Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.24, 2015

Effect of Aqueous Leaf Extract of *Borreria verticillata* Species of Sudano-Sahelian Savanna on CCl_4 Induced Hepatotoxicity

*[1]Murtala, Y., [1]Babandi, A.,[1] Sunusi, M. M., [2]Shehu, D. [1]Babagana, K. and [1]Alhassan, A. J.
[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Bayero University, Kano, Nigeria
[2]Institute of Biological Sciences, University of Malaya

Abstract
Preliminary phytochemical screening of *Borreria verticillata* species of Sudano-Sahelian savanna and the effect of aqueous leaf extract of the plant were studied in CCL_4-induced hepatotoxicity rats. Screening of the aqueous extract indicates the presence of alkaloids, flavonoids, tannins, glycosides, sterol and saponins. While athraquinone was absent. Serum levels of aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total protein (TP) and bilirubin (BL) were analysed in rats intraperitoneally administered with 100 mg/kg CCl_4 followed by oral treatment with 300mg/kg of aqueous leaf extract of *B. verticillata* for 48 and 96hrs. The rats treated for 48 hours after had serum AST, ALT, ALP, TP and BL levels not statistically different ($P>0.05$) compared to both normal control and positive control (treated with 100mg/kg standard drug) although the value of positive control was slightly lower than the test values. However, the test values were statistically lower compared to toxicity control at $P< 0.05$. The serum AST, ALT, ALP, TP and BL levels when the treatment was extended to 96 hours showed similar pattern to 48 hours treatment. Even though the test values with respect to the enzymes activity were slightly lower in the extended treatment. This result indicates the hepatocurative properties of aqueous leaf extract of *B. verticillata* on CCL_4-induced hepatotoxicity rats, which could be attributed to its phytochemical contents.
Keywords: *Borreria verticillata,* Hepatotoxicity, Hepatocurative, Aqueous leaf extract, CCL_4

1. Introduction

Nowadays people are being bombarded with thousand of unhealthy products, often with little or no pharmacological activities against the target diseases but promote the susceptibility to the toxic effects of these unhealthy products. Indeed, in recent years, there is increase in drug resistance by disease causing organisms (WHO, 2008). This dilemma made the use of medicinal plants a best solution and that traditional remedies are being sought by a cross section of scientists for various ailments (Chandresena, 1995; Heinrich *et al*., 2005). Moreover, therapeutic properties of medicinal plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural (Elvin-Lewis, 2001). There is resurgence in the use of herbal medicines worldwide. An estimated one third of adults in the western world use alternative therapies, including medicinal plants (Ernst, 2002). Nowadays, traditional medicine has brought to focus a wider coverage of primary healthcare delivery, not only in the African region but also, to various countries of the world (Acharya and Anshu, 2008). It is the first choice of healthcare treatment for at least 80% of Africans suffering from high fever and other common ailments (Oliver, 1959).

Liver is a multifunctional organ, largely responsible for nutrient and xenobiotics metabolism. It is the heart beat of biotransformation, altering a wide range of biochemical substances, depending on the physiological needs. It is also critical in the storage of a number of biochemical compounds such as excess iron, some vitamins and excess glucose as glycogen (Nduka, 1999). Carbon tetrachloride (CCl_4) has been reported to induce lipid peroxidation and liver damage (Nduka, 1999) as in viral hepatitis, cirrhosis and obstructive jaundice, leading to an increase in the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the blood. These enzymes are mostly contained in the liver, and when parenchymal cells of the liver are damage, the enzymes leak into the blood (Zimmerman, 1976). Silymarin was a flavonoid obtained from Silybum marianum or milk thistle and was composed of three isomers: silybinin, silydianin and silychristin (Wagner, 1986), silybinin being quantitatively the most important (Bosisio *et al.,* 1992). Wills and Asha (2006) showed standard drug Silymarin has a remarkable protection of serum AST, ALT and LDH levels towards CCl_4 induced hepatotoxicity.

