Research Journal of Chemical Sciences ISSN 2231-606X
Vol. 1(7), 1-7, Oct. (2011) *Res.J.Chem.Sci.*

Short Communication

Hepatoprotective activity of aqueous extract of the leaves of *Hyptis suaveolens* (l.) Poit on acetaminophen Induced hepatotoxicity in rabbits

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Available online at: www.isca.in
(Received 30th July 2011, revised 05th August 2011, accepted 06th September 2011)

Abstract

The study was designed to evaluate the possible hepatoprotective activity of the pretreatment with aqueous extract of the leaves of Hyptis suaveolens on acetaminophen induced hepatotoxicity in rabbits. Albumin, total protein, total globulin, alanine transaminase aspertate transaminase and catalase in the blood plasma and liver were assayed as biochemical markers of hepatotoxicity and possible associated oxidative stress. Plasma and total liver proteins, albumin, globulin concentrations were determined using standard spectrophotometric methods also the activities of alanine transaminase, aspertate transaminase and catalase in the plasma and liver homogenates were determined by standard techniques. The maker enzymes show significant elevation in acetaminophen treated animals; these were significantly reduced toward an almost normal level in animals pretreated with aqueous extract of the leaves of Hyptis suaveolens. The reduction was observed for the concentrations of total protein and albumin. This showed that aqueous extract of the leaves of Hyptis suaveolens in addition to many of its numerous reported bioactivity probably possesses hepatoprotective potentials on acetaminaphen induced liver damage.

Key words: Hyptis suaveolens, hepatoxicity, oxidative stress, toxicity, rabbit.

Introduction

Hyptis suaveolens has been reported to be widely distributed in the tropics[1], northeast India[2] and a native of Neotropics. *Hyptis suaveolens* is commonly used Nigeria traditional medicinal practices for the treatment of respiratory tract infections, colds, pain, fever, cramps and skin diseases[3]. The essential oils isolated from the leaves have been found to possess antifungal [2,4,5], antibacterial[3,6,7], and anticonvulsant activities The plant is also used as an appetizing agent, to combat indigestion, stomach pain, nausea and infection of the gall bladder[2,8,9]. In the present study s the possible hepatoprotective potentials of the aqueous extract of the leaves of *Hyptis suaveolens* was investigated.

Material and Methods

Plant collection: Fresh leaves of *Hyptis suaveolens* were collected (in the month of September) from Hezekiah Oluwasami road (Road 7 area) Ile-Ife Nigeria. The leaves were identified and authenticated by Dr F. A. Oloyede from the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of the Extract: The leaves of *Hyptis suaveolens* were air dried for 15 days after which they were grounded to fine powder. Fifty (50) g of the fine powder was suspended in 500ml of distilled water; this was stirred mechanically for 4 hours at room temperature for 3 consecutive days. The suspension was filtered with a muslin cloth and the filtrate was further concentrated in a water bath by boiling for 1hour at 100°C. This was later freeze dried at Central Science Laboratory Obafemi Awolowo University, Ile-Ife, Nigeria.

Animal: Twenty wiener, all male rabbits (0.6 – 0.9 Kg) were procured from the Obafemi Awolowo University School farm. The rabbits were fed with concentrates purchased from Adedeji Feed Mills, Ile-Ife and clean water throughout the experiment. The rabbits underwent a two weeks acclimatization period and were later randomly distributed into 4 treatment groups of five animals each as follows:

Group	Treatment
Group I	0.9% Normal Saline (Controls)
Group II	Acetaminophen Only
Group III	Acetaminophen + Extract
Group IV	Acetaminophen + Vitamin C (Positive Control)

The group 1 (control group) received 0.9% normal saline (2 ml/kg body weight), The rabbits in groups II, III, IV received toxic dose of acetaminophen 1000 mg/kg orally using a cannula ad lib. The rabbits in groups III and IV were pre-treated with the plant leaves extract and vitamin C respectively at the dose of 200mg/kg body weight. The animals were treated for 4 weeks (3 times per week). At the end of the treatment period the animals were sacrificed. The blood were collected and the liver samples were obtained from each of the animal

Preparation of Blood Plasma: The blood samples collected in heparinzed bottles was centrifuged at 3000 rpm for 10minutes in microfield table centrifuge (Model 800D) at room temperature. Supernatants were collected into sterile sample bottles, labeled and kept in the deep freezer for further analyses.

