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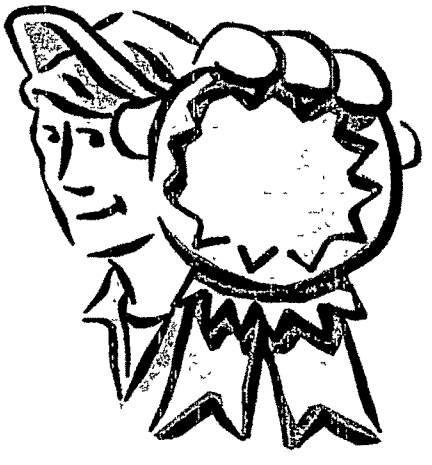

PUBLICATIONS

PAPERS PUBLISHED

1. Late Komal Mehta, R.Balaraman, A.H.Amin, P.A.Bafna, O.D.Gulati (2003) Effect of fruits of *Moringa oleifera* on the lipid profile of normal and hypercholesterolemic rabbits. ***Journal of Ethnopharmacology*** 86: 191-195.

2. Bafna P.A. and R.Balaraman (2004) Anti-ulcer and antioxidant activity of DHC-1, an herbal formulation. ***Journal of Ethnopharmacology*** 90: 123-127.

3. Bafna P.A. and R.Balaraman (2004) Antioxidant activity of Activit, a herbomineral formulation, in experimentally induced cardiac and renal damage. ***Ars Pharmaceutica*** 45:1; 45-57.

4. Bafna P.A. and R.Balaraman (2004) Anti-ulcer and antioxidant activity of Normacid, a herbomineral formulation. ***Indian Journal of Experimental Biology*** 42: 674-680.

PAPERS IN PRESS (ACCEPTED)

5. Bafna P.A. and R.Balaraman "Anti-ulcer and antioxidant activity of Pepticare, a herbomineral formulation" - ***Phytomedicine.***

6. Bafna P.A. and R.Balaraman "Antioxidant activity of DHC-1, a herbal formulation" - ***Journal of Ethnopharmacology.***

7. Bafna P.A. and R.Balaraman "Antioxidant activity of DHC-1, an herbal formulation, in experimentally induced cardiac and renal damage" ***Phytotherapy Research.***

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Journal of Ethnopharmacology 86 (2003) 191–195
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Effect of fruits of *Moringa oleifera* on the lipid profile of normal and hypercholesterolaemic rabbits

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Received 17 November 2001, accepted 12 February 2003

Abstract

Rabbits were fed *Moringa oleifera* (200 mg/kg/day, p.o.) or lovastatin (6 mg/kg/day, p.o.) in banana pulp along with standard laboratory diet and hypercholesterolaemic diet for 120 days. *Moringa oleifera* and lovastatin were found to lower the serum cholesterol, phospholipid, triglyceride, VLDL, LDL, cholesterol to phospholipid ratio and atherogenic index, but were found to increase the HDL ratio (HDL/HDL-total cholesterol) as compared to the corresponding control groups. Treatment with *M. oleifera* or lovastatin in normal rabbits decreased the HDL levels. However, HDL levels were significantly increased or decreased in *M. oleifera*- or lovastatin-treated hypercholesterolaemic rabbits, respectively. Lovastatin- or *M. oleifera*-treated hypercholesterolaemic rabbits showed decrease in lipid profile of liver, heart and aorta while similar treatment of normal animals did not produce significant reduction in heart. *Moringa oleifera* was found to increase the excretion of faecal cholesterol. Thus, the study demonstrates that *M. oleifera* possesses a hypolipidaemic effect.

Keywords: *Moringa oleifera*, Lovastatin, Cholesterol, Hypolipidaemic effect, Prevention of atherosclerosis

1. Introduction

Moringa pterygosperma or *Moringa oleifera* Lam. (family: Moringaceae) is a small- or middle-sized tree, which is cultivated throughout India and Myanmar (Burma). The fruit of this tree is edible and constitutes one of the common vegetable dishes in India.

Various parts of the *M. oleifera* tree have been studied for several pharmacological actions. The juice from the leaves and stem bark of *M. oleifera* was found to inhibit *Staphylococcus aureus* but not *Escherichia coli* (Bhawasar et al., 1965). The bark extract has been shown to possess anti-fungal and anti-tubercular activity (Bhatnagar et al., 1961). Ethanolic extract (50%) of *M. oleifera* (whole plant excluding roots) showed anti-cancer activity in mice (Dhawan et al., 1980). The hypotensive activity of ethanolic and aqueous extracts of pods of *M. oleifera* was also studied by Faizi et al. (1998).

The fruit of *M. oleifera* contains proteins, fats, carbohydrates, minerals, fibre, Vitamin A, β-nicotinic acid, ascorbic acid, tocopherol, oestrogenic substances and β-sitosterol. Pterygospermin, a bactericidal and fungicidal compound, isolated from *M. oleifera* (subcutaneously injected) has an LD_{50} in mice and rats of 350–400 mg/kg body weight (Verma et al., 1976).

Moringa oleifera is incorporated in various marketed formulations, such as Rumalaya and Septilin (The Himalaya Drug Company, Bangalore, India), Orthoherb (Walter Bushnell Ltd., Mumbai, India), Kupid Fort (Pharma Products Pvt. Ltd., Thayavur, India) and Livospin (Herbals APS Pvt. Ltd., Patna, India), which are available for a variety of disorders.

Hypercholesterolaemia is one of the important risk factors for coronary heart disease (CHD). In spite of the availability of various anti-hyperlipaemic agents, there is increase in the incidence of CHD and risk of congestive heart failure (CHF). Thus, there is still considerable interest in the evaluation of new anti-hyperlipaemic agents (synthetic and herbal). *Commiphora mukul* (Guggul) has been evaluated for its lipid lowering activity (Nityanand and Kapoor, 1971; Malhotra et al., 1977; Satyavati et al., 1969) and found to possess this activity. Several of its formulations are used in the treatment of atherosclerosis.

The leaves of *M. oleifera* were used by the Indians in their herbal medicine as a hypocholesterolaemic agent in obese

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0378-8741/03/$ – see front matter © 2003 Elsevier Science Ireland Ltd. All rights reserved.
doi:10.1016/S0378-8741(03)00075-8

patients The scientific basis for their use in hypercholesterolaemia was examined by Ghasi et al (2000). They tested the crude extract of leaves of *M oleifera* and showed that it possessed hypocholesterolaemic activity This led them to conclude that there is a valid pharmacological basis for employing the leaves for this purpose

As the fruit of *M oleifera* is widely consumed by the people of India, it was considered worthwhile to study it for its hypocholesterolaemic activity. Thus, the aim of the study was to evaluate the fruit of *M. oleifera* for its effect on the lipid profile in normal and hypercholesterolaemic rabbits

2. Materials and methods

2 1 *Plant material*

Tender and semi-ripe fruits of *M. oleifera* were cleaned externally with water. Any fruit with damaged outer skin or any other kind of visible defect was discarded The tips of both the ends were cut and discarded. The middle portion was cut into 2–3 cm length pieces and steamed in a pressure vessel for about 10–20 min. The cooked pieces were cooled to room temperature and slit vertically. The outer skin was separated from the covering of the pulp and seed, which were collected separately and were dried at 60 °C to a constant mass. The dried mass was sifted through #60 mesh. The powder was stored in an airtight container until further use The yield per 100 g of *M. oleifera* fruit varied between 3 and 7 g depending on the season

2.2. *Animals*

Healthy male adult rabbits weighing between 1 5 and 2.5 kg were used for the experiments The animals were housed individually in large cages with free access to food and water ad libitum during the course of experiment During the experimental period the rabbits were fed with standard laboratory diet (SLD) or hypercholesterolaemic diet (HCD) containing 5% cholesterol, 5% coconut oil and 90% SLD, both provided by Alembic Ltd., Baroda, India.

2.3. *Experimental procedure*

The animals were divided into six groups of six animals each:

Group 1: Control animals fed with SLD daily for 120 days

Group 2 Rabbits fed with SLD plus lovastatin (6 mg/kg/day, p.o.) in banana pulp for 120 days

Group 3: Rabbits fed with SLD plus *M. oleifera* powder (200 mg/kg/day, p.o.) in banana pulp for 120 days

Group 4: Rabbits fed with hypercholesterolaemic diet (HCD) daily for 120 days

Group 5. Rabbits fed with HCD plus lovastatin (6 mg/kg/day, p o) in banana pulp for 120 days

Group 6 Rabbits fed with HCD plus *M. oleifera* powder (200 mg/kg/day, p o.) in banana pulp for 120 days

Faecal samples were collected from individual rabbits 1 week before the end of 120-day treatment period After 120 days of treatment, blood was collected from the marginal ear vein and serum was separated. The animals were then sacrificed and organs, such as heart, liver and thoracic aorta, were removed

2 4 *Serum estimations*

Quantitative determination of total cholesterol and HDL-cholesterol was done by using kits, which employ enzymatic method of estimation [Sigma Diagnostics (India) Pvt Ltd., Baroda]. LDL- and VLDL-cholesterol were calculated by the method of Varley et al (1983). Phospholipid was estimated by using kits [Sigma Diagnostics (India) Pvt Ltd , Baroda]. Cholesterol to phospholipid (C/P) ratio was calculated by the method reported by Sharma et al (1995). Cholesterol balance was calculated according to Connor et al (1969) Triglycerides and total protein were estimated using kits [Sigma Diagnostics (India) Pvt. Ltd , Baroda] Total lipids were determined by the sulphophosphovanillin method (Frings et al , 1972) HDL ratio and atherogenic index were determined by the method of Sheela and Augusti (1995)

2 5 *Lipid content in tissue and faeces*

The procedure of Folch et al. (1957) modified by Suzuki (1965) was used for the extraction of lipids from tissues, such as heart, liver, thoracic aorta and faeces. The lipid extract obtained from tissues and faeces was used for the estimation of total cholesterol, phospholipid and triglyceride as described earlier The change in body weight was also recorded.

2 6 *Statistical analysis*

The mean ± S.E M values were calculated for each group In order to determine the significance of intergroup difference, each parameter was analysed separately by using one-way ANOVA with Huynn–Feldt epsilon modification of degree of freedom to correct the departure from sphericity.

3. Results and discussion

The results of this study showed that the administration of *M oleifera* (200 mg/kg/day, p.o.) or lovastatin (6 mg/kg/day, p o) to rabbits fed a SLD or HCD for a period of 120 days decreased the serum total cholesterol, phospholipid, triglycerides, LDL, VLDL, total lipids and

Table 1
Effect of *Moringa oleifera* and lovastatin on serum total cholesterol (Chol), triglyceride (TGL), phospholipid (PL), total lipid (TL), cholesterol balance (Chol balance), HDL, VLDL, LDL, total protein, cholesterol to phospholipid ratio (C/P ratio), atherogenic index (AI), HDL ratio and lipid/protein ratio in rabbits fed standard laboratory diet (group 1 control, group 2 lovastatin, group 3 *M oleifera*) and hypercholesterolaemic diet (group 4 control, group 5 lovastatin, group 6. *M oleifera*) for 120 days

Parameter	Group					
	1	2	3	4	5	6
Total Chol (mg/dl)	130 34 ± 3 61	−59 31	−56 11	3097 8 ± 37 46*	−95 42	−81.69
TGL (mg/dl)	105 70 ± 3 28	−16 13	−19 69	1765 53 ± 51 17*	−84 37	−81 44
PL (mg/dl)	153 04 ± 1 96	−31 14	−32 63	774 19 ± 4 14*	−79 10	−52 50
TL (mg/dl)	527 43 ± 6 10	−27 96	−29 05	12572 72 ± 17 37*	−90 90	−67 53
Chol balance (mg/dl)	106 58	−63 08	−80 88	3174 08*	−80 96	−87.41
HDL (mg/dl)	44 33 ± 1 34	−48 61	−37 55	93 57 ± 4 50*	−73 19	+197 08
VLDL (mg/dl)	21 14 ± 0 66	−16 13	−19 68	353 11 ± 1 23*	−84 37	−81 43
LDL (mg/dl)	64 88 ± 3 18	−80 53	−80 59	2651 04 ± 38 17*	−97 72	−91 56
Total protein (g%)	6 43 ± 0 17	+2 49	+0 78	10 98 ± 0 91*	−33.79	−28 42
C/P ratio	0 85	−41 18	−34 12	4.00	−78 00	−61 50
AI	2 94	−20 75	−29 59	33 11	−82 94	−93 84
HDL ratio	0.52	+44 23	+80 77	0 03	+633 33	+3100 00
Lipid/protein ratio	82 03	−29 71	−29 60	1145 06	−86 26	−54 64

Control values are expressed as mean ± S E M Group 4 is compared to group 1 Groups 2 and 3 are compared to group 1 Groups 5 and 6 are compared to group 4 Values in columns 2, 3, 5 and 6 indicate the percent change in relation to the corresponding controls, '+' denotes increase and '−' denotes decrease HDL ratio HDL/HDL-total cholesterol

* $P < 0.01$.

C/P ratio, cholesterol balance and atherogenic index as compared to the corresponding control groups Treatment of normal rabbits with *M. oleifera* or lovastatin decreased the HDL levels as compared to the normal control group. Lovastatin treatment reduced the HDL levels as compared to hypercholesterolaemic control rabbits. However, treatment of hypercholesterolaemic rabbits with *M. oleifera* showed a significant increase in HDL levels. The HDL ratio (HDL/HDL-total cholesterol) was increased in both *M. oleifera*- and lovastatin-treated groups as compared to the corresponding control groups (Table 1) Increase in HDL ratio is one of the desirable criteria of an ideal hypocholesterolaemic agent since the higher the ratio, the lower the atherosclerotic risk. The decrease in cholesterol balance, which indicates the total change in body pools of cholesterol, may be due to the compensatory mechanisms, such as a decrease in resorption of endogenous cholesterol or an increase in the rate of secretion into intestinal tract or both.

Treatment of normal rabbits with *M. oleifera* or lovastatin did not produce any significant change in the total proteins as compared to normal control group. However, total protein levels were significantly increased in hypercholesterolaemic control rabbits. Treatment with lovastatin or *M. oleifera* reduced the total protein levels as compared to hypercholesterolaemic control rabbits (Table 1).

The change in the lipid profile with *M oleifera* treatment may contribute to the decreased incidence of atherosclerosis and CHD similar to that with lovastatin. Generally high C/P ratio is associated with atherosclerosis (Sharma et al., 1991, 1995). Administration of *M. oleifera* was found to markedly reduce this ratio suggesting a possible role in reducing the incidence of atherosclerosis.

Lovastatin- or *M oleifera*-treated hypercholesterolaemic rabbits also showed decrease in the lipid profile of liver, heart and aorta as compared to the corresponding control group while similar treatment of normal animals did not produce significant reduction in the heart (Table 2). The reduction in liver cholesterol, triglyceride and phospholipid levels may be because of partial inhibition of cholesterol synthesis de novo or by inhibition of cholesterol absorption, thereby depleting critical intracellular pool of sterols in the liver. The reduction of lipid content of heart and aorta may serve as a useful index of the severity of atherosclerosis

Atherogenic index indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidneys The higher the atherogenic index, the higher is the risk of the above organs for oxidative damage. Atherogenic index was significantly reduced in the *M oleifera*-treated group.

Plant sterols inhibit the absorption of dietary cholesterol, but the resulting decrease in serum cholesterol has been slight (Lees et al., 1977; Grundy et al., 1969). A more recent study (Tatu et al., 1995) has found a 10.2% reduction in serum cholesterol. Although *M. oleifera* has been shown to contain β-sitosterol, the amount contained is unknown. The cholesterol lowering effect may be due to this inhibition in reabsorption of cholesterol from endogenous sources in association with a simultaneous increase in its excretion into faeces in the form of neutral steroids. The increased faecal excretion of cholesterol and phospholipid observed with *M. oleifera* (Table 2) may be related to the latter suggestion.