Borreria verticillata is a perennial shrub belonging to the family *Rubiaceae*. It is commonly called shrubby false button weed or shrubby false button wood (Burkill, 2000). It is distributed in tropical and subtropical America, Africa, Asia, and Europe (Dessein *et al.,* 2006). It is originated from South and Central America (Burger and Taylor, 1993; Chiquieri *et al.,* 2004). *Borreria verticillata* species is used medicinally in various manners and are reputed in traditional medicine of Latin America, Asia, Africa, and West Indies. In Brazil, the infusion of the flowers is used as antipyretic and analgesic (Vieira *et al.,* 1999; Moreira *et al.,* 2010). The roots extract is emetic, the leaves extracts as antidiarrheal, and the remedy against erysipelas and hemorrhoids (Lorenzi and Matos, 2002). It is also commonly used effectively to cure eczema (*Tinea versicolor)*, ring worm (*Tinea capitis),* scabies and other skin lesions (e.g. infectious dermatitis), toothache, headache, and dyspepsia (Chopra *et al.,* 1956). The

juice obtained from the aerial part is applied topically for the treatment of skin diseases. A lotion is prepared to relieve skin itches (Liogier, 1990). In West India, the decoction of this plant is used for diabetes and dysmenorrhea, and when prepared with *Cuscuta* and *Zebrina schnizlein* is used for amenorrhea (Ayensu, 1978). While in Senegal it is used to treat bacterial skin infections and leprosy (Maynart *et al.,* 1980).

In Nigeria, it is common to all vegetations particularly in Sudan savanna, normally found in wastelands, in non cultivated fields or within the vicinity of ponds, mainly in the rainy season. It is known as *Fasa- kaba* by the Hausa people of northern Nigeria, *Irawo-ile* by the Yoruba people and *Abia-ikana* by the Ibibio people of southern Nigeria. The leaves are used in some parts of Nigeria, as in many West African countries for curative purposes as one of the mainstream traditional medicines. Studies have confirmed that extracts from *Borreria* and *Spermacoce* species as well as their isolated compounds possessed diverse biological activities, including analgesic, anti-inflammatory, antitumor, antimicrobial, larvicidal, antioxidant, gastrointestinal, anti-ulcer, and hepatoprotective, with alkaloids and iridoids as the major active principles (Shajiselvin *et al.,* 2010; Conserva and Ferreira, 2012; Abdullahi *et al.,* 2014). The roots are used to treat malaria, the leaves as ophthalmic, inflammation of eye and gums, blindness, fever, spleen complaints, sore, hemorrhage, dysentery and diarrhea, and also for the treatment of liver ailment, kidney disorder and abortifacient (Sofowora, 2008).

It was established that liver problem is on the increase in Sudano-Sahelian region of the northern Nigeria, particularly in rural areas. However, majority of the inhabitants of this region gave preference to traditional than conventional medicine. Indeed, there is growing interest in the antioxidant properties of the phenolic compounds in vegetables, fruits, and shrubs due to their strong activity and low toxicity compared with those of synthetic phenolic antioxidants, such as BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) (Marinova and Yanishlieva, 1997). Thus, finding accessible and cost effective alternative is necessary. Therefore this research intends to unveil the potentiality of *Borreria verticillata* in curing liver diseases.

2. Materials and Methods

2.1 Sample Collection and Identification

The sample of *Borreria verticillata* was collected by hand picking at dump site in Kanya Babba village, Babura Local Government, Jigawa State, Nigeria. It was authenticated at the Department of Plant Science, Bayero University, Kano, Nigeria.

2.2 Sample Preparation and Extraction

The fresh leaves of *B. verticillata* were shade-dried and ground into a fine powder. The fine powder (50g) was added into distilled water and shaken gently for ten minutes using a shaker to make a homogenous mixture. The mixture was left to stand for 24 hours and then filtered. The filtrate concentration was 20mg/cm^3 and used for the study

2.3 Experimental Animals

Thirty five (35) albino rats (weighing 120-130g) were obtained from the Animal House of the Veterinary and Research Institute (VOMVET), Plateau State, Nigeria. They were housed in animal house, Department of Biological Sciences, Bayero University, Kano and allowed to acclimatized and fed with their normal starter feed and water *ad libitum.*