Preparation of Liver Homogenates: One gram each of liver was homogenized separately in 10 ml of 100 mM phosphate buffer (pH 7.4) and centrifuged at 12000 rpm for 30 minutes. Supernatants were collected into clean sterile bottles, labeled and kept in the deep freezer for further analyses.

Biochemical Investigation: Determination of Total Protein: The most commonly used method for total protein determination is based on biuret reaction[10], this was adopted for this study.

Determination of Albumin: The bromocresol purple (BCP) method for albumin determination used in this study is a modification of Pinnel and Northan[11].

Total Globulin: Total globulin was calculated as the difference between total protein and albumin:
Total globulin = Total Protein (g/dL) – Albumin (g/dL)

Determination of Alanine Transaminase: The assay of alanine transaminase (ALT) in the blood and liver homogenates was performed as described by Reitman and Frankel[12]

Determination of Aspertate Transaminase: The assay of aspertate transaminase (AST) in the blood and liver homogenates was performed as described by Reitman and Frankel[12].

Determination of Catalase Activity: Catalase activity was assayed in the blood and liver homogenate by the method of Claiborne[13].

Statistical Analysis: All the values were represented as mean ± standard deviation. These data were analyzed and the group means were compared using the Student t – test. A probability of $p < 0.05$ was considered as significant.

Results and Discussion

Tables 1, 2 and 3 show the comparison of the mean concentration/activity (± SD) of the total protein, albumin, total globulin, alanine transaminase, aspertate transaminase and catalase between the controls versus acetaminophen only treated group; the controls versus acetaminophen plus extract pre-treated group, and the controls versus acetaminophen plus vitamin C pre-treated group respectively.

Table-1
Comparisons of the Mean ± SD of Total protein, Albumin, Globulin, ALT, AST and Catalase between the Acetaminophen only treated group and the controls

Parameters	Controls N = 5	Acetaminophen Only N = 5	T	P
Total protein (Liver)	3.08 ± 0.11	1.52 ± 0.26	12.356	P < 0.001
Total protein (Plasma)	8.70 ± 1.56	4.36 ± 0.43	5.997	P < 0.001
Albumin(Liver)	0.86 ± 0.38	0.36 ± 0.46	1.874	P < 0.05
Albumin (Plasma)	1.18 ± 0.39	0.43 ± 0.16	3.978	P < 0.01
Globulin(Liver)	2.22 ± 0.27	1.16 ± 0.20	7.054	P < 0.001
Globulin (Plasma)	7.52 ± 1.17	3.93 ± 0.27	6.685	P < 0.001
ALT (Liver)	0.11 ± 0.025	0.14 ± 0.019	2.136	P < 0.05
ALT (Plasma)	0.02 ± 0.01	0.04 ± 0.02	2.000	P < 0.05
AST (Liver)	0.14 ± 0.21	0.32 ± 0.15	1.560	P < 0.05
AST (Plasma)	0.09 ± 0.70	0.14 ± 0.21	0.153	NS
Catalase (Liver)	4.73 ± 0.41	4.09 ± 0.15	3.278	P < 0.001
Catalase (Plasma)	6.13 ± 0.77	8.74 ± 0.21	3.274	P < 0.001

N = No. of animals in each group ALT = Alanine aminotransferase AST = Aspertate aminotransferase

Table-2
Comparisons of the Mean ± SD of Total protein, Albumin, Globulin, ALT AST and Catalase between the Acetaminophen + Extract treated group and the controls