Table 2
Effect of *Moringa oleifera* and lovastatin on total cholesterol (Chol), triglyceride (TGL), phospholipid (PL) and total lipid (TL) in heart, liver, aorta and faeces of rabbits fed standard laboratory diet (group 1 control, group 2 lovastatin, group 3 *M oleifera*) and hypercholesterolaemic diet (group 4 control, group 5 lovastatin, group 6 *M oleifera*) for 120 days

Sample	Group					
	1	2	3	4	5	6
Heart						
Chol. (mg/g tissue)	14 68 ± 1 57	−50 89	−53 13	309 16 ± 3 89*'	−83 71	−80 28
TGL (mg/g tissue)	11 26 ± 0 43	−12 88	−12 97	207 99 ± 3 54*	−42 89	−36 63
PL (mg/g tissue)	18 86 ± 0 50	−15 06	−15 54	261 55 ± 3.22*	−64 54	−55 95
TL (mg/g tissue)	45 47 ± 0 75	−39 45	−34 02	1025 05 ± 20 29*	−49 02	−28 78
Liver						
Chol. (mg/g tissue)	23 64 ± 0 85	−42 72	−45 30	506 40 ± 5 57*	−81.89	−76 93
TGL (mg/g tissue)	35 56 ± 1 10	−14 15	−14 48	346 55 ± 2 99*	−71 19	−62 02
PL (mg/g tissue)	29 81 ± 0 74	−14 26	−16 44	371 58 ± 5 10*	−66 85	−60 40
TL (mg/g tissue)	87 22 ± 1 05	−29 60	−27 88	1530 39 ± 6 34*	−70 02	−66 79
Aorta						
Chol (mg/g tissue)	19 18 ± 1 54	−38 95	−38 06	325 34 ± 7 09*	−71 63	−63 48
TGL (mg/g tissue)	16 26 ± 0 43	−10 76	−8 92	305 22 ± 4 50*	−54 96	−42 68
PL (mg/g tissue)	23 37 ±0 52	+6 12	−11 17	320 97 ± 7 08*	−56 45	−54 09
TL (mg/g tissue)	56 02 ± 1 62	−19 58	−3 57	1233 95 ± 10 07*	−45 03	−30 61
Faeces						
Chol (mg/g dry faeces)	23 76 ± 1 34	−42 38	+54 97	423 72 ± 1 97*	−91.20	+57 49
PL (mg/g dry faeces)	19 88 ± 1 56	−40 14	−14 59	13041 ± 3 36*	−61 99	−43 89
TL (mg/g dry faeces)	32.70 ± 1 04	−25 75	+73 30	497 00 ± 1 26*	−79 52	+32 76

Control values are expressed as mean ± S.E M Group 4 is compared to group 1 Groups 2 and 3 are compared to group 1 Groups 5 and 6 are compared to group 4 Values in columns 2, 3, 5 and 6 indicate the percent change in relation to the corresponding controls, '+' denotes increase and '−' denotes decrease

* $P < 0.001$

The present study suggests that *M. oleifera* has hypolipidaemic action. The present study has also shown that *M. oleifera* has anti-atherosclerotic effect together with a reduction in body weight (Table 3). Clinical work in human hypercholesterolaemic patients carried out recently correlates with the experimental data referred herein (Patel K., personal communication, 2001).

Table 3
Effect of *Moringa oleifera* and lovastatin on change in body weight in rabbits fed standard laboratory diet and hypercholesterolaemic diet daily for 120 days

Group	Change in body weight (kg)
1 (SLD (control))	0 317 ± 0 025
2 (SLD + lovastatin)	−42 27
3 (SLD + *M. oleifera*)	−18 61
4 (HCD)	0.992 ± 0 09*
5 (HCD + lovastatin)	−84.88
6 (HCD + *M. oleifera*)	−89.11

Control values are expressed as mean ± S E M Group 4 is compared to group 1. Groups 2 and 3 are compared to group 1 Groups 5 and 6 are compared to group 4 Values in rows 2, 3, 5 and 6 indicate the percent change in relation to the corresponding controls, '+' denotes increase and '−' denotes decrease SLD standard laboratory diet, HCD hypercholesterolaemic diet

* $P < 0.001$

Acknowledgements

We are extremely grateful to Alembic Ltd, Baroda, for sponsoring the project, generous financial assistance, including uninterrupted supply of rabbits, *Moringa oleifera* powder and lovastatin, for carrying out this work.

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Journal of Ethnopharmacology 90 (2004) 123–127
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Anti-ulcer and antioxidant activity of DHC-1, a herbal formulation

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Received 1 April 2003, accepted 22 September 2003

Abstract

DHC-1, a herbal formulation, was tested for its anti-ulcer and antioxidant activity in rats Effect of various doses (125, 250, 500, and 1000 mg/kg, p.o.) of DHC-1 was studied on gastric secretion and gastric ulcers in pylorus-ligation and on ethanol-induced gastric mucosal injury in rats. The reduction in ulcer index in both the models along with the reduction in volume and total acidity, and an increase in the pH of gastric fluid in pylorus-ligated rats proved the anti-ulcer activity of DHC-1. The increase in the levels of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and membrane bound enzymes like Ca^{2+}ATPase, Mg^{2+}ATPase, and $Na^{+}K^{+}$ATPase and decrease in lipid peroxidation (MDA) in both the models showed the antioxidant activity of the formulation. Thus, it can be concluded that DHC-1 possesses anti-ulcer activity, which can be attributed to its antioxidant mechanism of action.

Keywords. Anti-ulcer, Antioxidant, Lipid peroxidation, Superoxide dismutase, Catalase, Reduced glutathione

1. Introduction

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects (arrythmias, impotence, gynaecomastia, and haematopoeitic changes) of modern medicine (Akhtar et al., 1992), indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer.

There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies, and digestive system disorders such as gastrointestinal inflammation and gastric ulcer (Repetto and Llesuy, 2002). Studies have shown alterations in the antioxidant status following ulceration, indicating that free radicals seem to be associated with the pylorus ligation-induced (Rastogi et al., 1998) and ethanol-induced (Pihan et al., 1987; Mizui et al., 1987) ulceration in rats. Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward in minimizing tissue injury in human disease (Barry, 1991).

Many indigenous drugs are known to possess anti-ulcer activity. The anti-ulcer properties of *Emblica officinalis* (Rajeshkumar et al., 2001), *Glycyrrhiza glabra* (Al-Rehaily et al., 2002), and *Syzygium aromaticum* (Chaudhary, 1996) have been mentioned. The antioxidant properties of *Bacopa monnieri* (Tripathi et al., 1996), *Emblica officinalis* (Bhattacharya et al., 1999), *Glycyrrhiza glabra* (Hatano et al., 1991), *Mangifera indica* (Martinez et al., 2000), and *Syzygium aromaticum* (Deans et al., 1995) were earlier investigated and were found to possess free radical scavenging properties. Some of the ingredients were also found to produce significant increase in the levels of various endogenous antioxidant enzymes

The present study was, thus, aimed to investigate the anti-ulcer effects of DHC-1 along with its effect on the antioxidant enzymes to justify whether the formulation exerts an anti-ulcer action by means of its antioxidant activity.

2. Material and methods

2.1. Composition

Each gram of DHC-1 (manufactured by Himalaya Drug Company, Bangalore, India) contains methanolic extracts of *Bacopa monnieri* L. (Scrophulariaceae; whole plant, 200 mg), *Emblica officinalis* Gaertn. (Euphorbiaceae, fruit; 200 mg), *Glycyrrhiza glabra* L. (Papilionaceae; roots; 200 mg), *Mangifera indica* L. (Anacardiaceae; bark; 200 mg), and *Syzygium aromaticum* L. (Myrtaceae; flower bud; 200 mg).

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doi:10.1016/j.jep.2003.09.036

2 2. Animals

Female albino rats of wistar strain weighing between 150 and 225 g were used for the study. The animals were fed ad libitum with standard pellet diet and had free access to water

2.3. Experimental procedure

The animals were divided into five groups each consisting of six rats. Group 1 represented the control group, which received 5 ml/kg body weight of vehicle (1% gum acacia, p o.). Groups 2–5 received DHC-1 orally at the doses of 125, 250, 500, and 1000 mg/kg body weight, respectively.

2.3.1. Study of anti-ulcer and antioxidant activity using pylorus ligation method

The method of Shay rat ulcer described by Ghosh (1984) was adopted. Rats were fasted for 48 h. The drug, DHC-1 was administered to the animals. During the course of the experiment food was withdrawn. After the pretreatment period of 1 h, the animals were anaesthetized with anaesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process; pylorus portion of stomach was slightly lifted out and ligated. Precaution was taken to avoid traction to the pylorus or damage to its blood supply. The stomach was placed carefully in the abdomen and the wound was sutured by interrupted sutures. Nineteen hours after pylorus ligation the rats were sacrificed and the stomach was removed. The gastric content was collected and centrifuged. The volume, pH, and total acidity of gastric fluid was determined. The stomach was then incised along the greater curvature and observed for ulcers The number of ulcers was counted using a magnifying glass and the diameter of the ulcers was measured using a vernier caliper Ulcer index was determined by following the scoring method of Suzuki et al. (1976).

Score 1: Maximal diameter of 1 mm.
Score 2: Maximal diameter of 1–2 mm.
Score 3: Maximal diameter of 2–3 mm.
Score 4: Maximal diameter of 3–4 mm.
Score 5: Maximal diameter of 4–5 mm.
Score 10: An ulcer over 5 mm in diameter.
Score 25: A perforated ulcer.

The stomach was then weighed and homogenized in chilled Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000 × *g* at 0 °C for 20 min using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assays of lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase (SOD) and catalase (CAT)), and reduced glutathione (GSH). The sediment was resuspended in ice-cold Tris buffer (10 mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of different membrane bound enzymes (Na^+K^+ATPase, Ca^{2+}ATPase, and Mg^{2+}ATPase) and proteins.

2.3 2 Study of anti-ulcer and antioxidant activity using ethanol-induced ulcer method

The method described by Dhuley (1999) was adopted. DHC-1 was administered orally to the rats for a period of 10 days On the 10th day, 1 h after the final dose of DHC-1, 96% ethanol (5 ml/kg, p.o.) was administered to the overnight fasted rats of all groups. The animals were then sacrificed 1 h after the dose of ulcerogen. The stomach was then removed, incised along the greater curvature and its mucosal erosion was determined randomly by measuring the area of the lesions. The sum of the areas was expressed as ulcer index (mm^2). The stomach was then weighed and processed for antioxidant estimations as mentioned in previous section.

2.4. Biochemical estimations

Superoxide dismutase was determined by the method of Mishra and Fridovich (1972). Catalase was estimated by the method of Hugo Aebi as given by Colowick et al. (1984). Reduced glutathione was determined by the method of Moron et al. (1979). Lipid peroxidation or malondialdehyde formation was estimated by the method of Slater and Sawyer (1971). Membrane bound enzymes namely Na^+K^+ATPase, Ca^{2+}ATPase, and Mg^{2+}ATPase were assayed according to the methods of Bonting (1970), Hjerten and Pan (1983), and Ohnishi et al. (1982), respectively. The inorganic phosphorus was estimated by the method of Fiske and Subbarow (1925). Total proteins were determined by the method of Lowry et al. (1975)

2.5. Statistical analysis

Results of all the above estimations have been indicated in terms of mean ± S.E.M.. The difference between means was analyzed by Student's *t*-test. Minimum level of significance was fixed at $P \leq 0.05$ (95%).

3. Results

3.1. Study of anti-ulcer and antioxidant activity using pylorus ligation method

It was observed that in the vehicle treated control group, the ulcer index was 92.75 ± 10.40 and the maximum number of ulcers were of the ulcer score 4 and 5. In the rats of this group, a number of perforated ulcers (score 25) were also observed.

DHC-1 was found to produce significant decrease in ulcer index. All the ulcers were of scores 1 and 2 and no perforated ulcers were observed. The formulation also significantly reduced the volume and total acidity, and increased the pH of the gastric fluid, proving its anti-ulcer activity (Table 1).

As compared to normal rats, pylorus-ligation was found to increase lipid peroxidation and decrease SOD, catalase and reduced glutathione in the control group, thus leading

Table 1
Effect of DHC-1 on the various gastric parameters of pylorus-ligated rats

Groups	Ulcer index	Volume of gastric fluid (ml)	pH of gastric fluid	Total acidity (mEq /l per 100 g)
Control	92.75 ± 10 40	17 28 ± 1.18	1 20 ± 0 07	127 0 ± 3 63
DHC-1 (125 mg/kg)	53 62 ± 5 80* (42 19%)	13 78 ± 1 31^{NS}	2 18 ± 0 17**	90 75 ± 2 96**
DHC-1 (250 mg/kg)	21.33 ± 2 45** (77 63%)	11.58 ± 1 02*	3 12 ± 0.23**	36 50 ± 4.27**
DHC-1 (500 mg/kg)	11 25 ± 1.50** (87.87%)	4 50 ± 1.17**	3 30 ± 0.24**	20.65 ± 1.23**
DHC-1 (1000 mg/kg)	4 25 ± 1.75** (95 42%)	1 15 ± 0 31**	5 50 ± 0 87**	13 50 ± 1.94**

Values are expressed as mean ± S E.M DHC-1 treated groups were compared with control group *$P < 0\ 05$, **$P < 0\ 001$, NS· non significant. Values in parenthesis indicate the % reduction in ulcer index in relation to the control group

Table 2
Effect of DHC-1 on the antioxidant parameters in stomach of pylorus ligated rats

Parameters	Normal	Control	DHC-1			
			125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
SOD (unit/mg protein)	5 63 ± 0 33	2 37 ± 0 26*	2 49 ± 0 2^{NS}	2 63 ± 0 93*	3 03 ± 1 08*	4 95 ± 1 35**
Catalase (μmoles of H_2O_2 consumed/(min mg protein))	8 26 ± 0 27	5 93 ± 0 61*	5 99 ± 0 71^{NS}	6 25 ± 0 85*	7 09 ± 1 15**	8 25 ± 1 10**
Reduced glutathione (μg of GSH/mg protein))	3 21 ± 0 15	0 58 ± 0 14**	1 25 ± 0 32*	1 88 ± 1 04**	2.49 ± 0 43**	3.53 ± 0.36**
Lipid peroxidation (nmoles of MDA/mg protein)	3 57 ± 0 33	10 10 ± 0 43**	9 13 ± 0 63^{NS}	4 75 ± 0.41**	3 99 ± 0 13**	2 23 ± 0 17**
Na^+K^+ATPase (μmoles of inorganic phosphorus liberated/(min mg protein))	5 23 ± 0 23	1 49 ± 0 11**	2 17 ± 0 08*	3 61 ± 0 07**	4 63 ± 0 08**	6 70 ± 0 10**
Ca^{2+}ATPase (μmoles of inorganic phosphorus liberated/(min mg protein))	3 78 ± 0 12	1 63 ± 0 62*	1 98 ± 0 24*	2 73 ± 0 12*	2 83 ± 0 17**	3 80 ± 0 32**
Mg^{2+}ATPase (μmoles of inorganic phosphorus liberated/(min mg protein))	3 37 ± 0 36	1 53 ± 0 63*	2 78 ± 0 27**	3 13 ± 0 24**	3 43 ± 0 17**	3 56 ± 0 15**

Values are expressed as mean ± S E M Control group was compared with normal group DHC-1 treated groups were compared with control group *$P <$ 0 05, ** $P < 0\ 001$, NS non significant

to oxidative stress. Administration of DHC-1, at the doses of 250, 500, and 1000 mg/kg, brought about a significant reduction in lipid peroxidation and an increase in the activities of antioxidant enzymes namely, SOD and catalase. An increase in the level of reduced glutathione along with the enhancement in the membrane bound ATPases was also observed at all the dose levels of DHC-1 (Table 2).

3.2 Study of anti-ulcer and antioxidant activity using ethanol-induced ulcer method

Administration of ethanol produced significant ulcers (287.98 ± 17.79) in the control group. There was a significant reduction in ulcer index at all the four doses of DHC-1.

Table 3
Effect of DHC-1 on the ulcer index and antioxidant parameters in stomach of ethanol-treated rats

Parameters	Normal	Control	DHC-1			
			125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Ulcer index (mm^2)	–	287 98 ± 17 79	72 96 ± 5 87** (74 66%)	51 69 ± 4 50** (82 05%)	27 86 ± 3 30** (90 33%)	4 47 ± 1.75** (98 45%)
SOD (unit/mg protein)	5 63 ± 0 33	2 23 ± 0 03**	3 68 ± 0 04*	5 58 ± 0 54**	6 38 ± 0 79**	8 05 ± 1.25**
Catalase (μmoles of H_2O_2 consumed/(min mg protein))	8 26 ± 0 27	5 13 ± 0 24*	5 95 ± 1 17*	6 03 ± 0 83**	6 68 ± 1 55**	7 68 ± 1.13**
Reduced glutathione (μg of GSH/mg protein)	3 21 ± 0 15	0 79 ± 0 17**	0 98 ± 3 03*	1 28 ± 3 78*	2 26 ± 4 10**	4.65 ± 6 25**
Lipid peroxidation (nmoles of MDA/mg protein)	3 57 ± 0 33	6 75 ± 0 70**	2 20 ± 0 13**	1 86 ± 0 03**	1 69 ± 0.08**	1 43 ± 0 08**
Na^+K^+ATPase (μmoles of inorganic phosphorus liberated/(min mg protein))	5 23 ± 0 23	2 03 ± 0 19**	8 45 ± 0 53**	9 80 ± 0 83**	10 93 ± 0 59**	11 23 ± 0 64**
Ca^{2+}ATPase (μmoles of inorganic phosphorus liberated/min/mg protein)	3 78 ± 0 12	1 47 ± 0 03**	3 00 ± 0 11**	3 17 ± 0 18**	3 23 ± 0 27**	3 30 ± 0 22**
Mg^{2+}ATPase (μmoles of inorganic phosphorus liberated/(min mg protein))	3 37 ± 0 36	1 45 ± 0 09**	2 75 ± 0 17**	3 28 ± 0 15**	3 20 ± 0.29**	3.56 ± 0 25**

Values are expressed as mean ± S E M Control group was compared with the normal group DHC-1 treated groups were compared with control group *$P < 0\ 05$, ** $P < 0\ 001$; NS non significant Values in parenthesis indicate the % reduction in ulcer index in relation to the control group

Ethanol administration was found to increase lipid peroxidation and decrease SOD, catalase, and reduced glutathione in the control group when compared to normal rats. Administration of DHC-1 significantly decreased lipid peroxidation and increased the levels of SOD, catalase, reduced glutathione and all membrane bound ATPases at all the dose levels (Table 3).