2.4 Experimental Design

The animals were divided into four (4) groups: Group I (Normal control) contains five (5) albino rats which serve as normal control; liver damage was not induced and extract was also not administered. Group II contains ten (10) rats serving as toxicity control; the liver damage was induced but the extract was not administered. Group III contains also ten (10) animals, in which liver damage has been induced, followed by oral administration of the extract. Group III animals were further subdivided into two groups each containing five (5) animals of which one sub – group was given 300mg/kg of extract for 48 hrs and the other sub – group for 96 hours. Group IV (Positive control) also contains ten (10) animals, in which liver damage has been induced, followed by oral administration of 100mg/kg of Salymarin (standard drug). Group IV animals were further subdivided into two groups each containing five (5) animals of which one sub – group was given 100mg/kg of Salymarin for 48 hrs and the other sub – group for 96 hours. The extract and Salymarin were administered orally while 100mg/kg CCl_4 was administered intraperitoneally. High doses of CCl_4 (90-120mg/Kg) can induce massive liver damage and may persist for longer period, giving rise to ideal hepatotoxicity rats model (Alhassan, 2009). The animals were sacrificed after 48 and 96 hours respectively. Blood samples were collected and serum was collected for analysis.

2.5 Phytochemical analysis

The preliminary phytochemical screening of the *B. verticillata* leaves was carried out using standard procedures.

2.6 Enzyme assay

Serum AST and ALT were assayed using Reitman and Frankel (1957) method. The serum ALP was assayed using the method of Rec (1972). Serum bilirubin and total protein were assayed using Jendrassik and Grof, (1938) method and Biuret method (Chawla, 1990) respectively.

2.7 Statistical Analysis: Graph ad Instat 3 statistical software (2000) version 3.05 was used to analyze the data obtained.

3. Results and Discussion

Table 1: Preliminary phytochemical Screening of the Aqueous Extract of *Borreria verticillata*

Phytochemical Constituents	Result
Saponins	+
Anthraquinones	–
Alkaloids	+
Flavonoids	+
Glycoside	+
Tannins	+
Sterol	+

Key: + = Present – = Absent

The preliminary phytochemical screening of the aqueous leaf extract of *Borreria verticillata* species of Sudano-Sahelian savanna has indicated the presence of flavonoids, alkaloids, saponins, sterols, tannins and glycosides while anthraquinone was absent. This finding was in conformity with a previous finding reported by Abdullahi *et al* (2014). These compounds have a well known pharmacological activities including antioxidant, hepatocurative, analgesic and anti-inflammatory effects (Perez, 2001 and Park *et al.,* 2001).

The presence of alkaloids in Sudano-Sahelian *B. verticillata* species was in line with documented findings reported by Lucia and Jesu (2012) that a representative classes of alkaloids were present in *B. verticillata* from America, Europe and Africa but absent in Asian species, while flavonoids were found only in species from Asia. Thus, the common possession of alkaloids and flavonoids by the Sudano-Sahelian savanna species should be viewed as retention of an ancient characteristic and would be extremely helpful to clarify trends in the chemical evolution of the species. Indeed, variation in the phytochemical constituents within a single species is affected by location, environmental conditions and genotypes of the plant.

Albino rats with CCl_4 induced liver damage but no treatment (toxicity control) had serum AST, ALT, ALP, TP and BL levels significantly higher compared to those of both the normal and positive controls ($P<0.05$) after 48 and 96 hours of treatment. This increase in the levels of serum liver enzymes, total protein and bilirubin in toxicity control (group II) is in line with findings reported by Nduka (1999) and Alhassan *et al.,* (2009) that high doses of CCl_4 (90-120mg/kg) is toxic and can inflict acute necrosis upon liver causing clinical conditions like infective hepatitis. Carbon tetrachloride (CCl_4), a well-known model compound for producing chemical hepatic injury, requires biotransformation by hepatic microsomal cytochrome $P\text{-}_{450}$ to produce toxic metabolites, namely trichloromethyl free radicals ($CCl_3^{\bullet}$) and subsequent derivative $Cl_3COO^{\bullet}$ (Brautbar and Williams, 2002). The values of serum bilirubin in toxicity control indicate the presence of hyperbilirubunaemia, which may be an indication of liver dysfunction with consequent elevated levels of total serum bilirubin. The increase in serum total proteins is an indication of acute liver toxicity. Since albumin which is the most important protein synthesized by the liver, and reflects the extent of functioning liver cell mass, has a fairly long half life of 20 days, it is not a good indicator of acute liver diseases (Vasudevan and Sreekumari, 2005), the findings of this study have confirmed this observation.

TABLE 2: Serum Levels of AST, ALT, ALP, Total protein (TP) and Bilirubin (BL) in CCl_4 Hepatotoxicity Rats Orally Administered with 300mg/kg of Aqueous Leaf Extract of *B. verticillata* for 48 hours.

