rutin is by upregulating the expression of genes for antioxidant enzymes (Lores-Arnaiz et al., 1995). In this context, treatment with rutin probably increased the activity of enzymatic antioxidants and also levels on non-enzymatic antioxidants in the liver of acetaminophen-intoxicated rats.

Effect of rutin on hepatic malondialdehyde (MDA) and ascorbic acid levels of acetaminophen-intoxicated rats

Lipid peroxidation causes changes in the properties of biological membranes, thus altering their fluidity and permeability, leading to impairment in membrane signal transduction and ion exchange, resulting in lipid peroxidation, oxidation of proteins and DNA and eventually, cytotoxicity (Fang et al., 2002; Stehbens, 2003; Jaeschke et al., 2003; Teimouri et al., 2006). Generation of free radicals such as superoxide anion and peroxynitrite during acetaminophen metabolism results in the depletion of antioxidants such as glutathione, ascorbate and superoxide dismutase leading to oxidative stress and lipid peroxidation. In our study, an increase in hepatic MDA levels in the acetaminophen-only rats (Table 4) suggests enhanced lipid peroxidation leading to hepatic damage and failure of antioxidant defense mechanisms resulting in oxidative stress. The observed increase in levels of hepatic MDA correlates with the decrease in hepatic total SOD activity (Table 3). The rats co-treated
with acetaminophen and rutin (200 and 500 mg/kg) showed significantly (p < 0.05) lower levels of MDA (0.331 ± 0.05 and 0.323 ± 0.02 µmol/mg protein respectively) relative to the acetaminophen-only rats (0.471 ± 0.02 µmol/mg protein). Reduction of MDA levels in the groups co-treated with both of acetaminophen and rutin was possibly due to the ability of rutin to quench free radicals by transfer electrons to the free radicals (Ferrali et al., 1997) and possibly by activation of antioxidants enzymes (Elliott et al., 1992). Hepatic ascorbate levels were significantly reduced in the acetaminophen-only rats relative to the healthy control. Co-administration of rutin (200 and 500 mg/kg) significantly (p < 0.05) increased the ascorbate levels (6.6 ± 0.3 and 6.4 ± 0.7 mg/dL respectively) vis-à-vis the acetaminophen-only rats (4.5 ± 0.2 mg/dL) (Table 4). Low levels of ascorbate are associated with increase levels of free radicals and oxidative stress since much ascorbate would be utilized to quench radical. The present results show that rutin could help protect against the assault of free radical thereby stabilizing the oxidative status of the rats. The pharmacokinetics of rutin in humans is still under investigation. Studies have shown that about 17% of an ingested dose of rutin is absorbed mainly from the colon following the removal of the carbohydrate moiety by bacterial enzymes to form quercetin (Walle, 2004). Quercetin and glucuronide conjugates of quercetin are then transported to the liver via the portal circulation, where they undergo significant metabolism forming metabolites like isorhamnetin, kaempferol and tamarixetin (Walle, 2004). It could therefore be inferred that quercetin and its metabolites are most likely responsible for the in vivo antioxidant and hepatoprotective capabilities of rutin.

Table 3: Hepatic total superoxide dismutase (SOD) and catalase (CAT) activites of control and acetaminophen-intoxicated rats

<table>
<thead>
<tr>
<th></th>
<th>Total SOD Activity (U/mg protein)</th>
<th>CAT Activity (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.32 ± 1.50</td>
<td>90.43 ± 3.06</td>
</tr>
<tr>
<td>Acetaminophen Only</td>
<td>5.82 ± 0.37</td>
<td>81.62 ± 4.07</td>
</tr>
<tr>
<td>Acetaminophen + 100 mg/kg Rutin</td>
<td>8.69 ± 1.05</td>
<td>97.54 ± 5.02</td>
</tr>
<tr>
<td>Acetaminophen + 200 mg/kg Rutin</td>
<td>10.63 ± 1.58*</td>
<td>84.32 ± 4.04</td>
</tr>
<tr>
<td>Acetaminophen + 500 mg/kg Rutin</td>
<td>10.34 ± 1.50*</td>
<td>85.23 ± 2.03</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM; * p < 0.05 relative to the Acetaminophen-only group
Table 4: Hepatic malondialdehyde (MDA), protein carbonyls and ascorbate levels in control and acetaminophen-intoxicated rats

<table>
<thead>
<tr>
<th></th>
<th>MDA (µmol/mg protein)</th>
<th>Ascorbic acid (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.367 ± 0.08</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>Acetaminophen Only</td>
<td>0.471 ± 0.02</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>Acetaminophen + 100 mg/kg Rutin</td>
<td>0.409 ± 0.03</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>Acetaminophen + 200 mg/kg Rutin</td>
<td>0.331 ± 0.05*</td>
<td>6.6 ± 0.3*</td>
</tr>
<tr>
<td>Acetaminophen + 500 mg/kg Rutin</td>
<td>0.323 ± 0.02*</td>
<td>6.4 ± 0.7*</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SEM; *p < 0.05 compared to the Acetaminophen-only group (n = 5)

CONCLUSION

Many, if not most, of rutin's possible activities can be accounted for, in part, by rutin's antioxidant activity. The present study shows that rutin has the abilities to preserve the activity of antioxidant enzymes and hepatocyte membrane, which may be referred to its role in modulating the levels of superoxide anion associated with acetaminophen toxicity. Rutin could therefore be used as an antidote in combination with acetaminophen to protect the liver in case of an overdose. Further investigations are however, warranted to ascertain the feasibility of such combination.

ACKNOWLEDGEMENT

We are very grateful to Prof. Peter N. Uzoegwu of the Department of Biochemistry, University of Nigeria, Nsukka for encouragement, guidance, and financial support extended to us during the course of the study.

REFERENCES


Source of Support: Nil

Conflict of Interest: None Declared

Global Journal of Research on Medicinal Plants & Indigenous Medicine