4. Discussion and conclusion

Although in most of the cases the aetiology of ulcer is unknown, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism (Piper and Stiel, 1986). To regain the balance, different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production. The present study was undertaken to evaluate the anti-ulcerogenic effect of DHC-1, a herbal drug formulation consisting plants that are mentioned in Indian system of medicine (Ayurveda) for their remedial properties. The anti-ulcer effect of DHC-1 was tested against gastric lesions induced by pylorus-ligation and ethanol, the experimental models related to lesion pathogenesis with production of reactive species. DHC-1 prevented the mucosal lesions induced by pylorus-ligation and ethanol. DHC-1 was also found to increase the pH and decrease the acid volume and total acidity of gastric fluid. These effects of DHC-1 treatment on the parameters that influence the initiation and induction of ulceration may be considered as highly desirable property of anti-ulcerogenic agent.

Reactive oxygen species are involved in the pathogenesis of pylorus ligation-induced (Rastogi et al., 1998) and ethanol-induced (Pihan et al., 1987) gastric mucosal injury in vivo. Results in the present study also indicate similar alterations in the antioxidant status after pylorus ligation- and ethanol-induced ulcers. Preventive antioxidants, such as superoxide dismutase and catalase enzymes are the first line of defense against reactive oxygen species. Reduced glutathione is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation (Halliwell, 1995). Administration of DHC-1 resulted in a significant increase in the SOD, catalase, and reduced glutathione levels as compared to the control animals, which suggests its efficacy in preventing free radical-induced damage.

Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids which eventually results in destruction of membrane lipids. Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore, it is not surprising that membrane lipids are susceptible to peroxidative attack (Cheesman, 1993). The study has revealed a significant decrease in lipid peroxidation by DHC-1 in both the experimental models, which suggests its protective effect.

Na^+K^+ATPase, Ca^{2+}ATPase, and Mg^{2+}ATPase are membrane bound enzymes. The drug significantly increased the activity of all the ATPases in both the models.

It is thus concluded that DHC-1 is an effective anti-ulcer agent. Further, this study also proves that the anti-ulcer effect may be due to its antioxidant mechanism of action.

Acknowledgements

We are thankful to the Himalaya Drug Company, Bangalore and UGC for providing financial assistance for successfully carrying out this work.

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TRABAJOS ORIGINALES
ORIGINAL WORKS

Actividad antioxidante de activit, una formulación herbomineral, en daños renales y cardiacos inducidos experimentalmente

Antioxidant activity of activit, a herbomineral formulation, in experimentally induced cardiac and renal damage

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RESUMEN

Se administró Activit, una formulación herbomineral, a ratas por vía oral en dosis de 125, 250, 500 y 1000 mg kg^{-1} para investigar sus efectos en infartos de miocardio inducidos mediante isoproterenol y daños renales inducidos mediante cisplatina. El fármaco redujo los niveles de glutamato oxaloacetato transaminasa (GOT), lactato dehidrogenasa (LDH), ácido úrico y de creatina kinasa (CK)en el suero en casos de daño cardíaco inducido mediante isoproterenol. En los casos de daño renal inducido mediante cisplatina, Activit redujo los niveles séricos de creatinina, urea, nitrógeno ureico en sangre (NUS) y ácido úrico. Se descubrió además que la administración de Activit aumentó los niveles de superóxido dismutasa (SOD), catalasa (CAT); glutatión reducido (GSH) y enzimas ligadas a la membrana tales como la Ca^{2+}ATPasa y $Na^{+}K^{+}$ATPasa, y redujo la peroxidación lipídica (MDA) en el riñón y en el corazón en los casos de daño renal inducidos mediante cisplatina y en los de necrosis miocárdica inducida mediante isoproterenol, respectivamente. Por tanto, se puede concluir que Activit posee actividad antioxidante y que, en virtud de esa acción, puede proteger el corazón y el riñón de los daños causados por el isoproterenol y la cisplatina, respectivamente.
PALABRAS CLAVE Antioxidante Catalasa. Cisplatina Isoproterenol Peroxidación lipídica Glutatión reducido Superóxido dismutasa

ABSTRACT

Activit, a herbomineral formulation, was administered orally to rats at the dose levels of 125, 250, 500 and 1000 mg kg^{-1} to investigate its effect on isoproterenol-induced myocardial infarction and cisplatin-induced renal damage. The drug reduced the levels of serum creatine kinase (CK), glutamic oxaloacetate transaminase (GOT), lactate dehydrogenase (LDH) and uric acid in isoproterenol-induced cardiac damage. In cisplatin-induced renal damage, Activit reduced the serum levels of creatinine, urea, blood urea nitrogen (BUN) and uric acid. It was further found that administration of Activit increased the levels of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and membrane bound enzymes like Ca^{2+}ATPase, Mg^{2+}ATPase and $Na^{+}K^{+}$ATPase and decreased lipid peroxidation (MDA) in heart and kidney in isoproterenol-induced myocardial necrosis and cisplatin-induced renal damage, respectively. Thus it can be concluded that Activit possesses antioxidant activity and by virtue of this action it can protect the heart and kidney from damage caused by isoproterenol and cisplatin, respectively.
KEY WORDS: Antioxidant. Catalase Cisplatin Isoproterenol Lipid peroxidation. Reduced glutathione Superoxide dismutase

Aebi, tal y como lo describen Colowick et al.[17] El glutatión reducido se determinó mediante el método de Moran et al.[18] La peroxidación lipídica o la formación de malonil-dialdehído se determinó mediante el método de Slater y Sawyer[19]. Las enzimas ligadas a la membrana, concretamente la Na^+K^+ATPasa, la Ca^{2+}ATPasa y la Mg^{2+}ATPasa se estudiaron de acuerdo con los métodos de Bonting[20], Hjerten y Pan[21] y Ohinishi et al.[22], respectivamente. El fósforo inorgánico se determinó mediante el método de Fiske y Subbarow[23]. Las proteínas totales se determinaron mediante el método de Lowry et al.[24]

Análisis estadístico

Los resultados de las estimaciones indicadas anteriormente se han expresado de acuerdo con el promedio ± SEM. Las diferencias entre los grupos se determinaron estadísticamente mediante un análisis de varianza seguido por el test de comparaciones múltiples de Tukey-Kramer, con un nivel de significación establecido en $p < 0.05$

RESULTADOS

Infarto de miocardio inducido mediante isoproterenol

El tratamiento con isoproterenol (grupo II) provocó un aumento significativo de la actividad de GOT, LDH y CK séricas ($p<0,001$) y de los niveles de ácido úrico ($p<0,01$) en comparación con el grupo de control (grupo I) La administración de Activit provocó una disminución significativa de la actividad de GOT, LDH y CK séricas y del nivel de ácido úrico (Tabla I).

En el corazón, el isoproterenol hizo disminuir significativamente los niveles de SOD ($p<0,05$) y de catalasa ($p<0,001$) en el grupo II en comparación con el grupo I. También se observó un aumento significativo de la peroxidación lipídica ($p<0,01$) en el grupo II en comparación con el grupo I. Se observó que Activit había reducido significativamente la peroxidación lipídica (contenido de MDA) y había aumentado el nivel de glutatión y la actividad de la SOD y la catalasa en comparación con el grupo II (Tabla II).

RESULTS

Isoproterenol-induced myocardial infarction

The treatment with isoproterenol (group II) resulted in a significant elevation in serum CK, LDH and GOT activity ($p<0.001$) and uric acid levels ($p<0.01$) as compared to control (group I). The administration of Activit resulted in significant decrease in the activities of serum CK, LDH and GOT and level of uric acid (Table I).

In heart, isoproterenol significantly decreased the activities of SOD ($p<0.05$) and catalase ($p<0.001$) in group II as compared to group I. A significant ($p<0$ 01) increase in lipid peroxidation was also observed in group II as compared to group I. Activit was found to significantly decrease lipid peroxidation (MDA content) and increase the glutathione level and activities of SOD and catalase as compared to group II (Table II).

Indian Journal of Experimental Biology
Vol. 42, July 2004, pp. 674-680

Anti-ulcer and antioxidant activity of Normacid, a herbomineral formulation

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Received 19 November 2003; revised 5 May 2004

Effect of various doses (125, 250, 500 and 1000 mg/kg, po) of Normacid was studied on gastric secretion and gastric ulcers in pylorus-ligation and on ethanol-induced gastric mucosal injury in rats. The reduction in ulcer index in both the models along with the reduction in total acidity and an increase in the *p*H of gastric fluid in pylorus-ligated rats proved the anti-ulcer activity of Normacid. The increase in the levels of superoxide dismutase, catalase, reduced glutathione and membrane bound enzymes like Ca^{2+}ATPase, Mg^{2+}ATPase and $Na^{+}K^{+}$ATPase and decrease in lipid peroxidation in both the models showed the antioxidant activity of the formulation. Thus it can be concluded that the anti-ulcer activity shown by Normacid may be due to the modulation of defensive factors by improvement in gastric cytoprotection and partly due to antioxidant property.

Keywords: Anti-ulcer, Antioxidant, Lipid peroxidation, Superoxide dismutase, Catalase, Reduced glutathione
IPC Code: Int.Cl.[7] A61P

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side-effects (arrythmias, impotence, gynaecomastia and haematopoeitic changes) of modern medicine[1], indigenous drugs possessing fewer side-effects should be looked for as a better alternative for the treatment of peptic ulcer.

There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer[2]. Studies have shown alterations in the antioxidant status following ulceration, indicating that free radicals seem to be associated with the pylorus ligation-induced[3] and ethanol-induced[4,5] ulceration in rats. Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way of minimizing tissue injury in human disease[6].

Many indigenous drugs are known to possess anti-ulcer activity. The anti-ulcer property of *Solanum nigrum*[7] and Shankh bhasma[8] has been mentioned. Bhunimbadi Kwath, Mouktika bhasma and Kapardi bhasma are well-known for their antacid property and used in acid peptic disorders[9]. The antioxidant properties of *Solanum nigrum*[10], Swarnabhasma[11,12] and Shankh bhasma[8] were earlier investigated and were found to possess free radical scavenging properties. Some of the ingredients were also found to produce significant increase in the levels of various endogenous antioxidant enzymes.

The present study has been aimed to investigate the anti-ulcer effects of Normacid along with its effect on the anti-oxidant enzymes to justify whether the formulation exerts an anti-ulcer action by means of its antioxidant activity.

Materials and Methods

Composition—Each gram of Normacid (manufactured by Ayur Herbals Pvt. Ltd., Baroda, India) contains Bhunimbadi Kwath (500 mg), *Solanum nigrum* (200 mg), Mouktika bhasma (60 mg), Shuddha Gairika bhasma (60 mg), Kapardi bhasma (120 mg), Swarnabhasma (20 mg), Praval bhasma (30 mg) and Shankh bhasma (10 mg).

Animals—Female albino rats of Wistar strain weighing between 150-225 g were used in the study. The animals were housed in an air-conditioned room at 23°± 1°C. They were fed *ad libitum* with standard pellet diet and had free access to water. Animal experiments were approved by the Social Justice and Empowerment Committee for the purpose of control and supervision of experiments on animals, Ministry of Government of India, New Delhi.

Experimental procedure—The animals were divided into five groups of six rats each. Group I

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represented the control group, which received 5 ml/kg body weight of vehicle (1% gum acacia, po). Groups 2 to 5 received Normacid orally at the doses of 125, 250, 500 and 1000 mg/kg body weight, respectively. Group 6 received the standard drug, cimetidine orally at the dose of 50 mg/kg body weight.

Study of anti-ulcer activity using pylorus ligation — The method of Shay rat ulcer[13] was adopted. Rats were fasted for 48 hr. The drug, Normacid or cimetidine was administered to the animals. During the course of the experiment food was withdrawn. After the pretreatment period of 1 hr, the animals were anaesthetised with anaesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process; pylorus portion of stomach was slightly lifted out and ligated. Precaution was taken to avoid traction to the pylorus or damage to its blood supply. The stomach was placed carefully in the abdomen and the wound was sutured by interrupted sutures. After 19 hr of pylorus ligation the rats were sacrificed and the stomach was removed. The gastric content was collected and centrifuged. The volume, *p*H and total acidity of gastric fluid was determined. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass and the diameter of the ulcers was measured using a vernier caliper. Ulcer index was determined by following the scoring method of Suzuki *et al.*[14]

Score 1: maximal diameter of 1mm
Score 2: maximal diameter of 1-2mm
Score 3: maximal diameter of 2-3mm
Score 4: maximal diameter of 3-4mm
Score 5: maximal diameter of 4-5mm
Score 10: an ulcer over 5mm in diameter
Score 25: a perforated ulcer

Study of anti-ulcer activity using ethanol-induced ulcer method—The method described by Dhuley[15] was adopted. Normacid or cimetidine was administered orally to the rats for 10 days. On the 10th day, 1 hr after the final dose of the drug, 96% ethanol (5 ml/kg, po) was administered to the overnight fasted rats of all groups. The animals were then sacrificed 1 hr after the dose of ulcerogen. The stomach was removed, incised along the greater curvature and its mucosal erosion was determined randomly by measuring the area of the lesions. The sum of the areas was expressed as ulcer index (mm^2).

Study of anti-oxidant activity of Normacid—The stomach of rats of Group 1 (control) and Groups 2 to 5 (Normacid-treated groups) was then weighed and homogenized in chilled Tris buffer (10 m*M*, *p*H 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000×g at 0°C for 20 minutes using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assays of lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione (GSH). The sediment was resuspended in ice cold Tris buffer (10 m*M*, *p*H 7.4) to get a final concentration of 10% and was used for the estimation of different membrane bound enzymes (Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase) and proteins.

Superoxide dismutase (SOD)[16], catalase[17], reduced glutathione[18], lipid peroxidation or malondialdehyde formation[19], inorganic phosphorus[20] and total proteins[21] were determined. Membrane bound enzymes namely, Na^+K^+ATPase[22], Ca^{2+}ATPase[23] and Mg^{2+}ATPase[24] were assayed.

Statistical analysis—Results were presented as mean ± SE. Difference between the groups (Normacid and control groups) was statistically determined by analysis of variance followed by Tukey-Kramer Multiple Comparisons test, with the level of significance set at $P<0.05$. Ranitidine group was compared with the control group by using unpaired Student's *t*-test.

Results

Study of anti-ulcer activity using pylorus ligation method—In the vehicle treated control group the ulcer index was 92.75 ± 10.40 and the maximum number of ulcers were of the ulcer score 4 and 5. In the rats of this group a number of perforated ulcers (score 25) were also observed.

Normacid was found to produce a significant ($P<0.001$) decrease in ulcer index at the doses of 500 and 1000 mg/kg; the percentage reduction being 66.31 and 80.59%, respectively. All the ulcers were of score 1 and 2 and no perforated ulcers were observed. The formulation at the same two doses significantly ($P<0.001$) increased the *p*H of the gastric fluid from 1.20±0.07 to 2.78±0.17 and 2.83±0.18, respectively. It also significantly reduced the total acidity at all the four doses, but however did not affect the volume of gastric fluid (Table 1).

Cimetidine (50 mg/kg) was found to produce a significant reduction in ulcer index, the percentage reduction being 58.13%. It also reduced the total acidity and increased the volume of gastric fluid

significantly (Table 2) and increased the *p*H to 3.73 ± 0.26.

Study of anti-ulcer activity using ethanol-induced ulcer method—Administration of ethanol produced significant ulcers (287.98 ± 17.79) in the control group. There was a significant reduction in ulcer index at all the four doses of Normacid (Table 3). Cimetidine (50 mg/kg) produced a percentage reduction of 78.75% in ulcer index (Table 2).

Study of anti-oxidant activity of Normacid—Pylorus-ligation was found to increase lipid peroxidation and decrease SOD, catalase and reduced glutathione in the control group, thus leading to oxidative stress. Administration of Normacid brought about a significant reduction in lipid peroxidation and an increase in the level of reduced glutathione as compared to control. An increase in the activities of antioxidant enzymes namely, SOD and catalase along with the enhancement in the membrane bound ATPases was also observed in the drug treated groups (Table 4).

Ethanol administration was found to increase lipid peroxidation and decrease SOD, catalase and reduced glutathione in the control group when compared to normal rats. Administration of Normacid significantly decreased lipid peroxidation and increased the levels of SOD, catalase, reduced glutathione and all membrane bound ATPases (Table 3).

