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Antiperoxidative Effect of Livex, a Herbal Formulation against Erythromycin Estolate Induced Lipid Peroxidation in Rats

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The present study was carried out to investigate the antiperoxidative effect of Livex, a compound herbal formulation, against erythromycin estolate induced lipid peroxidation. The oral administration of Livex to rats along with erythromycin estolate caused a significant reversal in lipid peroxidation, enzymatic leakage and produced enhancement of cellular antioxidant defence, revealing that the antioxidative action of Livex is responsible for its protective activity. These observations were supplemented by histopathological examination of liver and kidney sections. The results of this study revealed that Livex could afford significant protection against erythromycin estolate induced lipid peroxidation. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Erythromycin estolate, a commonly used antibiotic, in high doses causes severe hepatic injury both in humans (Zafrani, *et al.*, 1979) and experimental animals (Nicholas, 1977). Cardiovascular dysfunction has been observed in experimental animals during exposure to erythromycin (Richard and Adams, 1976). Erythromycin estolate has been shown to be mediated by an electrophilic reactive metabolite (nitrosoalkane derivative) which binds covalently to thiol groups of proteins and other cellular macromolecules (Pessayre *et al.*, 1985). Herbs are known to play a vital role in the management of various hepatic and renal disorders. Ayurveda, the ancient system of Indian medicine, identified hepatic and renal disease quite early and recommended a number of herbal drugs.

A number of herbal formulations that are currently used as hepatoprotective agents or those that possess antioxidative activity are believed to exert their effect by reducing the formation of the ultimate active metabolite of the drug or by scavenging the reactive molecular species to prevent their reaching the target site. One such herbal formulation is Livex, which contains the extract of nine medicinal plants whose composition and concentration are given in Table 1.

Further, during the course of aerobic metabolic reactions, considerable amounts of reactive oxygen species such as superoxide anion ($O_2\cdot$) and hydrogen peroxide (H_2O_2) are generated (McCord *et al.*, 1971). H_2O_2 and $O_2\cdot$ further undergo a variety of chain reactions and produce free radicals such as $OH\cdot$. These hydrogen species attack polyunsaturated fatty acids and thereby initiate the process of lipid peroxidation (Pryor, 1976–82) resulting in oxidative degradation and inactivation of various important bimolecules. Superoxide dismutase (SOD) is the principle enzyme which dismutase $O_2\cdot$ to H_2O_2 and oxygen (Fridovich, 1975). H_2O_2 is detoxified by catalase (CAT) and glutathione peroxidase (GSH-PX). GSH-PX is further capable of reacting with lipid peroxides to control the process of peroxidation (Christophersen, 1968). There is no report available in the literature of the protective effect of Livex on the status of cellular antioxidants.

The present study was undertaken to investigate the effect of Livex on the status of antioxidants, lipid peroxidation in the liver and kidney and release of marker enzymes of hepatotoxicity in the serum during erythromycin estolate induced toxicity.

MATERIALS AND METHODS

Animals. Male Wistar albino rats of 180–220 g body weight were used for our study. All animals were purchased from the Central Animal House, Rajah Muthiah Medical College, Annamalai University and were fed on standard pellet diet (Hindustan Lever Ltd, Bombay) and water *ad libitum.*

Protective agent. The protective agent used in this study is Livex, a commercially available herbal formulation purchased from Ban Laboratories, Rajkot, India.

Toxic agent. Erythromycin estolate, purchased from

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Table 1. Composition and concentration of Livex

Serial No.	Botanical name	Family	Concentration (mg/mL)
1.	*Tephrosia purpurea*	*Leguminosae*	1.00
2.	*Aconitum heterophyllum*	*Ranunculaceae*	0.75
3.	*Solanum nigrum*	*solanaceae*	0.50
4.	*Cichorium intybus*	*Asteraceae*	3.00
5.	*Cassia occidentalis*	*Caesalpiniaceae*	4.00
6.	*Tamarix gallica*	*Tamaricaceae*	3.00
7.	*Embelia ribes*	*Myrsinaceae*	5.00
8.	*Andrographis Paniculata*	*Acanthaceae*	5.00
9.	*Piper longum*	*Piperaceae*	2.00

Alembic Chemical works, Gujarat, India, was used to produce toxicity in liver and kidney of rats.

All other biochemicals and chemicals used for the experiments were of analytical grade.

Experimental procedure. The animals were randomly divided into three groups each consisting of ten rats. The first group was normal untreated rats. The animals in the second group received only erythromycin estolate (800 mg/kg/day) (Amacher *et al.*, 1991) for 10 days, while the third group received Livex (5 mL (121.25 mg)/kg/day) and erythromycin estolate (800 mg/kg/day) orally as an aqueous solution daily using an intragastric tube for 10 days.