	DOSE(mg/kg)	AST(U/L)	ALT(U/L)	ALP(U/L)	TP(g/L)	BL(Umol/L)
GROUP I (Normal control)	_	$21.30\pm2.02^{a,}$	35.26 ± 1.59^{e}	31 ± 4.72^{i}	43.50 ± 3.70^{m}	12.76 ± 1.30^{q}
GROUP II (Toxicity control)	100mg/kg (CCl_4)	$102.15\pm2.09^{a,c,d}$	$70.29\pm0.25^{e,f,g,h}$	$60.5\pm3.47^{i,j,k,l}$	$70.5\pm1.41^{m,n,o,p}$	$21.74\pm0.28^{q,r,s,t}$
GROUP III 100mg/kg (CCl_4) +	300mg/kg (extract)	25.45 ± 3.06^{c}	38.23 ± 2.66^{g}	39.00 ± 5.39^{k}	$52.2\pm1.41^{o,m}$	15.39 ± 3.16^{r}
GROUP IV (Positive control) 100mg/kg (CCl_4) +	100mg/kg (silymarin)	24.05 ± 2.07^{d}	37.50 ± 2.05^{h}	35.5 ± 2.58^{l}	48 ± 0.00^{p}	14.03 ± 0.09^{s}

Values are expressed as MEAN±S.D. Values within the same column, bearing the same superscript are statistically different at $p<0.05$.

TABLE 3: Serum Levels of AST, ALT, ALP, Total protein (TP) and Bilirubin (BL) in CCl_4 Hepatotoxicity Rats Orally Administered with 300mg/kg of Aqueous Leaf Extract of *B. verticillata* for 96 hours.

	DOSE(mg/kg)	AST(U/L)	ALT(U/L)	ALP(U/L)	TP(g/L)	BL(Umol/L)
GROUP I (Normal control)	_	$21.30\pm2.02^{a,}$	35.26 ± 1.59^{e}	31 ± 4.72^{i}	43.50 ± 3.70^{m}	12.76 ± 1.30^{q}
GROUP II (Toxicity control)	100mg/kg (CCl_4)	$79.51\pm2.33^{a,b,c}$	$52.27\pm0.24^{d,e,f}$	$43.20\pm1.38^{g,h,i}$	$60.51\pm2.10^{j,k,l}$	$18.71\pm0.26^{m,n,o}$
GROUP III 100mg/kg (CCl_4) +	300mg/kg (extract)	24.05 ± 3.05^{b}	36.17 ± 1.09^{e}	36.5 ± 2.12^{h}	48.00 ± 0.00^{k}	$15.00\pm0.14^{m,n}$
GROUP IV (Positive control) 100mg/kg (CCl_4) +	100mg/kg (silymarin)	22.65 ± 2.77^{c}	36.04 ± 0.14^{f}	33.5 ± 1.21^{i}	45.00 ± 0.70^{l}	13.91 ± 0.85^{o}

Values are expressed as MEAN±S.D. Values within the same column, bearing the same superscript are statistically different at $p<0.05$.

The serum levels of enzyme markers in toxicity control have decreased substantially after 96 hours (Table 3) compared to 48 hours even without treatment. This is probably due to the ability of hepatocytes to regenerate rapidly following chemically induced cytotoxicity.

However, the rats treated with 300mg/kg aqueous leaf extract of *B. verticillata* (group III) and 100mg/kg of Silymarin (positive control) (group IV) for 48 and 96 hours had serum AST, ALT, ALP, TP and BL not statistically different from normal control but significantly lower than the toxicity control at $P>0.05$. This would probably be due to hepatocurative effect of *B. verticillata* aqueous extract on chemically induced hepatocellular damage. This dramatic lowering effect could however be attributed to extract-enhanced rapid regeneration of hepatocytes due to the presence of phytochemicals, particularly flavonoids which have antioxidant effect that could terminate the lipid peroxidation damage induced by CCl_4 and promote the healing process. As previously demonstrated by s number of investigators that antioxidants prevent CCl_4 toxicity, particularly hepatotoxicity, by inhibiting lipid peroxidation (Teselkin *et al.,* 2000), suppressing alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (Lin and Huang, 2000), and increasing antioxidant enzyme activity (Kumaravelu *et al.,* 1995). Furthermore, some alkaloids detected in the *B. verticillata* species were reported to display some *in vivo* or *in vitro* biological activities (Lucia and Jesu, 2012). Thus, the hepatocurative effect of this plant could be associated with these biological activities.