Parameters	Controls N = 5	Acetaminophen + Extract treated N = 5	T	P
Total protein (Liver)	3.08 ± 0.11	2.66 ± 0.01	8.503	P < 0.001
Total protein (Plasma)	8.70 ± 1.56	9.45 ± 2.78	0.526	NS
Albumin(Liver)	0.86 ± 0.38	1.27 ± 0.08	2.361	P < 0.05
Albumin (Plasma)	1.18 ± 0.39	1.88 ± 0.00	4.013	P < 0.001
Globulin(Liver)	2.22 ± 0.27	1.39 ±0 .08	6.591	P < 0.001
Globulin (Plasma)	7.52 ± 1.17	7.57 ± 2.78	0.037	NS
ALT (Liver)	0.11 ± 0.02	0.13 ± 0.01	2.000	P < 0.05
ALT (Plasma)	0.02 ± 0.01	0.03 ± 0.01	1.581	NS
AST (Liver)	0.14 ± 0.21	0.12 ± 0.43	0.093	NS
AST (Plasma)	0.09 ± 0.70	0.08 ± 1.01	0.018	NS
Catalase (Liver)	4.73 ± 0.41	3.98 ± 0.75	1.962	P < 0.05
Catalase (Plasma)	6.13 ± 0.77	7.55 ± 0.55	3.356	P < 0.001

N = No. of animals in each group ALT = Alanine aminotransferase AST = Aspertate aminotransferase

Table-3
Comparisons of the Mean ± SD of Total protein, Albumin, Globulin, ALT AST and Catalase between the Acetaminophen + Vitamin C treated group and the controls

Parameters	Controls N = 5	Acetaminohen + Vitamin C N = 5	T	P
Total protein (Liver)	3.08 ± 0.11	2.36 ± 0.396	3.917	P < 0.01
Total protein (Plasma)	8.70 ± 1.56	9.73 ± 3.42	0.613	NS
Albumin(Liver)	0.86 ± 0.38	1.32 ± 0.24	2.286	P < 0.01
Albumin (Plasma)	1.18 ± 0.39	1.28 ± 0.55	0.332	NS
Globulin(Liver)	2.22 ± 0.27	1.04 ± 0.16	8.407	P < 0.001
Globulin (Plasma)	7.52 ± 1.17	8.45 ± 2.87	0.671	NS
ALT (Liver)	0.11 ± 0.02	0.10 ± 0.02	0.791	NS
ALT (Plasma)	0.02 ± 0.01	0. 11 ± 0.09	2.222	P < 0.01
AST (Liver)	0.14 ± 0.21	0.16 ± 1.02	0.043	NS
AST (Plasma)	0.09 ± 0.70	0. 09 ± 1.11	0.000	NS
Catalase (Liver)	4.73 ± 0.41	3.86 ± 0.37	3.523	P < 0.01
Catalase (Plasma)	6.13 ± 0.77	14.13 ±9.01	1.976	P < 0.05

N = No. of animals in each group ALT = Alanine aminotransferase AST = Aspertate aminotransferase

Liver is the key organ in metabolism, detoxification and secretary function in the body. It also regulates important metabolic functions. Hepatic damage is therefore associated with the distortion of all of these functions. The present study was conducted to investigate the possible hepatoprotective effect of the aqueous extract of the leaves of *Hyptis suaveolens* on acetaminophen induced toxicity and oxidative stress in rabbits. The local traditional medicinal practices in this part of the world use water as solvent for extraction of the plant; this informed the use of crude aqueous extract of the leaves of the plant in this study. Additionally vitamin C, a well established antioxidant vitamin was used as positive control. The investigation revealed that the aqueous extract of *Hyptis suaveolens* probably possesses protective effect on hepatotoxicity and oxidative stress induced by acetaminophen. Standard indices of hepatotoxicity and oxidative stress namely albumin, total protein, total globulin, alanine transaminase, aspertate transaminase, and catalase in both plasma and the liver were assayed.

In this study the albumin and total protein concentration in both the plasma and liver homogenate decrease significantly in the acetaminophen only treated animals when compare with the control. This is not unexpected, since acetaminophen is a well established hepatotoxin which decreases synthesis of albumin and total protein, being normally associated with liver pathology.