Table 1—Effect of Normacid on various gastric parameters of pylorus-ligated rats
[Values are mean ± SE. Figures in parentheses are % reduction in ulcer index]

Groups	Ulcer Index	Volume of gastric fluid (ml/100g)	Total acidity (mEq/l/100g)
Control	92.75±10.40	9.22±0.63	127.0±3.63
Normacid (125mg/kg)	86.50±5.14[a,s] (6.74)	8.56±0.36[NS]	103.75±7.17[a]
Normacid (250mg/kg)	77.75±3.66[NS] (16.17)	9.81±0.13[NS]	89.00±6.19[b]
Normacid (500mg/kg)	31.25±4.25[b] (66.31)	8.72±1.11[NS]	75.75±4.15[b]
Normacid (1000mg/kg)	18.00±2.94[b] (80.59)	6.95±0.84[NS]	47.5±2.78[b]
F value	62.399	2.169	34.786
P value	<0.0001	<0.001	<0.0001

Normacid treated groups were compared with control group
P values: [a]<0.05; [b]<0.001; [NS]non-significant

Table 2—Effect of cimetidine on various gastric parameters of pylorus-ligated and ethanol-treated rats
[Values are mean ± SE. Figures in parentheses are % reduction in ulcer index]

Gastric Parameters	Control	Cimetidine (50mg/kg)
Ulcer Index		
Pylorus-ligated rats	92.75±5.20	38.83±5.36[a] (58.13)
Ethanol-treated rats	287.98±17.79	61.19±21.22[a] (78.75)
Volume of gastric fluid (ml/100g)	9.22±0.63	7.05±3.53[a]
Total acidity (mEq/l/100g)	127.01±3.63	63.56±8.56[a]

Cimetidine treated groups were compared with control group.
[a]*P* <0.001

Discussion

Although in most of the cases the aetiology of ulcer is unknown, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism[25]. To regain the balance, different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production. The anti-ulcer effect of Normacid was tested against gastric lesions induced by pylorus-ligation and ethanol, the experimental models related to lesion pathogenesis with production of reactive species. Normacid prevented the mucosal lesions induced by pylorus-ligation and ethanol. It also increased the *p*H and decreased the total acidity of gastric fluid. These effects of Normacid treatment on the parameters that influence the initiation and induction of ulceration may be considered as highly desirable property of anti-ulcerogenic agent. Normacid was found to be 10 and 5 times less potent than the standard drug, cimetidine in protecting against pylorus-ligation and ethanol-induced ulcers, respectively.

Reactive oxygen species are involved in the pathogenesis of pylorus ligation-induced[3] and ethanol-induced[4] gastric mucosal injury *in vivo*. Results of the present study also indicate similar alterations in the antioxidant status after pylorus ligation and ethanol induced ulcers. Preventive antioxidants, such as superoxide dismutase (SOD) and catalase (CAT) enzymes are the first line of defense against reactive oxygen species. Reduced glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation[26]. Administration of Normacid resulted in a significant increase in the SOD, catalase and reduced glutathione levels as compared to control animals, which suggests its efficacy in preventing free radical induced damage.

Table 3—Effect of Normacid on the ulcer index and biochemical parameters in stomach of ethanol-treated rats
[Values are mean ± SE. Figures in parenthesis are % reduction in ulcer index]

Groups	Ulcer Index	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/ mg protein)	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated/min/ mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/ mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/ mg protein)
Normal	-	3.45 ± 0.24	3.31 ± 0.13	5.64 ± 0.32	8.27 ± 0.22	5.29 ± 0.18	3.65 ± 0.30	3.52 ± 0.20
Control	287.98 ± 17.79	6.75 ± 0.72^c	0.79 ± 0.17^c	2 23 ± 0 14^c	5.12 ± 0 21^c	2.03 ± 0.11^c	1.50 ± 0.21^c	1.49 ± 0.13^c
Normacid (125 mg/kg)	85.20 ± 4.93^c (70.41)	6.40 ± 0.44^{NS}	1.14 ± 0.08^{NS}	2.24 ± 0.12^{NS}	5.02 ± 0.17^{NS}	2.13 ± 0.05^{NS}	1.75 ± 0.09^{NS}	1.91 ± 0.11^{NS}
Normacid (250 mg/kg)	56.83 ± 2.37^c (80.27)	5.79 ± 0.22^{NS}	2.72 ± 0.18^c	3.13 ± 0.25^{NS}	5.72 ± 0.27^{NS}	2.57 ± 0.08^a	2.18 ± 0.08^{NS}	2.39 ± 0.09^b
Normacid (500 mg/kg)	37.59 ± 3.61^c (86.95)	3.97 ± 0.29^c	3.21 ± 0.08^c	3.47 ± 0.13^b	6.94 ± 0.08^c	4.21 ± 0.10^c	2.46 ± 0.17^a	3.04 ± 0.11^c
Normacid (1000 mg/kg)	5.75 ± 1.11^c (98.00)	3.97 ± 0.08^c	3.96 ± 0.32^c	4.32 ± 0.16^c	7.23 ± 0.13^c	5.12 ± 0.11^c	3.01 ± 0.08^c	3.23 ± 0.12^c
F *value*	173.49	13.524	49.985	42.458	45.802	174.16	21.341	37.669
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Control group was compared with normal group. Normacid groups were compared with control group.
P values: ^{a}<0.05; ^{b}<0.01; ^{c}<0.001; ^{NS}non-significant

Table 4—Effect of Normacid on the biochemical parameters in stomach of pylorus ligated rats
(Values are mean ± SE)

Groups	Lipid Peroxidation (nmoles of MDA/ mg protein)	Reduced Glutathione (µg of GSH/ mg protein)	Superoxide Dismutase (Units/mg protein)	Catalase (µmoles of H_2O_2 consumed/ min/mg protein)	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated/min/ mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/ mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/ mg protein)
Normal	3.45 ± 0.24	3.31 ± 0.14	5.64 ± 0.32	8.27 ± 0.22	5.29 ± 0.18	3.65 ± 0.30	3.52 ± 0.20
Control	10.09 ± 0.44[c]	0.58 ± 0.14[c]	2.37 ± 0.26[c]	5.93 ± 0.60[b]	1.49 ± 0.10[c]	1.62 ± 0.17[c]	1.54 ± 0.13[c]
Normacid (125mg/kg)	5.38 ± 0.48[c]	1.43 ± 0.18[NS]	2.34 ± 0.26[NS]	5.73 ± 0.38[NS]	1.53 ± 0.10[NS]	2.59 ± 0.23[a]	2.42 ± 0.18[a]
Normacid (250mg/kg)	4.00 ± 0 17[c]	2.44 ± 0.19[c]	2.73 ± 0.10[NS]	6.52 ± 0.36[NS]	2.06 ± 0 12[NS]	2.65 ± 0.15[a]	2.75 ± 0.17[c]
Normacid (500mg/kg)	3.35 ± 0.43[c]	3.36 ± 0 20[c]	3.17 ± 0.08[NS]	6.93 ± 0.15[NS]	2.50 ± 0.40[NS]	3.06 ± 0.10[c]	3.17 ± 0.17[c]
Normacid (1000mg/kg)	2.89 ± 0.15[c]	4.45 ± 0.34[c]	4.55 ± 0.20[c]	8 23 ± 0.14[b]	3.64 ± 0.29[c]	3.56 ± 0.05[c]	3.52 ± 0.07[c]
F value	61.058	45.725	36.698	9.991	42.265	16.318	22.819
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Control group was compared with normal group. Normacid groups were compared with control group.
P values: [a]<0.05; [b]<0.01; [c]<0 001; [NS]non-significant

Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids which eventually results in destruction of membrane lipids. Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore, it is not surprising that membrane lipids are susceptible to peroxidative attack[27]. The study revealed a significant decrease in lipid peroxidation by Normacid in both the experimental models, which suggests its protective effect.

Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase are membrane bound enzymes. The drug significantly increased the activity of all the ATPases in both the models.

One of the constituents of Normacid, namely *Solanum nigrum*, has been reported to possess hepatoprotective effect due to its ability to suppress the oxidative degradation of DNA in the tissue debris[10]. Another constituent of Normacid, Swarnabhasma, has been used since ancient times in several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness and incidence of early aging. According to modern concept, the scientific basis for its application in degenerative diseases may arise, atleast in part, through enhancement in free radical concentrations. It was found to induce enhanced activity of SOD and catalase. Swarnabhasma was found to produce no signs of toxicity indicating that the drug can be used safely in oral route for atleast a few months[11]. Shah and Vohora have also reported the antioxidant effects of calcined gold preparations used in Indian systems of medicine against global and focal models of ischemia[12]. Pandit *et al.*[8] have suggested that Shankh bhasma may act as gastric cytoprotective agent by modulating scavenging of free radicals. It is thus apparent that the antioxidant effect of Normacid may be due to the antioxidant property of its constituents mentioned above.

On the basis of the present results and available reports, it can be concluded that the anti-ulcer activity elucidated by Normacid could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition and free radical scavenging activity.

Acknowledgement

Thanks are due to Ayur Herbals Pvt. Ltd., Baroda and UGC, New Delhi for financial assistance.

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Journal of Ethnopharmacology.

Antioxidant Activity of DHC-1 – A Herbal Formulation

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Abstract

DHC-1, a multiherbal formulation, was tested for its antioxidant activity in rats. DHC-1 was investigated at dose levels of 100 mg/kg, p.o. and 200 mg/kg, p.o., once daily, for 30 days in normal rats. The levels of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), lipid peroxidation, membrane bound enzymes like Ca^{2+}ATPase, Mg^{2+}ATPase, $Na^{+}K^{+}$ATPase, lipids like phospholipid, cholesterol, triglyceride and total proteins were estimated in liver, kidneys and heart. Liver Glucose-6-Phosphate-Dehydrogenase was also determined. The serum levels of GOT, GPT, alkaline phosphatase, lactate dehydrogenase and bilirubin were also estimated. The decrease in the serum enzymes may be due to the membrane stabilizing action of DHC-1. The inhibition of lipid peroxidation and enhancement of antioxidant enzymes (SOD and catalase) along with reduced glutathione by DHC-1 may be attributed to the antioxidant potential of various ingredients present in the formulation. Thus, it can be concluded that DHC-1 exhibits an antioxidant activity and could prove beneficial in the treatment of various disorders associated with the involvement of reactive oxygen species.

Key words: Antioxidant; Ca^{2+}ATPase; Mg^{2+}ATPase; $Na^{+}K^{+}$ATPase; DHC-1

1. Introduction

Free radical reactions have been implicated in the pathology of many human diseases including atherosclerosis, ischaemic heart disease, ageing process, inflammation, diabetes, immunodepression, neurodegenerative condition and other disease conditions (Maxwell, 1995). These free radicals, which are atoms or molecules with an unpaired electron, are capable of reversibly or irreversibly damaging compounds of all biochemical classes, including nucleic acids, proteins and free amino acids, lipids and lipoproteins, carbohydrates and connective tissue macromolecules (Hemnani and Parihar, 1998).

Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward in minimizing tissue injury in human disease (Barry, 1991). A number of plants have been reported to possess antioxidant effects. The antioxidant properties of *Bacopa monnieri* (Bhattacharya *et al.*, 2000; Tripathi *et al.*, 1996), *Emblica officinalis* (Bhattacharya *et al.*, 1999; Mathur *et al.*, 1996), *Glycyrrhiza glabra* (Hatano *et al.*, 1991), *Mangifera indica* (Ghosal *et al.*, 1996) and *Syzygium aromaticum* (Deans, *et al.*, 1995) were earlier investigated and were found to possess free radical scavenging property. Some of the ingredients were also found to produce significant induction in the levels

of various endogenous antioxidant enzymes.

The present study was aimed to investigate the effects of DHC-1, an herbal formulation, on the anti-oxidant enzymes and markers of free radical generation in normal rats.

2. Material and methods

2.1 Plant Materials

Bacopa monnieri, Emblica officinalis, Glycyrrhiza glabra, Mangifera indica and *Syzygium aromaticum* were procured from a local supplier and identified by Dr. Kannan, Ph.D., Botanist, The Himalaya Drug Company, Bangalore. Samples were retained for reference purpose at the R & D herbarium.

2.2 Composition

Each gram of DHC-1 contains:

Herbs

Bacopa monnieri Linn. (Scrophulariaceae) HDHB-157
Emblic officinalis Gaertn. (Euphorbiaceae) HDHB-143
Glycyrrhiza glabra Linn. (Papilionaceae)
Mangifera indica Linn. (Anacardiaceae)
Syzygium aromaticum Linn. (Myrtaceae)

2.3 Sources of Fine Chemicals

1,1,3,3-tetraethoxypropane, crystalline beef liver catalase and superoxide dismutase were obtained from Sigma Chemicals, St. Louis, M.O., U.S.A. Thiobarbituric acid, tris buffer, sucrose, ATP, reduced glutathione, 1-amino-2-naphthol-4-sulphonic acid, 5,5'-dithiobis (2-nitro benzoic acid) and bovine serum albumin were obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. Trichloroacetic acid, ammonium molybdate, citric acid monohydrate, sodium nitrate, sulphanilic acid, hydrogen peroxide, copper sulphate, sodium potassium tartarate, sodium metaperiodate, ethanol and Folin's phenol reagent were obtained from S.D. Fine Chemicals, Mumbai, India. Sodium hydroxide, sodium carbonate, sodium bicarbonate, magnesium chloride, sodium chloride, potassium chloride, calcium chloride, disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, chloroform, ether, hydrochloric acid and conc. sulphuric acid were purchased from Qualigens Chemicals Ltd., Mumbai, India. Ethylenediaminetetraacetic acid disodium salt and epinephrine bitartarate were obtained from BDH Chemicals, Mumbai, India.

2.4 Animals

Forty eight Wistar rats of either sex weighing between 150-225 gms were used for the study. During the course of the experiment the animal were fed with standard pellet diet *ad libitum* and had free access to water. Animal experiments were approved by the Social Justice and Empowerment Committee for the purpose of control and supervision of experiments on animals, Ministry of Government of India, New Delhi.

2.5 Experimental Protocol

The animals were divided into two groups of 24 each for the first 200 mg of experiment, the animals were divided into three groups of eight animals each, which are as follows:

Group 1: Represented control that received 5 ml/kg b.wt. of vehicle (1% CMC) orally for 30 days.

Group 2: Rats received the drug DHC-1 at a dose of 100 mg/kg b.wt. as an aqueous suspension, once daily, orally for 30 days.

Group 3: Rats received DHC-1 at a dose of 200 mg/kg b.wt., once daily, orally for 30 days.

After completion of 30 days of drug administration the rats were anaesthetized with ether and blood was collected from the orbital plexus. Serum was separated and kept at 4°C until use. The animals

were then sacrificed and organs such as liver, kidneys and heart were removed, processed and homogenized in Tris buffer (10 mM, pH 7.4) at a concentration of 10% w/v.

The homogenates were centrifuged at 10000 X g at 4°C for 20 minutes using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assays of lipid peroxidation, endogenous antioxidant enzymes and G-6-P-D. The sediment was resuspended in ice cold Tris buffer (10 mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of different membrane bound enzymes and proteins.

2.5.1 Tissue estimations

2.5.1.1 Determination of Lipid Peroxidation (MDA content)

It was estimated using the method described by Slater and Sawyer (1971). 2.0ml of the tissue homogenate (supernatant) was added to 2.0ml of freshly prepared 10% w/v trichloroacetic acid (TCA) and the mixture was allowed to stand in an ice bath for 15 minutes. After 15 minutes, the precipitate was separated by centrifugation and 2.0ml of clear supernatant solution was mixed with 2.0ml of freshly prepared 0.67% thiobarbituric acid (TBA). The resulting solution was heated in a boiling water bath for 10 minutes. It was then immediately cooled in an ice bath for 5 minutes. The colour developed was measured at 532nm against reagent blank. Different concentrations (0-23nM) of standard malondialdehyde were taken and processed as above for standard graph. The values are expressed as nM of MDA/mg protein.

2.5.1.2 Determination of Superoxide Dismutase (SOD)

Superoxide dismutase was estimated using the method developed by Misra and Fridovich (1972). 0.5ml of tissue homogenate was diluted with 0.5ml of distilled water, to which 0.25ml of ice-cold ethanol and 0.15ml of ice-cold chloroform were added. The mixture was mixed well using cyclo mixer for 5 minutes and centrifuged at 2500 rpm. To 0.5ml of supernatant, 1.5ml of carbonate buffer (0.05M, pH 10.2) and 0.5ml of EDTA solution (0.49M) were added. The reaction was initiated by the addition of 0.4ml of epinephrine (3mM) and the change in optical density/minute was measured at 480nm against reagent blank. SOD activity was expressed as units/mg protein. Change in optical density per minute at 50% inhibition of epinephrine to adrenochrome transition by the enzyme is taken as the enzyme unit. Calibration curve was prepared by using 10-125 units of SOD.

2.5.1.3 Determination of Catalase (CAT)

Catalase was estimated by the method of Hugo Aebi as given by Colowick *et al* (1984). To 2ml of diluted sample 1ml of hydrogen peroxide (30 mmol/l) was added to initiate the reaction. The blank was prepared by mixing 2ml of diluted sample (similar dilution) with 1ml of phosphate buffer (50mmol/l; pH 7.0). The dilution should be such that the initial absorbance should be approximately 0.500. The decrease in absorbance was measured at 240nm. Catalase activity was expressed as μmoles of H_2O_2 consumed/min/mg protein.

2.5.1.4 Determination of Reduced glutathione

Reduced glutathione was determined by the method of Moron *et al* (1979). Equal volumes of tissue homogenate (supernatant) and 20% trichloroacetic acid were mixed. The precipitated fraction was centrifuged and to 0.25ml of supernatant, 2ml of 0.6mM 5,5'-dithiobis (2-nitro benzoic acid) reagent was added. The final volume was made upto 3ml with phosphate buffer (0.2M, pH 8.0). The colour developed was read at 412nm against reagent blank. Different concentrations (10-50μg) of standard glutathione were taken and

processed as above for standard graph. The amount of reduced glutathione was expressed as μg of GSH/mg protein.