4. Conclusion

Based on the findings of this study, high dose (300mg/kg) of aqueous leaf extract of *B. verticillata* from Sudano-Sahelian savanna enhanced the regeneration of hepatocytes inflicted with CCl_4 induced hepatotoxicity within 96 hours of treatment. And the healing effect of (300mg/kg) leaf extract is lower than that of 100mg/kg standard drug (Silymarin).

Acknowledgement: We humbly register our acknowledgement to Kamaluddeen Babagana of the Department of Biochemistry, Bayero University, Kano, Nigeria for his technical and statistical efforts. Our gratitude also goes to Mrs. Amina Abubakar for typing and organizing this manuscript. All sources used therein were dully acknowledged.

References

Abdullahi, H.S., Ahmed, A., Umar, Z. A., and Hussaini, I. M. (2014). Preliminary evaluation of ethanol leaf extract of Borreria verticillata Linn (Rubiaceae) for analgesic and anti-inflammatory effects. *Journal of Medicinal Plant Research.* 8 (**20**): 736-747.

Acharya, D. and Anshu, S. (2008). *Indigenous Herbal Medicines: Tribal Formulations and Traditional Herbal Practices* Aavishkar Publishers, Jaipur. Pp. 45 – 60

Alhassan, A.J., Sule, M.S., Aliyu, S.A., and Aliyu, M.D. (2009). Ideal Hepatotoxicity Model in Rats using CCl4. *Bayero J. Pure Appl. Sci.* 2 (2): 185 – 187.