Albumin is a single polypeptide chain. It is synthesized in liver where it amounts to 60% of hepatic protein synthesis though less than one a third of hepatocytes appear to synthesize albumin at any one time. In any form of hepatocellular damage, there is an increase in the plasma acute phase proteins and a fall in the plasma concentration of albumin[14]. The finding in this study is consistence with this. Albumin here is used to assess the synthesizing function of the liver and both total protein and albumin may also be used for the estimation of nutritional status of the subjects. Similarly, ALT is an enzyme located in the liver cells and leak out to make their way into the blood plasma when liver cells are injured. Elevations in the activities of ALT and AST in rabbits treated with toxic dose of acetaminophen have been demonstrated and have proven to be sensitive indices of hepatotoxicity and oxidative stress 15. The ALT is thought to be a more specific indicator of liver inflammation. The increased level of ALT in the blood plasma during liver damage should lead to a corresponding decrease in ALT activity in hepatocytes. This is also consistence with the results obtained in this study

Catalase activity though not significantly different in the liver, it is however significantly elevated in the blood of the animals suggesting acetaminophen induces oxidative stress. Table 2 also shows that the decrease concentration of total protein and albumin in both the liver and blood between the control and acetaminophen only were significantly reversed in the extract pretreated animals .

Consequently It is probable that the aqueous extract of the leaves of *Hyptis suaveolens* protects liver tissues against oxidative damages and acetaminophen induced toxicity. Further works are needed to fully characterize the active principles present in the plant and to elucidate the possible mode of action of these active principles.

References

1. **Fun C.E. and** Svendsen A. B., The essential oil of *Hyptis suaveolens, Poit grown on Aruba Flav Fragr J.*, **5**, 161-163 **(1990)**

2. Singh H.B. and A.K., Handique, Antifungal activity of the essential oil of *Hyptis suaveolens* and its efficacy in biocontrol measures in combination with *Trichoderma harzianum*, **9**, 683-687 **(1997)**

3. Iwu M.M., Handbook of African medicinal plants CRC Press Inc., Boca Raton, USA **(1993)**

4. Zollo P.H.A., Biyiti L., Tchoumbougnang F., Menut C., Lamaty G. and Bouchet P.H., Aromatic plants of tropical Central Africa, Part XXXII, Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon, *Flav Fragr J.*, **13**, 107-114 **(1998)**

5. Malele R.S., Mutayabarwa C.K., Mwangi J.W., Thoithi, G.N., Lopez A.G., Lucini E.I. and Zygadlo J.A., Essential oil of *Hyptis suaveolens* (L.) Poit from Tanzania: composition and antifungal activity, *Journal of Essential Oil Research*, **15**, 438–440 **(2003)**

6. Jain S.R., Jain P.R. and Jain M.R., Antibacterial evaluation of some indigenous volatile oils, *Planta Med.*, **26**, 196-199 **(1974)**

7. Asekun O.T., Ekundayo O. and Adeniyi B.A., Antimicrobial activity of the essential oil of *Hyptis suaveolens* leaves, *Fitoterapia*, **70**, 440–442 **(1999)**

8. Akah P.A. and Nwambie A.I., Nigerian plants with anticonvulsant properties, *Fitoterapia*, **64**, 42–44 **(1993)**

9. Ahmed M. Scora R.W. and Ting I.P., Composition of leaf oil of *Hyptis suaveolens* (L.) Poit, *J. Essent, Oil Res.*, **6**, 571-575 **(1994)**

10. Cannon D.C, Olitzky I. and Inkpen J.A Protein, In Clinical Chemistry and Techniques, 2nd Edition, Henry R.J, Cannon D.C and Winkleman W (eds). Harper and Row, New York, 407-421**(1974)**

11. Pinnel A.E. and Northam B.E., New automated dye-binding method for serum albumin determination with bromocresol purple, *Clin Chem.*, 24-80 **(1978)**

12. Reitman S. and Frankel A., colorimetric method for aspertate and alanine aminotransferase in the serum, *J. Clin Pathol*, **28**, 56-58**(1957)**

13. Claiborne A., Catalase activity In: Greenwald R.A. (Ed.), Handbook of Methods for Oxygen Free Radical Research CRC Press, Boca Raton, FL **(1985)**

14. Sies H. Strategies of antioxidant defense, *Eur J Biochem.* **(215)** 213 -219 **(1993)**

15. Harper H.A., The functions and tests of the liver. Review of Physiological Chemistry Lange Medical Publishers, Los Atlos, CA, 271– 283 **(1961)**