2.5.1.5 Determination of Membrane Bound Enzymes and Inorganic Phosphorus

Membrane bound enzymes namely Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase were assayed according to the methods of Bonting (1970), Hjerken and Pan (1983) and Ohinishi *et al.* (1982) respectively. The inorganic phosphorus was estimated by the method of Fiske and Subbarow (1925).

Total proteins were determined by the method of Lowry *et al* (1951). Liver G-6-P-D was estimated by the method of Gowenlock *et al* (1988a).

2.5.2 Serum estimations

SGPT and SGOT levels were estimated by Reitman and Frankel (1957) method. Serum alkaline phosphatase was determined by the method devised by King (1965). Total bilirubin estimation was done using the method of Jendrassik and Grof as mentioned by Shull *et al* (1980). Lactate Dehydrogenase activity was estimated by the method of Gowenlock (1988b).

2.5.3 Lipid estimations

In the second set of experiment, the animals were divided into three groups as previously mentioned and were given similar treatment for 30 days. After 30 days of drug administration the animals were sacrificed and organs such as liver, kidneys and heart were removed. Lipids were extracted from these tissues by the method of Folch *et al* (1955). Triglyceride was estimated by the method of Foster and Dunn (1973). Phospholipid and cholesterol were determined by the method of Stewart (1980) and Zlatkis *et al* (1953) respectively.

2.6 Statistical analysis

Results of all the above estimations have been indicated in terms of mean ± SEM. The difference between means was analyzed by Student's '*t*' test. Minimum level of significance was fixed at $p \leq 0.05$.

3. Results

3.1 Effect of DHC-1 on tissue parameters

Administration of the drug DHC-1 (200 mg/kg) rats resulted in a significant ($p<0.05$) increase in superoxide dismutase and catalase enzymes in liver. It also significantly ($p<0.05$) elevated the levels of reduced glutathione in liver. The drug produced a decrease in the level of lipid peroxidation in all organs but a significant ($p<0.01$) decrease was produced only in the heart. Amongst the three membranes bound enzymes, the drug DHC-1 (200 mg/kg) produced a significant ($p<0.01$) increase in the level of Ca^{2+}ATPase in liver. The drug (200 mg/kg) also significantly ($p<0.05$) increased the protein content of liver, heart and kidneys (Tables 1a-1c).

3.2 Effect of DHC-1 on serum parameters

The drug (200 mg/kg) significantly ($p<0.05$) reduced the levels of SGPT, SGOT and alkaline phosphatase. The drug also significantly ($p<0.01$) reduced the levels of bilirubin at both the doses. However, there was no significant change produced in the levels of lactate dehydrogenase enzyme (Table 2).

3.3 Effect of DHC-1 on lipids

The drug (100 mg/kg and 200 mg/kg) significantly ($p<0.05$ and $p<0.01$) reduced the level of triglyceride in heart. However, the levels of cholesterol and phospholipid were not much affected by the drug (Table 3).

There was no significant difference in body weight gain between the three different groups.

3.4 Chemical analysis

Chemical analysis of DHC-1 is also proving quality of DHC-1 (Figures 1-6).

4. Discussion and Conclusions

Superoxide dismutase (SOD) and catalase (CAT) enzymes are the important endogenous antioxidant enzymes. Superoxide dismutases superoxide radical to form hydrogen peroxide and water. At high concentrations, hydrogen peroxide is removed by the enzyme catalase whereas at lower concentrations it is removed by reacting with reduced glutathione (Hemnani and Parihar, 1998). Glutathione is also an important inhibitor of free radical mediated lipid peroxidation (Meistor, 1983). DHC-1 (200 mg/kg) resulted in a significant increase in the liver SOD, catalase and reduced glutathione levels as compared to the control animals, which suggests its antioxidant activity. DHC-1 has also been reported to exhibit antioxidant activity in ulcerated animals using pylorus-ligation and ethanol-induced ulcer models, the experimental models related to lesion pathogenesis with production of reactive oxygen species (Bafna and Balaraman, 2004).

Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids that eventually results in destruction of membrane lipids. Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore, it is not surprising that membrane lipids are susceptible to peroxidative attack (Cheesman, 1993). The study revealed a significant decrease in lipid peroxidation in heart, which suggests its protective effect against myocardial necrotic changes.

Glucose 6-phosphate dehydrogenase (G-6-P-D) is one of the several hepatic carbohydrate metabolizing enzymes, which is important in providing reduced nicotinamide adenine dinucleotide phosphate (NADPH) for lipogenesis (Fitch and Chaikoff, 1960; Rohnstad and Katz, 1966). The activity of this enzyme is correlated with the rate of lipogenesis. Changes in the activity of this enzyme therefore may affect the utilization of ingested carbohydrates or the process of lipogenesis.

$Na^{+}K^{+}$ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase are membrane bound enzymes. Depending on the alteration in lipid composition; the fluidity and thus the activities of these enzymes vary (Floreani *et al.*, 1981). Increased enzyme activity was reported with decreased phospholipid activity. A change in membrane cholesterol content affects its fluidity (Kumari *et al.*, 1990). The drug increased the activity of Ca^{2+}ATPase, which may be due to the reduction in lipid content.

Serum transaminases such as GOT and GPT are liberated into the serum after extensive tissue damage. The heart muscle is rich in SGOT whereas liver is rich in both the enzymes. Increased levels are thus indicators of myocardial ischemia and liver damage (Reitman and Frankel, 1957). The reduction in these enzymes by the drug may be due to the prevention of release of these enzymes by virtue of its membrane stabilizing activity.

Serum alkaline phosphatase estimations are of interest in the diagnosis of hepatobiliary diseases and bone diseases associated with increased osteoblastic activity (King, 1965). The drug significantly reduced the levels of this enzyme at the higher dose (200 mg/kg). The drug was also found to significantly ($p<0.01$) reduce serum bilirubin as compared to normal rats. This shows the protective effect of the drug in liver.

Elevated levels of cholesterol and triglycerides are associated with atherosclerosis, nephrosis, diabetes

mellitus, obstructive jaundice and myxedema. Hypertriglyceridemia is associated with metabolic consequences of hypercoagulability, hyperinsulinaemia, insulin resistance and glucose tolerance (Godkar, 1996). Higher the lipid higher is the risk for oxidative damage. The drug DHC-1 (200 mg/kg) showed decrease in triglyceride levels.

With all these findings it can be concluded that DHC-1 exhibits an antioxidant activity by inhibition of lipid peroxidation and enhancement of antioxidant enzymes and reduced glutathione and thus can be used in the treatment of various disorders where reactive oxygen species are involved.

Acknowledgements

We are thankful to The Himalaya Drug Company, Bangalore, India, for providing financial assistance and the drug, DHC-1 for successfully carrying out this work.

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Table 1a
Effect of DHC-1 on the levels of tissue parameters in liver.

Tissue parameters	Group		
	Control	DHC-1 (100 mg/kg)	DHC-1 (200 mg/kg)
SOD (Unit/min/mg protein)	2.66 ± 0.63	2.78 ± 0.76	3.45 ± 0.88*
Catalase (μmoles of H_2O_2 consumed/min/mg protein)	404.25 ± 9.89	463.01 ± 11.37	505.23 ± 13.35*
Reduced Glutathione (μg of GSH/mg protein)	2.02 ± 0.09	2.09 ± 0.08	3.06 ± 0.05*
Lipid peroxidation (mmoles of MDA/min/mg protein)	52.59 ± 3.46	51.42 ± 2.93	48.00 ± 3.13
Ca^{2+}ATPase (μmoles of inorganic phosphorus liberated/mg protein)	3.15 ± 0.12	3.32 ± 0.05	4.10 ± 0.02#
Mg^{2+}ATPase (μmoles of inorganic phosphorus liberated/mg protein)	2.34 ± 0.10	2.36 ± 0.06	2.56 ± 0.23
$Na^{+}K^{+}$ATPase (μmoles of inorganic phosphorus liberated/mg protein)	4.75 ± 0.26	4.96 ± 0.11	5.27 ± 0.13
Liver G-6-P-D (U/mg protein)	5.15 ± 0.64	4.85 ± 0.42	4.60 ± 0.54
Proteins (mg/gm of tissue)	123.20 ± 3.09	129.58 ± 3.66	134.48 ± 2.87*

[a] Values are given as mean ± SEM for groups of eight animals each.
Experimental groups were compared with the corresponding values of Control group.
*$p<0.05$; # $p<0.01$

Table 1b
Effect of DHC-1 on the levels of tissue parameters in kidneys.

Tissue parameters	Group		
	Control	DHC-1 (100 mg/kg)	DHC-1 (200 mg/kg)
SOD (Unit/min/mg protein)	2.59 ± 0.36	2.63 ± 0.99	2.77 ± 0.66
Catalase (μmoles of H_2O_2 consumed/min/mg protein)	244.77 ± 6.23	253.23 ± 25.88	258.24 ± 18.22
Reduced Glutathione (μg of GSH/mg protein)	1.97 ± 0.14	1.98 ± 0.11	2.05 ± 0.11
Lipid peroxidation (mmoles of MDA/min/mg protein)	55.91 ± 2.41	53.24 ± 2.95	46.88 ± 4.75
Ca^{2+}ATPase (μmoles of inorganic phosphorus liberated/mg protein)	4.55 ± 0.20	4.62 ± 0.17	4.77 ± 0.26
Mg^{2+}ATPase (μmoles of inorganic phosphorus liberated/mg protein)	3.63 ± 0.06	3.82 ± 0.16	3.97 ± 0.17
$Na^{+}K^{+}$ATPase (μmoles of inorganic phosphorus liberated/mg protein)	10.21 ± 0.31	10.31 ± 0.43	10.47 ± 0.33
Proteins (mg/gm of tissue)	72.82 ± 0.16	77.68 ± 1.44	84.45 ± 1.45*

[a] Values are given as mean ± SEM for groups of eight animals each.
Experimental groups were compared with the corresponding values of Control group.
*$p<0.05$; #$p<0.01$

Table 1c
Effect of DHC-1 on the levels of tissue parameters in heart.

Tissue parameters	Group		
	Control	DHC-1 (100 mg/kg)	DHC-1 (200 mg/kg)
SOD (Unit/min/mg protein)	2.23 ± 0.72	2.36 ± 0.98	2.53 ± 0.22
Catalase (μmoles of H_2O_2 consumed/min/mg protein)	46.91 ± 5.79	53.91 ± 5.09	64.37 ± 12.53
Reduced Glutathione (μg of GSH/mg protein)	1.05 ± 0.07	1.09 ± 0.08	1.14 ± 0.15
Lipid peroxidation (mmoles of MDA/min/mg protein)	33.47 ± 2.56	25.41 ± 2.82	17.49 ± 1.62$^{\#}$
Ca^{2+}ATPase (μmoles of inorganic phosphorus liberated/mg protein)	2.57 ± 0.19	2.67 ± 0.19	2.73 ± 0.16
Mg^{2+}ATPase (μmoles of inorganic phosphorus liberated/mg protein)	2.38 ± 0.12	2.53 ± 0.20	2.72 ± 0.12
$Na^{+}K^{+}$ATPase (μmoles of inorganic phosphorus liberated/mg protein)	4.85 ± 0.20	5.10 ± 0.45	5.10 ± 0.13
Proteins (mg/gm of tissue)	123.76 ± 0.27	138.45 ± 8.18	241.22 ± 10.95*

[a] Values are given as mean ± SEM for groups of eight animals each.
Experimental groups were compared with the corresponding values of Control group.
$^{*}p<0.05$; $^{\#}p<0.01$

Table 2
Effect of DHC-1 on the levels of serum parameters of control and experimental animals[a]

Parameters	Treatment		
	Control	DHC-1 (100 mg/kg)	DHC-1 (200 mg/kg)
SGPT (U/ml)	21.33 ± 1.12	17.67 ± 1.15	16.33 ± 0.73*
SGOT (U/ml)	66.33 ± 2.09	60.50 ± 1.10	56.00 ± 2.93*
Alkaline phosphatase (IU/l)	135.03 ± 11.22	108.74 ± 4.81	87.92 ± 5.33*
LDH (IU/L)	305.75 ± 21.64	255.75 ± 9.54	231.50 ± 5.54
Bilirubin (mg/dl)	0.38 ± 0.03	0.23 ± 0.01#	0.16 ± 0.02#

[a] Values are given as mean ± SEM for groups of eight animals each.
Experimental groups were compared with the corresponding values of Control group.
*$p<0.05$ and #$p<0.01$.

Table 3
Effect of DHC-1 on the levels of lipid parameters in various organs of control and experimental animals[a]

Parameters	Organs		
	Liver	Kidneys	Heart
Control			
Phospholipid (mg/gm of tissue)	5.90 ± 0.05	5.86 ± 0.03	5.19 ± 0.04
Triglyceride (mg/gm of tissue)	7.18 ± 0.16	6.08 ±0.21	12.93 ± 0.12
Cholesterol (mg/gm of tissue)	4.44 ± 0.44	6.83 ± 0.24	1.10 ± 0.07
DHC-1 (100 mg/kg)			
Phospholipid (mg/gm of tissue)	5.88 ± 0.03	5.76 ± 0.05	5.35 ± 0.15
Triglyceride (mg/gm of tissue)	5.15 ± 0.07	8.25 ± 0.13	3.39 ± 0.52*
Cholesterol (mg/gm of tissue)	3.02 ± 0.24	5.76 ± 0.33	1.07 ± 0.04
DHC-1 (200 mg/kg)			
Phospholipid (mg/gm of tissue)	5.88 ± 0.01	5.75 ± 0.04	5.53 ± 0.04
Triglyceride (mg/gm of tissue)	4.40 ± 0.20	10.76 ±0.21	1.58 ± 0.05#
Cholesterol (mg/gm of tissue)	2.88 ± 0.41	4.71 ± 0.21	0.90 ± 0.03

[a] Values are given as mean ± SEM for groups of eight animals each.
Experimental groups were compared with the corresponding values of Control group.
*$p<0.05$; #$p<0.01$

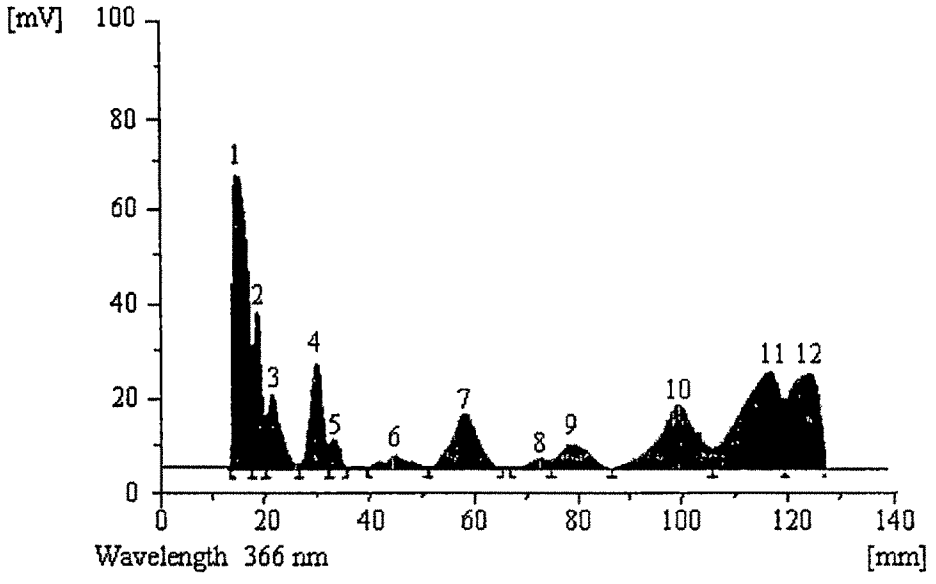

Figure 1. High performance thin layer chromatogram fingerprint of *Bacopa monnieri*.

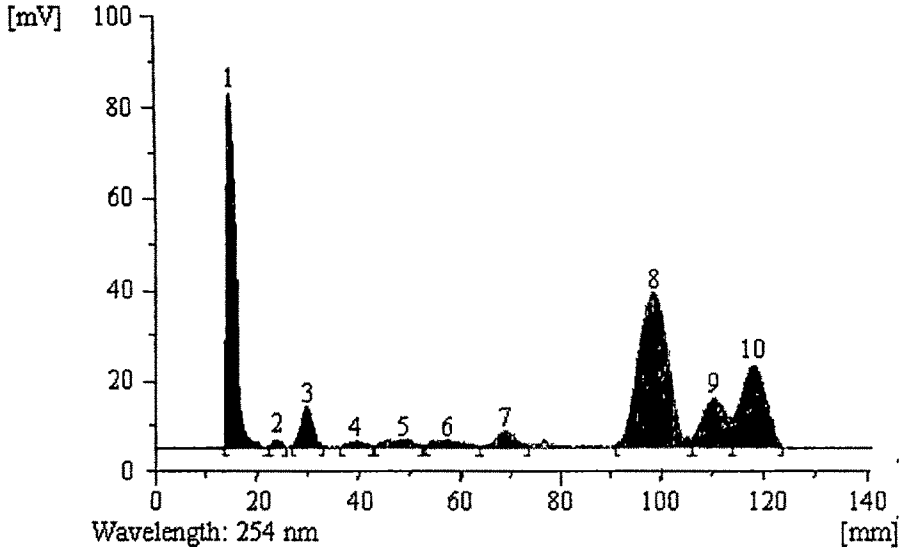

Figure 2. High performance thin layer chromatogram fingerprint of *Emblica officinalis*.