After 10 days, the rats in different groups were killed by decapitation. The blood was collected and serum obtained after centrifugation was used for various biochemical estimations. The liver and kidney were also collected in ice-cold containers for various estimations and for study of histopathology.

Biochemical estimations. The activities of serum aspartate transaminase (AST E.C. 2.6.1.1) and alanine transaminase (ALT E.C. 2.6.1.2) were assayed by the method of Reitmann and Frankel as described by Bergmeyer and Bernt (1974). The level of lipid peroxides in terms of thiobarbituric acid reactive substances (TBARS) was estimated by the method of Nichans and Samuelson (1968). The level of conjugated diene (CD) was estimated by the method of Beuje and Aust (1978). The activities of SOD (E.C.1.15.1.1), CAT (E.C.1.11.1.6) and GSH-PX (E.C.1.11.1.9) were estimated by the methods of Kakkar *et al.* (1984), Sinha (1972) and Rotruck *et al.* (1973), respectively. The content of glutathione (GSH) in liver and kidney was estimated by the method of Beutler and Kelley (1963). The level of serum ceruloplasmin was estimated by the method of Ravin (1961).

Histopathology. After the blood was collected, the liver and kidney specimens were *excised* immediately, fixed in 10% neutral formalin for 7–10 days, dehydrated in graded alcohol (80%–100%), cleared in xylene, and embedded in paraffin. Then the liver and kidney tissues were sliced into 5 μm pieces with a microtome, deparaffinated in xylene, passed through 80% to 100% alcohol, and stained with haematoxylin and eosin (HE) for photomicroscopic assessment.

Statistical analysis. All data were expressed as mean ± SD. Student's *t*-test was applied for detecting the significance of difference between different groups.

RESULTS

Effect of Livex on AST and ALT activity in serum

Treatment of rats with erythromycin estolate caused significant ($p < 0.001$) hepatic damage as observed from elevated serum levels of hepato-specific enzymes such as AST and ALT (Table 2). The treatment of Livex along with erythromycin estolate afforded significant ($p < 0.001$) protection against the erythromycin estolate induced increase in the level of serum AST and ALT (Table 2).

Effect of Livex on erythromycin estolate induced lipid peroxidation

The content of lipid peroxides in liver and kidney of rats treated with erythromycin estolate were significantly increased (Table 3).

The content of lipid peroxides (TBARS and CD) were significantly increased in liver ($p < 0.001$) and kidney

Table 2. Activities of serum AST and ALT

Group	AST (IU/L)	ALT (IU/L)
1. Normal	74.03 ± 2.53	21.38 ± 1.38
2. Normal + erythromycin estolate	97.68 ± 3.25[a]	48.04 ± 2.83[a]
3. Normal + erythromycin estolate + Livex	83.97 ± 2.72[b]	36.11 ± 1.65[b]

Values are mean ± SD from six rats in each group.
[a] $p < 0.001$ group 2 compared with normal.
[b] $p < 0.001$ group 3 compared with group 2.

Table 3. Levels of lipid peroxides in liver and kidney

Group	TBARS (mM/100 g tissue) Liver	TBARS (mM/100 g tissue) Kidney	CD (mM/100 g tissue) Liver	CD (mM/100 g tissue) Kidney
1. Normal	0.853 ± 0.023	1.062 ± 0.068	66.13 ± 2.45	17.68 ± 1.50
3. Normal + erythromycin estolate	1.202 ± 0.085[a]	1.408 ± 0.088[a]	85.37 ± 4.30[a]	21.13 ± 1.42[b]
3. Normal + erythromycin estolate + Livex	0.985 ± 0.052[c]	1.179 ± 0.096[d]	76.40 ± 2.81[d]	19.30 ± 0.75[e]

Values are mean ± SD from six rats in each group.
[a] $p < 0.001$ [b] $p < 0.01$ group 2 compared with normal.
[c] $p < 0.001$ [d] $p < 0.01$ [e] $p < 0.05$ group 3 compared with group 2.

Table 4. Activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx) in liver

Group	SOD (units[a]/mg protein)	CAT (units[b]/mg protein)	GSHPx (units[c]/mg protein)
1. Normal	5.58 ± 0.41	206.10 ± 12.24	9.37 ± 0.87
3. Normal + erythromycin estolate	4.46 ± 0.22[d]	137.75 ± 11.25[d]	6.35 ± 0.56[d]
3. Normal + erythromycin estolate + Livex	5.19 ± 0.32[e]	174.35 ± 8.00[e]	8.26 ± 0.72[e]

Values are mean ± SD from six rats in each group.
[a] One unit of activity was taken as the enzyme reaction which gave 50% inhibition of NBT reduction in 1 min.
[b] μmol hydrogen peroxide consumed/min.
[c] μg glutathione consumed/min.
[d] $p < 0.001$ group 2 compared with normal.
[e] $p < 0.001$ group 3 compared with group 2.