Ayensu ES. Medicinal plants of the West Indies. Unpublished Manuscript. 1978:110.
Bosisio, E., Benelli, C. and Pirola, O. (1992). Effect of the flavanolignans of Silybum marianum L. on lipid peroxidation in rat liver microsomes and freshly isolated hepatocytes. *Pharmacol. Res.* 25 : 147.
Brautbar, N. and Williams, J. (2002). 2nd industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. *Inter. J. Hygiene Enviro. Health* 205 : 479.
Chandresena, J.P.C. (1995). *The Chemistry and Pharmacology of Ceylon and Indian Medicinal Plants.* (Ceylon): H and C Press, Sri lanka. P.163
Chiquieri A, Di Maio FR, Peixoto AL. A distribuição geográfica da família Rubiaceae Juss. na Flora Brasiliensis de Martius. Rodriguésia. 2004;55:47–57.
Chopra, R.N., Nayar, S.L. and Chopra, I.C. (1956). *Glossary of Indian Med Plants*; CSIR: *New-Delhi*. Pp. 39.
Clawla, R. (1990). Practical Clinical Biochemistry (Methods and Interpretations). Second edition. Jaypee Brothers Medical Publishers, New Delhi, India. Pp. 106-118.
Conserva, L.M. and Ferreira, J.C. (2012). *Borreria and Spermacoce species* (Rubiaceae): A review of their ethnomedicinal properties, chemical constituents, and biological activities. *Pharmacogn Rev*. 6, 11 ; 46-55
Dessein, S. Robbrecht, E. and Smets, E. (2006). A new heterophyllous Spermacoce species (Rubiaceae) from the Marungu highlands (D. R. Congo) *Novon*. 16:231–4.
Elvin-Lewis, M. (2001). Should we be concerned about herbal remedies?*Journal of Ethnopharmacology* 75: 141-164.
Ernst, E. (2002). Toxic effects of undeclared drugs in Asian herbal medicines. *Trends in Pharmacological Sciences 23* (3): 136-139.
Heinrich, M., Pieroni, A. and Bremner, P. (2005). *Plants as medicines*. *in* : Prance, G. and Nesbitt, M. *The Cultural history of plants*. W.B Saunders, Philadelphia. Pp. 205–238.
Kumaravelu, P., Dakshinamoorthy, D. P., Subramaniam, S., Devaraj, H. and Devaraj, N. S. (1995). Effect of eugenol on drug-metabolizing enzymes of carbon tetrachloride-intoxicated rat liver. *Biochem. Pharmacol*. 49 (11) : 1703.
Lin, C. C. and Huang, P. C. (2000). Antioxidant and hepatoprotective effects of Acathopanax senticosus. *Phytother. Res*. 14 (17): 489.
Liogier, H.A. (1990).Description Flora of Puerto Rico and Adjacent Island, *editorial Dela Universidad de Puerto, Rio piedras*. **5**:436.
Lorenzi H, Matos FJ. Plantas medicinais do Brasil. São Paulo: Nova Odessa; 2002.
Lucia, M. C. and Jesu, C. F. J. (2012). *Borreria* and *Spermacoce* species (Rubiaceae): A review of their ethnomedicinal properties, chemical constituents, and biological activities. *Pharmacogn. Rev*. 6(**11**) 46-55
Marinova, E. M. and Yanishlieva, N. V. (1997). Antioxidative activity of extracts from selected species of the family Laminacae in sunflower oil. *Food Chem.* 58: 245.
Maynart, G., Pousset, J. L., Mboup, S., Denis, F. (1980). Antibacterial activity of borreverine, an alkaloid isolated from *Borreria* verticillata (Rubiaceae) C R Seances *Soc. Biol Fil*. 174:925–8.
Moreira, V.F., Oliveira, R. R., Mathias, L., Braz-Filho, R. and Vieira, I.J. (2010). New chemical constituents from *Borreria* verticillata (Rubiaceae) *Helv Chim Acta*. 93:1751–7.
Nduka, N. (1999). *Clinical Biochemistry for Students of Pathology*. Longman Nigeria Plc. Lagos. P. 236
Oliver, A. (1959). *Medicinal Plant in Nigeria*. College of Arts, Science and Technology, Ibadan. New York University Press Pp. 11-14
Park, E.H., Kahng, J.H., Lee, S.H. and Shin, K.H. (2001). An Anti-inflammatory Principle from *Caetus. fetoterapia.* 72(**288**):290.
Perez, G.R.M. (2001). Anti-inflammatory Activity of Compounds Isolated from Plant. *Sci. World.* **1**:713-784.
Rec, G.S.C.C. (1972). A Colorometric Method for the Determination of Serum Alkaline Phosphate Determination. *J. clin-chem. clin.Biochem.* **10(2):**172.
Reitman, S. and Frankel, S. (1957). A Colorimetric method for the Determination of Serum Glutamic Pyruvate Transaminase. *Am. J. clin. Pathol.* **28**:56.
Shajiselvin, C. D., Kottai Muthu, A. and Suresh, K. (2010). Evaluation of *in vivo* antioxidant and lipid peroxidation effect of various extracts of the whole plant of *Borreria hispida (Linn)* on rat fed with high fat diet. *Intern. J. Pharma. Sci. Review and Research*. 3, 1 ; 66-69.
Sofowora , A.(2008). *Medicinal Plants and Traditional Medicines in Africa* .Third edi. Spec Books Limited, Ibadan. Pp.197-204, 306-310.
Teselkin, Y. O., Babenkova, I. V., Kolhir, V. K., Baginskaya, A. I., Tjukavkina, N. A., Kolesnik, Y. A., Selivanova, I. A. and Eichholz, A. A. (2000). Dihydroquercetin as a means of antioxidative defence in rats with tetrachloromethane hepatitis. *Phytother. Res.* 14 (3): 160.
Vasudevan, D. M. and Sreekumari, S. (2005). *Biochemistry for Medical Students.* 4th ed. Jaypee Brothers Medical Publishers, New Delhi. Pp. 55-225

Vieira, I.J., Mathias, L., Braz-Filho, R., Schripsema, J. (1999). Iridoids from *Borreria verticillata*. *Org Lett.* 1:1169–71.

Wagner, H. (1986). *Antihepatotoxic flavonoids* . In: "Plant Flavonoids in Biology and Medicine" (Eds. Cody,V.; Middleton, E. Jr. and Hardborne, J.B.), Biochemical, Pharmacological and Structure-Activity Relationships, New York, Alan R. Ed. Liss Inc, Pp. 545 - 558.

Wills, P. J. and Asha, V. V. (2006). Protective effect of Lygodium flexuosum (L.) Sw. extract against carbon tetrachloride-induced acute liver injury in rats. *J. Ethnopharmacol*. 108: 320.

World Health Organisation (2008). Traditional medicine Fact sheet no. 134.

Zimmerman, H.J. (1976). Various Form of Chemically Induced Liver Injury and their Detection by Diagnostic Procedures. *Environ. Health Persp.* 15: 3 – 12