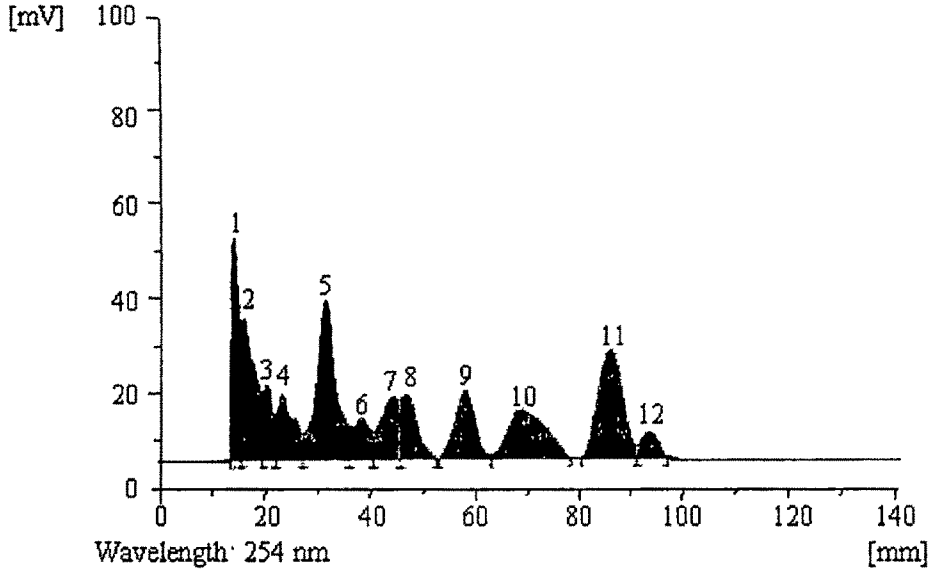

Figure 3. High performance thin layer chromatogram fingerprint of *Glycyrrhiza glabra.*

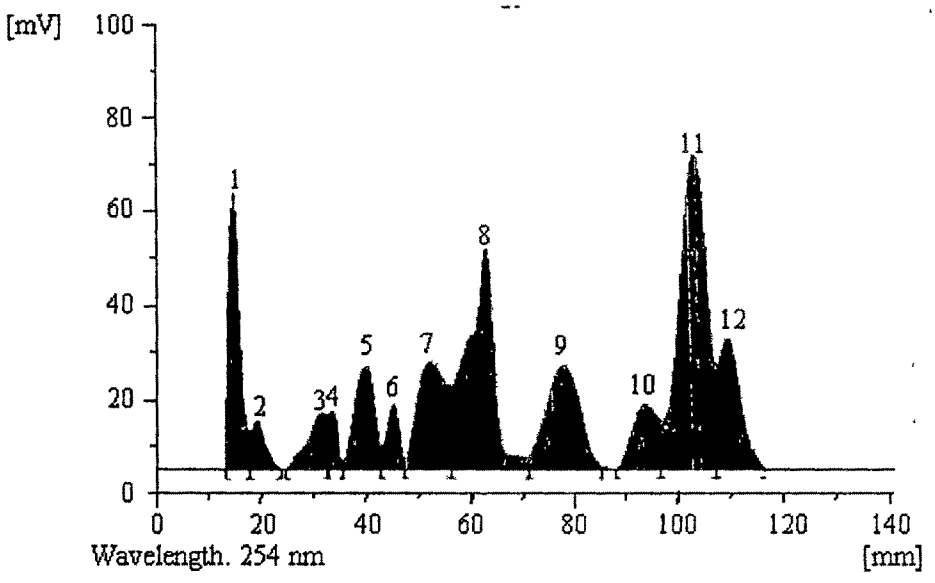

Figure 4. High performance thin layer chromatogram fingerprint of *Mangifera indica.*

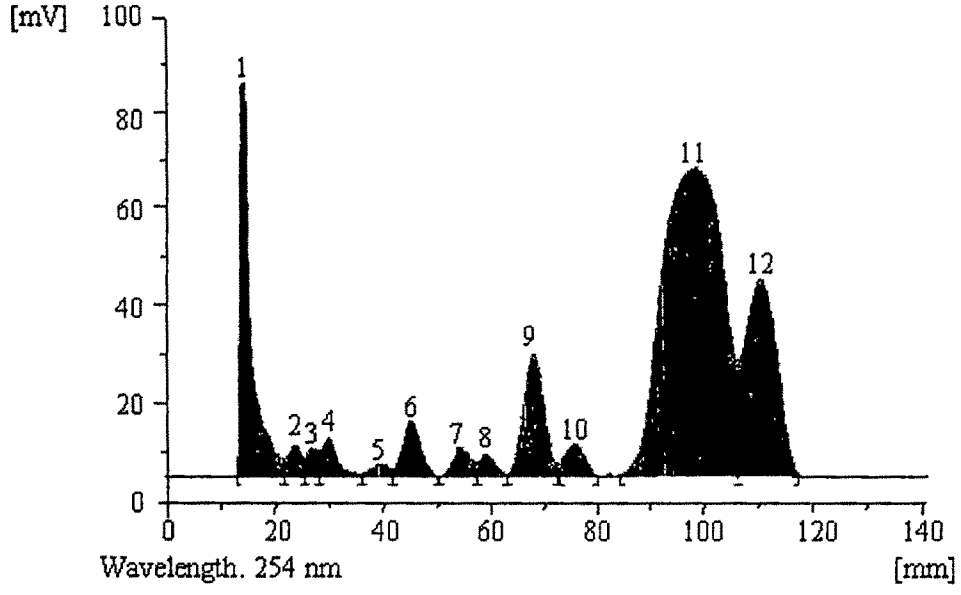

Figure 5. High performance thin layer chromatogram fingerprint of *Syzygium aromaticum.*

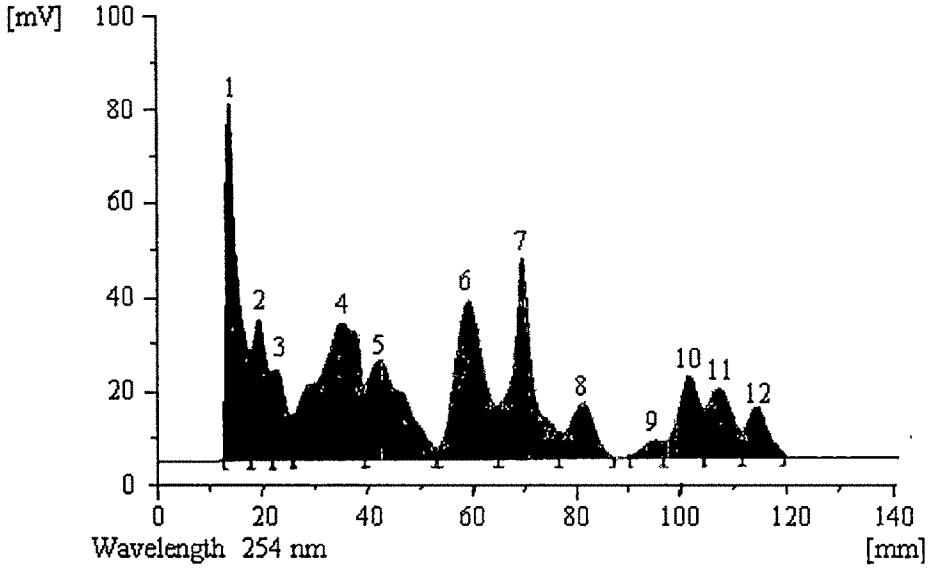

Figure 6. High performance thin layer chromatogram fingerprint of DHC-1.

Phytotherapy Research

ANTIOXIDANT ACTIVITY OF DHC-1, AN HERBAL FORMULATION, IN EXPERIMENTALLY INDUCED CARDIAC AND RENAL DAMAGE.

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ABSTRACT

DHC-1, an herbal formulation derived from popular plants like *Bacopa monniera, Emblica officinalis, Glycyrrhiza glabra, Mangifera indica* and *Syzygium aromaticum* was studied for its antioxidant activity. In this study, we studied the protective effect of DHC-1 in isoproterenol-induced myocardial infarction and cisplatin-induced renal damage. A significant reduction in the serum markers of heart and kidney damage and the extent of lipid peroxidation with a concomitant increase in the enzymatic (SOD and CAT) and non-enzymatic antioxidants (reduced glutathione) were observed in DHC-1 pretreated animals compared with the isoproterenol or cisplatin alone treated animals. Thus it can be concluded that DHC-1 possesses a protective effect against both damaged heart and kidneys in rats. This beneficial effect may be attributed, atleast in part, to its antioxidant activity.

Keywords: Antioxidant, isoproterenol, cisplatin.

INTRODUCTION

The role of reactive oxygen species in several diseases and the potential antioxidant protective effect of natural compounds on affected tissues are topics of high current interest. Many medical and scientific researchers are convinced that uncontrolled free radical activity in the body is directly associated with a number of health problems. Free radical reactions have been implicated in the pathology of many human diseases including atherosclerosis, ischemic heart disease, the aging process, inflammation, diabetes, immunodepression, the neurodegenerative condition and other disease conditions (Maxwell, 1995).

Free radicals are continuously produced in our body. However, these are rigorously controlled by antioxidants. When this precarious balance is broken, in favour of free radicals, it causes an oxidative stress. This oxidative stress can attack lipids, which constitute the cellular membranes, bases of the DNA, and amino acids of proteins. Antioxidants and other phytochemicals are Mother Nature's protection from assaults by free radicals. Antioxidants (free radical scavengers) fight free radicals, and therefore may be able to help prevent the diseases that free radicals promote.

Antioxidant compounds must be constantly replenished since they are used up in the process of neutralizing free radicals. Therefore these have to be continuously ingested in diet or by supplementation. Antioxidant supplements were once thought to be harmless but increasingly we are becoming aware of potential interactions and potential toxicity. As an example, in normal concentrations found in humans,

vitamin C and beta-carotene are antioxidants; but at higher concentrations they are pro-oxidants and can be harmful. It is also possible that unforeseen metabolic disturbances may occur after prolonged use of highly bioavailable pure compounds; such effects may not be apparent when antioxidants are obtained from natural foods. Thus, supplementation of antioxidants using natural sources seems to be a safe approach.

As plants produce a lot of antioxidants to control the oxidative stress, they can represent a source of new compounds with antioxidant activity. A number of plants and plant isolates have been reported to protect free-radical induced damage in various experimental models.

The antioxidant properties of *Bacopa monnieri* (Bhattacharya *et al.*, 2000; Tripathi *et al.*, 1996), *Emblica officinalis* (Scartezzini and Speroni, 2000; Bhattacharya *et al.*, 1999; Mathur *et al.*, 1996), *Glycyrrhiza glabra* (Hatano *et al.*, 1991), *Mangifera indica* (Ghosal *et al.*, 1996; Martinez *et al.*, 2000) and *Syzygium aromaticum* (Deans *et al.*, 1995; Lee *et al.*, 2001) were earlier investigated and were found to possess free radical scavenging property. Some of the ingredients were also found to produce significant induction in the levels of various endogenous antioxidant enzymes.

Many chemical compounds and herbal formulations have been studied for their antioxidant activity by using *in vivo* models such as isoproterenol-induced myocardial infarction (Sathish *et al.*, 2003; Mitra *et al.*, 1999) and cisplatin -induced nephrotoxicity (Antunes *et al.*, 2001; Rao and Rao, 1998).

Thus the aim of the present study was to evaluate DHC-1, an herbal formulation (manufactured by Himalaya Drug Company, Bangalore, India) containing extracts of *Bacopa monnieri, Emblica officinalis, Glycyrrhiza glabra, Mangifera indica* and *Syzygium aromaticum* for its antioxidant activity by using the above experimental models.

MATERIALS AND METHODS

Composition

Each gram of DHC-1 (manufactured by Himalaya Drug Company, Bangalore, India) contains extracts of *Bacopa monnieri* Linn. (Scrophulariaceae; Whole plant; 200mg), *Emblica officinalis* Gaertn. (Euphorbiaceae; Fruit; 200mg), *Glycyrrhiza glabra* Linn. (Papilionaceae; Roots; 200mg), *Mangifera indica* Linn. (Anacardiaceae; Bark; 200mg) and *Syzygium aromaticum* Linn. (Myrtaceae; Flower bud; 200mg).

Experimental Procedure

Animals

Albino rats of Wistar strain weighing 150-200gms were used for the study. The animals were fed *ad libitum* with standard pellet diet and had free access to water.

Isoproterenol-induced myocardial infarction

The rats were divided into six groups of six animals each. Group I served as the control. Group II received isoproterenol (25mg/kg body weight, s.c. twice at an interval of 24 hours) in sterile saline. Group III received DHC-1 (1000 mg/kg) orally for a period of one month. Groups IV, V, VI and VII were administered DHC-1 (once daily) at the doses of 125, 250, 500 and 1000 mg/kg body weight p.o., respectively for one month. On day 30, Groups IV, V, VI and VII received isoproterenol (25mg/kg

body weight, s.c. twice at an interval of 24 hours) in sterile saline. After 24 hours of the last dose of isoproterenol, blood was collected and serum was separated for estimations of creatine kinase (CK), lactate dehydrogenase (LDH), uric acid and SGOT. CK and LDH were determined by diagnostic kits (Reckon Diagnostics Ltd., Baroda, India). Uric acid and SGOT were estimated by using kits of Span Diagnostics (India) Pvt. Ltd. The animals were then sacrificed and the heart was dissected out, weighed and homogenized in chilled Tris buffer (10mM, pH 7.4) at a concentration of 10% w/v. The homogenates were centrifuged and the supernatant was used for the assays of lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione (GSH). The sediment was resuspended in ice-cold Tris buffer (10mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of different membrane bound enzymes (Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase).

Cisplatin-induced nephrotoxicity

The rats were divided into six groups of six animals each. Group I served as control. Group II received cisplatin (3mg/kg, i.p.) every week for 28 days (days 1, 7, 14, 21 and 28). Group III received DHC-1 (1000 mg/kg) orally every week for 28 days (days 1, 7, 14, 21 and 28). Groups IV, V, VI and VII received both cisplatin (3mg/kg, i.p.) and DHC-1 at the doses of 125,250, 500 and 1000 mg/kg b.wt. p.o., respectively every week for 28 days (days 1, 7, 14, 21 and 28). Cisplatin was injected 1 h after the drug administration. After 24 hours of the last dose of cisplatin, blood was collected and serum was separated for estimations of creatinine, urea, uric acid and blood urea nitrogen (BUN). These values were determined with kits of Span Diagnostics (India) Pvt. Ltd. The animals were then sacrificed and the kidney was dissected out, weighed and processed for antioxidant estimations as mentioned in previous section.

Biochemical Estimations

Superoxide dismutase (SOD) was determined by the method of Mishra and Fridovich (1972). Catalase was estimated by the method of Hugo Aebi as given by Colowick *et al.* (1984). Reduced glutathione was determined by the method of Moron *et al.* (1979). Lipid peroxidation or malondialdehyde (MDA) formation was estimated by the method of Slater and Sawyer (1971). Membrane bound enzymes namely Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase were assayed according to the methods of Bonting (1970), Hjerken and Pan (1983) and Ohnishi *et al.* (1982), respectively. The inorganic phosphorus was estimated by the method of Fiske and Subbarow (1925). Total proteins were determined by the method of Lowry *et al.* (1975).

Statistical Analysis

Results of all the above estimations have been indicated in terms of mean ± SEM. Group receiving DHC-1 alone (Group III) was compared with the control group (Group I) by using Student's *t*-test. Difference between the control (Group I), isoproterenol control (Group II) and DHC-1-treated groups (Groups IV, V, VI and VII) was statistically determined by analysis of variance followed by Tukey-Kramer Multiple Comparisons test, with the level of significance set at $p < 0.05$.

RESULTS

Isoproterenol-induced myocardial infarction

The treatment with isoproterenol (group II) resulted in a significant elevation ($p<0.001$) in serum CK, LDH and GOT activity and uric acid levels as compared to control (Group I).

DHC-1-pretreated, isoproterenol-administered rats showed a significant decrease in the activities of serum CK, LDH and GOT and level of uric acid in a dose-dependent manner (Table I).

In heart, a significant decrease in the activities of SOD ($p<0.01$) and catalase ($p<0.001$) was observed in group II as compared to group I. A significant ($p<0.001$) increase in lipid peroxidation and decrease in reduced glutathione in group II was also found as compared to group I. Administration of DHC-1, followed by isoproterenol treatment, was found to significantly increase the glutathione level and activities of SOD and catalase as compared to group II. The drug was also found to significantly decrease lipid peroxidation (Table II).

Isoproterenol-administered rats (group II) showed a significant decrease in the activities of Na^+K^+ATPase and Ca^{2+}ATPase enzymes as compared to group I; however the decrease in Mg^{2+}ATPase activity was not found to be significant. Administration of DHC-1 in isoproterenol-treated rats significantly increased the activities of Na^+K^+ATPase and Ca^{2+}ATPase while the increase in Mg^{2+}ATPase was not found to be significant as compared to group II (Table II).

Rats pretreated with DHC-1 alone (Group III) neither showed any significant decrease in the levels of serum CK, LDH, GOT and uric acid nor any significant change in the levels of endogenous antioxidants (SOD, catalase and reduced glutathione), lipid peroxidation and membrane bound enzymes in heart.

Cisplatin-induced nephrotoxicity

Administration of cisplatin (group II) resulted in a significant ($p<0.001$) elevation in serum creatinine, urea, uric acid and BUN levels, the markers of renal injury, as compared to control group (group I). Treatment with DHC-1 followed by cisplatin administration significantly decreased these levels in rats. The effects of DHC-1 were found to be dose-related (Table III).

In the kidney, significant ($p<0.001$) reduction in the levels of SOD, catalase and reduced glutathione was observed in group II as compared to group I. In group II a significant increase ($p<0.01$) in lipid peroxidation was also found as compared to group I. Administration of DHC-1 in cisplatin-treated rats significantly increased the activities of SOD and catalase as compared to group II. A significant decrease in lipid peroxidation and an increase in reduced glutathione levels were observed in the drug treated groups as compared to group II (Table IV).

Cisplatin-administered rats (group II) showed a significant ($p<0.001$) decrease in the activities of membrane bound enzymes, namely Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase enzymes as compared to group I. DHC-1-treated, cisplatin administered rats showed an increase in the activity of all the ATPases as compared to cisplatin control (Table IV).

Rats pretreated with DHC-1 alone (Group III) neither showed any

significant decrease in the levels of serum creatinine, urea, uric acid and BUN nor any significant change in the levels of endogenous antioxidants (SOD, catalase and reduced glutathione), lipid peroxidation and membrane bound enzymes in the kidneys.

DISCUSSION

Free radicals have been implicated in many disease conditions. Herbal drugs containing radical scavengers are gaining importance in treating such diseases. Isoproterenol-induced myocardial infarction serves as a well-standardized model to study the beneficial effects of many drugs. Isoproterenol, a non-selective ß-adrenergic agonist, has been reported to cause oxidative stress in the myocardium resulting in infarct like necrosis of the heart muscle (Wexler and Greenberg, 1978). During isoproterenol-induced myocardial infarction enhanced free radical formation and lipid peroxide accumulation have been proposed as one of the possible biochemical mechanism for myocardial damage (Sushmakumari *et al.*, 1989).

Myocardial infarction is accompanied by the disintegration of membrane polyunsaturated fatty acids expressed by increase of thiobarbituric acid reactive substance (TBARS), a measure of lipid peroxides and by the impairment of natural scavenging, characterized by the decrease in the levels of superoxide dismutase, catalase and reduced glutathione (Nirmala and Puvanakrishnan, 1996).

Serum levels of creatine kinase, lactate dehydrogenase and GOT are the diagnostic indicators of myocardial infarction. The increased levels of serum enzymes in myocardial ischemia are due to the leakage of enzymes into blood (Mitra *et al*, 1999). Increase in serum uric acid could be due to excessive degradation of purine nucleotides and proteolysis (Iriama, 1987).

DHC-1 pretreatment significantly reduced lipid peroxidation and increased the levels of glutathione, catalase and SOD, which suggests its efficacy in preventing free-radical induced damage. Thus, the observations made in the present study showed that DHC-1 administration prevents oxidative stress induced by isoproterenol myocardial injury.

Cisplatin [cis-diamminedichloroplatinum (II): CDDP] is a widely used cancer chemotherapeutic agent whose clinical use is limited by its renal toxicity (Goldstein and Mayor, 1983). Previous reports suggest that cisplatin-induced nephrotoxicity is by increase in lipid peroxidation (Hanneman and Baumann, 1991) and depletion of cellular thiols (Levi *et al.*, 1980) in kidney tissues following cisplatin treatment. Cisplatin inhibits activities of antioxidant enzymes (SOD and catalase) in rat kidneys suggesting that cisplatin nephrotoxicity results from generation of reactive oxygen species (Sdzuka *et al.*, 1992). The results obtained in this study correlates with these previous reports, which mention that enhancement in lipid peroxidation, and decrease in reduced glutathione and antioxidant enzymes (SOD and catalase) contributes to cisplatin-induced nephrotoxicity.

Effects of several antioxidants like butylated hydroxyl anisole (BHA) and glutathione (GSH) on cisplatin-induced renal injury have been studied and these compounds prevent cisplatin-induced lipid peroxidation and depletion of glutathione (Kim *et al.*, 1997) and antioxidant enzymes, which is in

agreement with the results obtained in the present study. The nephrotoxicity of cisplatin, characterized by the elevation of serum creatinine, urea, uric acid and BUN, was also reversed to a significant extent by DHC-1.

Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase are membrane bound enzymes. It has been reported that administration of isoproterenol (Chernysheva *et al.*, 1980; Manjula and Devi, 1993) and cisplatin (Devi Priya and Shyamala Devi, 1999) alone resulted in decrease in the activities of these membrane bound ATPases. The results obtained in this study also correlates with the above reports. The drug, DHC-1 was found to significantly increase the activity of ATPases in both the models.

In conclusion, the results obtained from this study indicate that DHC-1 pretreatment offers significant protection to heart (cardioprotective effect) and kidney (nephroprotective effect) and reduces the risk of isoproterenol-induced cardiac damage and cisplatin-induced nephrotoxicity by inhibiting lipid peroxidation and activating antioxidant defense enzymes in the organ.

Acknowledgement:

We are thankful to Himalaya Drug Company, Bangalore and UGC for providing financial assistance for successfully carrying out this work.

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Table I: Effect of DHC-1 on the serum levels of creatine kinase, lactate dehydrogenase, uric acid and GOT in isoproterenol-induced myocardial infarction in rats.

GROUPS	Creatine Kinase (U/L)	Lactate Dehydrogenase (U/L)	Uric acid (mg/dl)	SGOT (U/ml)
Group I	326.67 ± 11.89	370.33 ± 13.74	0.67 ± 0.04	21.91 ± 3.28
Group II	1198.00 ± 209.13***	1056.30 ± 115.15***	1.92 ± 0.25***	74.29 ± 6.80***
Group III	323.55 ± 12.36 NS	367.39 ± 10.28 NS	0.64 ± 0.05 NS	20.29 ± 3.37 NS
Group IV	903.67 ± 45.18NS	714.33 ± 48.55*	1.56 ± 0.11NS	74.05 ± 3.04 NS
Group V	407.67 ± 15.45***	488.67 ± 54.81***	1.33 ± 0.07*	55.13 ± 4.68**
Group VI	346.00 ± 7.37***	416.67 ± 5.61***	1.18 ± 0.10*	44.72 ± 4.22**
Group VII	330.67 ± 8.51***	360.33 ± 14.31***	0.85 ± 0.05***	38.34 ± 2.85***
F value	18.079	23.394	13.789	22.441
P value	<0.0001	<0.0001	<0.0001	<0.0001

Group I: Control; Group II: Isoproterenol control; Group III: DHC-1 (1000 mg/kg)

Group IV: DHC-1 (125mg/kg) + Isoproterenol;

Group V: DHC-1 (250mg/kg) + Isoproterenol;

Group VI: DHC-1 (500mg/kg) + Isoproterenol;

Group VII: DHC-1 (1000mg/kg) + Isoproterenol

Values are expressed as mean ± SEM.

Groups II and III were compared with group I.

Groups IV, V, VI and VII were compared with group II.

*p<0.05; **p<0.01; ***p<0.001; NS = Non Significant

Table II: Effect of DHC-1 on the levels of lipid peroxidation (MDA content), reduced glutathione, superoxide dismutase, catalase, Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase in heart of rats in isoproterenol-induced myocardial infarction.

GROUPS	Lipid Peroxidation (nmoles of MDA/mg protein)	Reduced Glutathione (µg of GSH/mg protein)	Superoxide Dismutase (Units/mg protein)	Catalase (µmoles of H_2O_2 consumed/min/ mg protein)	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated/min/ mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/ mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/ mg protein)
Group I	3.43 ± 0.15	3.27 ± 0.55	2.77 ± 0.31	4.61 ± 0.05	5.03 ± 0.30	2.76 ± 0.16	2.39 ± 0.06
Group II	7.15 ± 0.54***	0.61 ± 0.09***	1.69 ± 0.08**	1.87 ± 0.14***	2.46 ± 0.21***	2.03 ± 0.20*	1.99 ± 0.15NS
Group III	3.40 ± 0.24NS	3.30 ± 0.58NS	2.79 ± 0.22NS	4.66 ± 0.09NS	5.09 ± 0.24NS	2.80 ± 0.18NS	2.38 ± 0.09NS
Group IV	4.92 ± 0.40**	0.98 ± 0.13 NS	1.86 ± 0.06 NS	2.06 ± 0.15 NS	2.67 ± 0.16 NS	2.31 ± 0.10NS	1.97 ± 0.12 NS
Group V	4.18 ± 0.36***	1.87 ± 0.16 NS	2.01 ± 0.08 NS	2.60 ± 0.18*	3.33 ± 0 07 NS	2.32 ± 0.05 NS	2.18 ± 0.08 NS
Group VI	3.57 ± 0.29***	2.72 ± 0.17**	2.21 ± 0.06 NS	3.68 ± 0.18***	4.12 ± 0.08***	2.98 ± 0.16**	2.40 ± 0.14 NS
Group VII	3.49 ± 0.34***	3.69 ± 0.30***	2.63 ± 0.08**	4.27 ± 0.15***	4.36 ± 0.22***	3.14 ± 0.08***	2.58 ± 0.20 NS
F value	15.502	19.879	9.071	62.217	28.022	10.497	3.516
P value	<0.0001	<0.0001	0.0009	<0.0001	<0.0001	0.0005	0.0345

Values are expressed as mean ± SEM. Group I: Control; Group II: Isoproterenol control; Group III: DHC-1 (1000mg/kg)

Group IV: DHC-1 (125mg/kg) + Isoproterenol; Group V: DHC-1 (250mg/kg) + Isoproterenol;

Group VI: DHC-1 (500mg/kg) + Isoproterenol; Group VII: DHC-1 (1000mg/kg) + Isoproterenol

Groups II and III were compared with group I. Groups IV, V, VI and VII were compared with group II.

*p<0.05; **p<0.01; ***p<0.001; NS = Non Significant

Table III: Effect of DHC-1 on the serum levels of creatinine, urea, uric acid and BUN in cisplatin-induced nephrotoxicity in rats.

GROUPS	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	BUN (mg/dl)
Group I	0.46 ± 0.03	26.60 ± 3.66	0.55 ± 0.02	15.32 ± 0.36
Group II	2.26 ± 0.14***	81.02 ± 3.85***	2.21 ± 0.18***	37.84 ± 1.79***
Group III	0.44 ± 0.07NS	25.57 ± 2.82NS	0.50 ± 0.06NS	15.13 ± 0.29NS
Group IV	1.53 ± 0.15***	59.47 ± 2.34***	1.45 ± 0.11***	27.77 ± 1.09***
Group V	0.93 ± 0.02***	49.93 ± 1.03***	1.05 ± 0.04***	23.32 ± 0.48***
Group VI	0.74 ± 0.04***	39.93 ± 1.46***	0.89 ± 0.04***	18.65 ± 0.68***
Group VII	0.68 ± 0.02***	33.47 ± 0.71***	0.73 ± 0.04***	15.63 ± 0.33***
F value	60.486	62.877	44.224	84.110
P value	<0.0001	<0.0001	<0.0001	<0.0001

Group I: Control; Group II: Cisplatin control; Group III: DHC-1 (1000 mg/kg)

Group IV: DHC-1 (125mg/kg) + Cisplatin;

Group V: DHC-1 (250mg/kg) + Cisplatin;

Group VI: DHC-1 (500mg/kg) + Cisplatin;

Group VII: DHC-1 (1000mg/kg) + Cisplatin

Values are expressed as mean ± SEM.

Groups II and III were compared with group I.

Groups IV, V, VI and VII were compared with group II.

*p<0.05; **p<0.01; ***p<0.001; NS = Non Significant

Table IV: Effect of DHC-1 on the levels of lipid peroxidation (MDA content), reduced glutathione, superoxide dismutase, catalase, Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase in kidney of rats in cisplatin-induced nephrotoxicity.

GROUPS	Lipid Peroxidation (nmoles of MDA/mg protein)	Reduced Glutathione (µg of GSH/mg protein)	Superoxide Dismutase (Units/mg protein)	Catalase (µmoles of H_2O_2 consumed/min/mg protein)	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated/min/ mg protein)	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/ mg protein)	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/min/ mg protein)
Group I	2.50 ± 0.08	1.71 ± 0.13	2.59 ± 0.36	3.11 ± 0.07	10.98 ± 0.34	4.41 ± 0.09	6.57 ± 0.18
Group II	$5.64 \pm 0.27^{**}$	$0.61 \pm 0.03^{***}$	$1.94 \pm 0.11^{***}$	$2.00 \pm 0.14^{***}$	$3.07 \pm 0.70^{***}$	$1.35 \pm 0.14^{***}$	$1.43 \pm 0.24^{***}$
Group III	2.46 ± 0.18^{NS}	1.73 ± 0.11^{NS}	2.62 ± 0.28^{NS}	3.10 ± 0.09^{NS}	10.89 ± 0.26^{NS}	$4.44 \pm 0\ 07^{NS}$	6.63 ± 0.28^{NS}
Group IV	4.52 ± 0.59^{NS}	0.69 ± 0.03^{NS}	2.06 ± 0.08^{NS}	1.97 ± 0.06^{NS}	$5.26 \pm 0.19^{**}$	$2.46 \pm 0.10^{***}$	$2.82 \pm 0.08^{***}$
Group V	$3.73 \pm 0.57^{*}$	$1.12 \pm 0.07^{*}$	2.11 ± 0.02^{NS}	2.21 ± 0.08^{NS}	$5.89 \pm 0.21^{**}$	$3.22 \pm 0.14^{***}$	$4.17 \pm 0.11^{***}$
Group VI	$2.93 \pm 0.34^{**}$	$1.32 \pm 0.10^{***}$	2.27 ± 0.07^{NS}	2.33 ± 0.04^{NS}	$7.10 \pm 0.18^{***}$	$3.63 \pm 0.13^{***}$	$4.54 \pm 0.12^{***}$
Group VII	$2.53 \pm 0.27^{**}$	$1.39 \pm 0.09^{***}$	$2.43 \pm 0.03^{**}$	$2.78 \pm 0.07^{***}$	$7.59 \pm 0.20^{***}$	$4.03 \pm 0.10^{***}$	$6.01 \pm 0.11^{***}$
F value	10.058	25.132	10.935	30.469	54.918	93.596	169.76
P value	0.0006	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	<0.0001

Values are expressed as mean ± SEM. Group I: Control; Group II: Cisplatin control; Group III: DHC-1 (1000mg/kg)

Group IV: DHC-1 (125mg/kg) + Cisplatin; Group V: DHC-1 (250mg/kg) + Cisplatin;

Group VI: DHC-1 (500mg/kg) + Cisplatin; Group VII: DHC-1 (1000mg/kg) + Cisplatin

Groups II and III were compared with group I. Groups IV, V, VI and VII were compared with group II.

$^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; NS = Non Significant

Phytomedicine

ANTI-ULCER AND ANTIOXIDANT ACTIVITY OF PEPTICARE, A HERBOMINERAL FORMULATION.

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ABSTRACT

Pepticare, a herbomineral formulation, was tested for its anti-ulcer and antioxidant activity in rats. Effects of various doses (125, 250, 500 and 1000mg/kg, p.o.) of Pepticare were studied on gastric secretion and gastric ulcers in pylorus-ligation and on ethanol-induced gastric mucosal injury in rats. The reduction in ulcer index in both the models along with the reduction in volume and total acidity, and an increase in the pH of gastric fluid in pylorus-ligated rats proved the anti-ulcer activity of Pepticare. It was also found that Pepticare was more potent than *Glycyrrhiza glabra* alone in protecting against pylorus-ligation and ethanol-induced ulcers, respectively. The increase in the levels of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and membrane bound enzymes like Ca^{2+}ATPase, Mg^{2+}ATPase and $Na^{+}K^{+}$ATPase and decrease in lipid peroxidation (MDA) in both the models proved the antioxidant activity of the formulation. Thus it can be concluded that Pepticare possesses anti-ulcer activity, which can be attributed to its antioxidant mechanism of action.

Keywords: Anti-ulcer, antioxidant, lipid peroxidation, superoxide dismutase, catalase, reduced glutathione.

1. Introduction

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects (arrythmias, impotence, gynaecomastia and haematopoeitic changes) of modern medicine, indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer (Akhtar et al., 1992).

There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer (Repetto and Llesuy, 2002). Studies have shown alterations in the antioxidant status following ulceration, indicating that free radicals seem to be associated with the pylorus ligation-induced (Rastogi et al., 1998) and ethanol-induced (Pihan et al., 1987; Mizui et al., 1987) ulceration in rats. Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward in minimizing tissue injury in human disease (Barry, 1991).

Since decades many indigenous drugs have been known to possess anti-ulcer activity. The anti-ulcer properties of *Emblica officinalis* (Rajeshkumar et al., 2001; Al-Rehaily et al., 2002) and *Glycyrrhiza glabra* (De et al., 1997) have been mentioned. The antioxidant properties of *Tinospora cordifolia* (Mathew and Kuttan, 1997; Prince and Menon, 2001.), *Emblica officinalis* (Scartezzini and Speroni, 2000; Bhattacharya et al., 1999; Mathur et al., 1996) *Glycyrrhiza glabra* (Hatano et al., 1991) and Suvarna Makshik bhasma (Shah and Vohora, 2002; Mitra et al., 2002) were earlier investigated and were found to possess free

radical scavenging property. These ingredients were also found to produce significant induction in the levels of various endogenous antioxidant enzymes.

It has been demonstrated that many drugs or formulations possess potent antioxidant actions and are effective in healing experimentally induced gastric ulcers. From these findings, Pepticare is expected to be effective in preventing ulcer formation and in ulcer healing. In addition in order to clarify whether or not Pepticare exerts an anti-ulcer action by means of its antioxidant activity, we examined the effect of this formulation on the content of thiobarbituric acid-reactive substances, an index of lipid peroxidation and on the activities of endogenous antioxidant enzymes.

The present study was thus aimed to investigate the anti-ulcer effects of Pepticare along with its effect on the anti-oxidant enzymes to justify whether the formulation exerts an anti-ulcer action by means of its antioxidant activity.

2. Material and Methods

2.1 Composition

Each gram of Pepticare (manufactured by Ayur Herbals Pvt. Ltd., Baroda, India) contains powders of *Tinospora cordifolia* Miers (Menispermaceae; Whole plant; 300mg), *Emblica officinalis* Gaertn. (Euphorbiaceae; Fruit; 200mg), *Glycyrrhiza glabra* Linn. (Papilionaceae; Root; 300mg) alongwith Sootshekhar ras (40mg), Praval bhasma (Corallium rubram; 30mg), Suvarna Makshik bhasma (Ferri sulphuratum; 20mg), Kapardi bhasma (calcium; 80mg) and Shodhit gairik (silicate of alumina and oxide of iron; 30mg).

2.2 Animals

Female albino rats of wistar strain weighing between 150-225gms were used for the study. The animals were fed *ad libitum* with standard pellet diet and had free access to water.

2.3 Experimental Procedure

The animals were divided into nine groups, each consisting of six rats. Group 1 represented the control group, which received 5ml/kg body weight of vehicle (1% gum acacia, p.o.). Groups 2 to 5 received Pepticare orally at the doses of 125, 250, 500 and 1000 mg/kg body weight, respectively. Groups 6 received powder of *Glycyrrhiza glabra* orally at the dose of 150 mg/kg body weight.

2.3.1 *Study of anti-ulcer activity using pylorus ligation method*

The method of Shay rat ulcer (Shay et al., 1945) was adopted. Rats were fasted for 48 hours. The drug, Pepticare or *Glycyrrhiza glabra* was administered to the animals. During the course of experiment food was withdrawn. After the pretreatment period of 1 h, the animals were anaesthetised with anaesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process; pylorus portion of stomach was slightly lifted out and ligated. Precaution was taken to avoid traction to the pylorus or damage to its blood supply. The stomach was placed carefully in the abdomen and the wound was sutured by interrupted sutures. Nineteen hours after pylorus ligation the rats were sacrificed and the stomach was removed. The gastric content was collected and centrifuged. The volume, pH and total acidity of gastric fluid was determined. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass and the diameter of the ulcers was measured using a vernier caliper. Ulcer index was determined by following the scoring method of Suzuki et al. (1976).

Score 1: maximal diameter of 1mm
Score 2: maximal diameter of 1-2mm
Score 3: maximal diameter of 2-3mm
Score 4: maximal diameter of 3-4mm
Score 5: maximal diameter of 4-5mm
Score 10: an ulcer over 5mm
Score 25: a perforated ulcer

2.3.2 *Study of anti-ulcer activity using ethanol-induced ulcer method*

Pepticare or *Glycyrrhiza glabra* was administered orally to the rats for a period of 10 days. On the 10^{th} day, 1 h after the final dose of Pepticare or *Glycyrrhiza glabra*, 96% ethanol (5ml/kg, p.o.) was administered to the overnight fasted rats of all groups. The animals were then sacrificed 1 h after the dose of ulcerogen. The stomach was then removed, incised along the greater curvature and its mucosal erosion was determined randomly by measuring the area of the lesions. The sum of the areas was expressed as ulcer index (mm^2).

2.3.3 *Study of anti-oxidant activity of Pepticare*

The stomach of rats of Group 1 (Control) and Groups 2 to 5 (Pepticare-treated groups) was then weighed and homogenized in chilled Tris buffer (10mM, pH 7.4) at a concentration of 10% w/v. The homogenates were centrifuged at 10,000 X g at 0°C for 20 minutes using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assays of lipid peroxidation (MDA content), endogenous antioxidant enzymes (superoxide dismutase and catalase) and reduced glutathione (GSH). The sediment was resuspended in ice cold Tris buffer (10mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of different membrane bound enzymes (Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase) and proteins.

Superoxide dismutase (SOD) was determined by the method of Mishra and Fridovich (1972). Catalase was estimated by the method of Hugo Aebi as given by Colowick et al. (1984). Reduced glutathione was determined by the method of Moron et al (1979). Lipid peroxidation or malondialdehyde (MDA) formation was estimated by the method of Slater and Sawyer (1971). Membrane bound enzymes namely Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase were assayed according to the methods of Bonting (1970), Hjerten and Pan (1983) and Ohnishi et al. (1982), respectively. The inorganic phosphorus was estimated by the method of Fiske and Subbarow (1925). Total proteins were determined by the method of Lowry et al. (1975).

2.5 Statistical analysis

Results of all the above estimations have been indicated in terms of mean ± SEM. Difference between the groups (Pepticare and control groups) was statistically determined by analysis of variance followed by Tukey-Kramer Multiple Comparisons test, with the level of significance set at $p<0.05$. *Glycyrrhiza glabra* group was compared with the control group by using unpaired Student's t-test.

3. Results

3.1 *Study of anti-ulcer activity using pylorus ligation method*

It was observed that in the vehicle treated control group the ulcer index was 92.75 ± 5.20 and the maximum number of ulcers were of the ulcer score 4 and 5. In the rats of this group a number of perforated ulcers (score 25) were also observed.

Pepticare was found to produce a decrease in ulcer index at all the four doses; the percentage reduction being 16.77%, 48.46%, 70.46% and 92.24%, respectively. Significant reduction ($p<0.001$) in ulcer index was observed at the doses of 250, 500 and 1000 mg/kg. All the ulcers were of score 1, 2, 3 and 4 and no perforated ulcers were observed. The formulation also significantly reduced the volume and total acidity, and increased the pH of the gastric fluid, proving its anti-ulcer activity (Table I).

Glycyrrhiza glabra (150mg/kg) was not found to produce a significant reduction in ulcer index. It also did not reduce the total acidity and volume significantly neither increased the pH of gastric fluid at the dose of 150mg/kg (Table IV).

3.2 *Study of anti-ulcer activity using ethanol-induced ulcer method*

Administration of ethanol produced significant ulcers (287.98 ± 17.79) in the control group. There was a significant ($p<0.001$)

reduction in ulcer index at all the four doses of Pepticare by 72.57%, 85.93%, 94.31% and 98.44%, respectively (Table III); whereas *Glycyrrhiza glabra* at the dose of 150 mg/kg did not produce a significant reduction in ulcer index (Table IV).

3.3 *Study of anti-oxidant activity of Pepticare*

Pylorus-ligation was found to increase lipid peroxidation and decrease SOD, catalase and reduced glutathione in the control group, thus leading to oxidative stress. Administration of Pepticare, at the doses of 250, 500 and 1000 mg/kg, brought about a significant reduction in lipid peroxidation and an increase in the content of reduced glutathione. The activities of antioxidant enzymes namely, SOD and catalase were also found to be significantly increased at the dose of 1000mg/kg. An enhancement in the membrane bound ATPases was also observed by Pepticare (Table II).

Ethanol administration was found to increase lipid peroxidation and decrease SOD, catalase and reduced glutathione in the control group. Administration of Pepticare at the doses of 250, 500 and 1000 mg/kg significantly decreased lipid peroxidation and increased the glutathione content and the activities of SOD and catalase. All the membrane bound ATPases were also found to be elevated in the drug treated groups (Table III).

4. Discussion and Conclusion

The present study demonstrates that Pepticare exhibits both gastroprotective and ulcer healing properties, probably as a result of the antioxidant action of the drug.

Although in most of the cases the aetiology of ulcer is unknown, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defence mechanism (Piper and Stiel, 1986). To regain the balance, different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanism by increasing mucus production.

Pepticare, a herbal drug formulation, consists of plants that are mentioned in Indian system of medicine (Ayurveda) for their remedial properties. The anti-ulcer effect of Pepticare was tested against gastric lesions induced by pylorus-ligation and ethanol, the experimental models related to lesion pathogenesis with production of reactive species. Pepticare prevented the mucosal lesions induced by pylorus-ligation and ethanol. Pepticare was also found to increase the pH and decrease the acid volume and total acidity of gastric fluid. These effects of Pepticare treatment on the parameters that influence the initiation and induction of ulceration may be considered as highly desirable property of anti-ulcerogenic agent.

Considering the amount of *Glycyrrhiza glabra* powder present in the formulation i.e. after comparing the ulcer index of 500mg/kg of Pepticare with that of 150 mg/kg of *Glycyrrhiza glabra* in both the models, it was found that Pepticare was 8.5 and 51 times more potent than *Glycyrrhiza glabra* in protecting against pylorus-ligation and ethanol-induced ulcers, respectively. Thus the study proves the therapeutic advantage of Pepticare over *Glycyrrhiza glabra* alone. It can thus be said that the herbal mixture with the minerals has a much better anti-ulcer effect than *Glyzyrrhiza* alone, due to potentiated synergistic effects.

Reactive oxygen species are involved in the pathogenesis of pylorus ligation-induced (Rastogi et al, 1998) and ethanol-induced (Pihan et al, 1987) gastric mucosal injury in vivo. Results in the present study also indicate similar alterations in the antioxidant status after pylorus ligation and ethanol induced ulcers.

Emblica officinalis and *Tinospora cordifolia* are categorized as 'rasayanas' (rejuvenatives). Rasayanas are non-toxic Ayurvedic complex herbal preparations or individual herbs used to rejuvenate or attain the complete potential of an individual in order to

prevent diseases and degenerative changes that leads to disease. Various activities of rasayanas have been reviewed by Vayalil et al. 2002 to support the above concept, its role as a prophylactic medication and significance in the prevention of diseases in both healthy as well as diseased individuals. The emerging data suggest that the possible mechanisms may be by immunostimulation, quenching free radicals, enhancing cellular detoxification mechanisms, repair damaged non-proliferating cells, inducing cell proliferation and self-renewal of damaged proliferating tissues, and replenishing them by eliminating damaged or mutated cells with fresh cells. The clinical efficacy of the fruits of *E.officinalis* is held in high esteem in Ayurveda and amla is referred to as a maharasayana. By virtue of their properties and clinical use in Ayurveda, the rasayanas may provide potential therapeutic intervention against oxidative threats, both in health and disease.

Preventive antioxidants, such as superoxide dismutase (SOD) and catalase (CAT) enzymes are the first line of defence against reactive oxygen species. Reduced glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation (Halliwell, B., 1995). Administration of Pepticare resulted in a significant increase in the SOD, catalase and reduced glutathione levels as compared to the control animals, which suggests its efficacy in preventing free radical induced damage.

Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids which eventually results in destruction of membrane lipids. Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore it is not surprising that membrane lipids are susceptible to peroxidative attack (Cheesman, 1993). The study has revealed a significant decrease in lipid peroxidation by Pepticare in both the experimental models, which suggests its protective effect.

Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase are membrane bound enzymes. The drug significantly increased the activity of all the ATPases in both the models.

It is thus concluded that Pepticare is an effective anti-ulcer agent. Further, this study also proves that the anti-ulcer effect may be due to its antioxidant mechanism of action.

ACKNOWLEDGEMENT

We are thankful to Ayur Herbals Pvt. Ltd., Baroda and UGC for providing financial assistance for successfully carrying out this work.

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TABLE I: Effect of Pepticare on the various gastric parameters of pylorus-ligated rats.

Groups	Ulcer Index	Volume of gastric fluid (ml)	pH of gastric fluid	Total acidity (mEq/l/100g)
Control	92.75 ± 5.20	17.28 ± 1.18	1.20 ± 0.07	127.01 ± 3.63
Pepticare (125mg/kg)	77.21 ± 3.93^{NS} (16.77%)	17.25 ± 0.87^{NS}	1.58 ± 0.07^{NS}	112.52 ± 3.52*
Pepticare (250mg/kg)	47.83 ± 7.40*** (48.46%)	14.88 ± 1.82^{NS}	1.85 ± 0.56*	89.03 ± 2.68***
Pepticare (500mg/kg)	27.44 ± 3.53*** (70.46%)	11.50 ± 0.68*	2.05 ± 0.19**	61.06 ± 1.68***
Pepticare (1000mg/kg)	7.22 ± 1.50*** (92.24%)	10.50 ± 0.32**	3.25 ± 0.11***	31.74 ± 3.21***
F value	53.332	7.739	36.423	161.93

Values are expressed as mean ± SEM.

Pepticare treated groups were compared with control group.

$^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; NS = Non Significant

Values in parenthesis indicate the % reduction in ulcer index in relation to the control group.

TABLE III: Effect of Pepticare on the ulcer index and antioxidant parameters in stomach of ethanol-treated rats.

Parameters	Normal	Control	Pepticare				F value
			125mg/kg	250mg/kg	500mg/kg	1000mg/kg	
Ulcer Index (mm^2)	-	287.98 ± 17.79	78.98 ± 14.9*** (72.57%)	40.53 ± 12.7*** (85.93%)	16.39 ± 10.9*** (94.31%)	4.54 ± 5.6*** (98.44%)	149.76
SOD (Unit/mg protein)	5.64 ± 0.33	2.23 ± 0.14***	2.64 ± 0.14^{NS}	3.70 ± 0.17**	4.27 ± 0.36***	5.37 ± 0.19***	34.032
Catalase (µmoles of H_2O_2 consumed/min/mg protein)	8.27 ± 0.27	5.12 ± 0.21***	5.45 ± 0.19^{NS}	6.21 ± 0.25*	6.96 ± 0.29***	7.80 ± 0.20***	30.168
Reduced glutathione (µg of GSH/ mg protein)	3.31 ± 0.15	0.79 ± 0.17***	1.13 ± 0.16^{NS}	2.87 ± 0.40**	3.35 ± 0.45***	4.56 ± 0.58***	20.584
Lipid peroxidation (nmoles of MDA/ mg protein)	3.45 ± 0.24	6.75 ± 0.72***	5.93 ± 0.33^{NS}	4.52 ± 0.27**	3.58 ± 0.28***	3.24 ± 0.13***	14.659
Na^+K^+ATPase (µmoles of inorganic phosphorus liberated/ min/mg protein)	5.29 ± 0.23	2.03 ± 0.11***	2.13 ± 0.06^{NS}	2.38 ± 0.11^{NS}	4.33 ± 0.216***	5.49 ± 0.08***	142.04
Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/ min/mg protein)	3.65 ± 0.12	1.50 ± 0.21***	1.54 ± 0.12^{NS}	1.76 ± 0.14^{NS}	1.90 ± 0.09^{NS}	2.87 ± 0.05***	25.666
Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/ min/mg protein)	3.52 ± 0.36	1.49 ± 0.13***	1.73 ± 0.12^{NS}	2.52 ± 0.15**	3.28 ± 0.13***	3.46 ± 0.17***	34.955

Values are expressed as mean ± SEM.

Control group was compared with the normal group; Pepticare treated groups were compared with control group.

*p<0.05; ** p<0.01; ***p<0.001; NS = Non Significant

Values in parenthesis indicate the % reduction in ulcer index in relation to the control group.

TABLE IV: Effect of *Glycyrrhiza glabra* powder on the various gastric parameters of pylorus-ligated and ethanol-treated rats.

Gastric Parameters	Control	Glycyrrhiza glabra (150mg/kg)
Ulcer Index		
Pylorus-ligated rats	92.75 ± 5.20	85.02 ± 5.36^{NS} (8.33%)
Ethanol-treated rats	287.98 ± 17.79	282.65 ± 21.22^{NS} (1.85%)
Volume of gastric fluid	17.28 ± 1.18	17.05 ± 0.77^{NS}
pH of gastric fluid	1.20 ± 0.07	1.38 ± 0.11^{NS}
Total acidity (mEq/l/100g)	127.01 ± 3.63	119.32 ± 2.56^{NS}

Values are expressed as mean ± SEM.

Glycyrrhiza glabra treated groups were compared with control group.

$^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; NS = Non Significant

Values in parenthesis indicate the % reduction in ulcer index in relation to the control group.