Table 5. Activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx) in kidney

Group	SOD (units[a]/mg protein)	CAT (units[b]/mg protein)	GSHPx (units[c]/mg protein)
1. Normal	5.97 ± 0.40	196.28 ± 12.90	7.30 ± 0.58
3. Normal + erythromycin estolate	4.92 ± 0.42[e]	168.98 ± 9.87[e]	4.78 ± 0.34[d]
3. Normal + erythromycin estolate + Livex	5.37 ± 0.24[g]	183.35 ± 8.42[g]	6.14 ± 0.47[f]

Values are mean ± SD from six rats in each group.
[a] One unit of activity was taken as the enzyme reaction which gave 50% inhibition of NBT reduction in 1 min.
[b] μmol hydrogen peroxide consumed/min.
[c] μg glutathione consumed/min.
[d] $p < 0.001$ [e] $p < 0.01$ group 2 compared with normal.
[f] $p < 0.001$ [g] $p < 0.05$ group 3 compared with group 2.

Table 6. Level of serum ceruloplasmin and reduced glutathione content in liver and kidney

Group	Ceruloplasmin (mg/dL)	Reduced glutathione (mg/100 g tissue) Liver	Reduced glutathione (mg/100 g tissue) Kidney
1. Normal	43.70 ± 2.00	157.10 ± 7.42	127.50 ± 8.84
3. Normal + erythromycin estolate	54.70 ± 2.80[a]	118.10 ± 6.31[a]	108.70 ± 7.10[b]
3. Normal + erythromycin estolate + Livex	46.90 ± 1.90[c]	138.91 ± 5.00[c]	116.96 ± 5.70[d]

Values are mean ± SD from six rats in each group.
[a] $p < 0.001$ [b] $p < 0.01$ group 2 compared with normal.
[c] $p < 0.001$ [d] $p < 0.05$ group 3 compared with group 2.

Table 7. Histopathological changes of liver

Microscopic observation	Normal	Erythromycin estolate	Erythromycin estolate + Livex
Congestion of vessels	Absent	Present	Reduced
Dilated blood vessels	Absent	Present	Reduced
Sinusoidal dilation	Absent	Present	Decreased
Inflammatory cell infiltrate	Absent	Present	Limited

Table 8. Histopathological changes of kidney

Microscopic observation	Normal	Erythromycin estolate	Erythromycin estolate + Livex
Congestion of vessels	Absent	Present	Reduced
Cloudy swelling of tubules	Absent	Present	Reduced

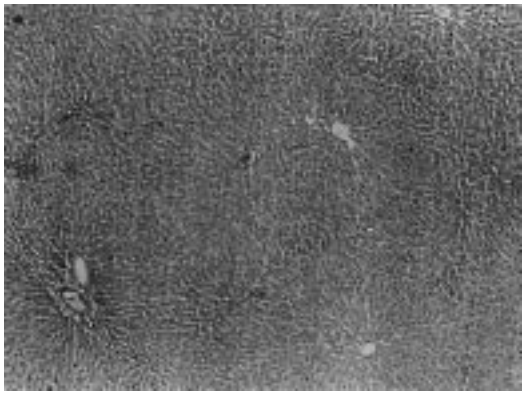

Figure 1. Control animals liver: H&E × 4.

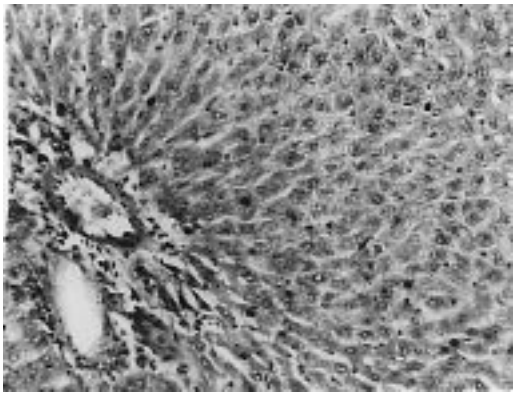

Figure 2. Control animals liver: H&E × 10.

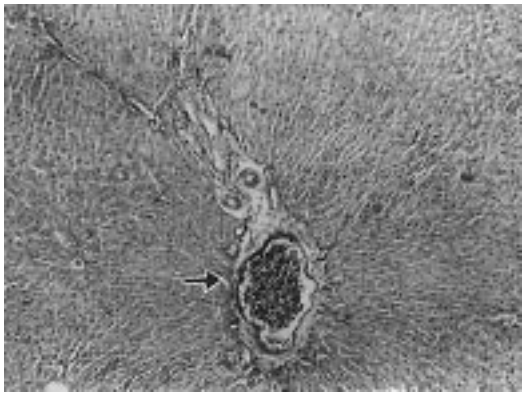

Figure 3. Erythromycin estolate treated animals liver: H&E × 4. Congested portal vessels (→).

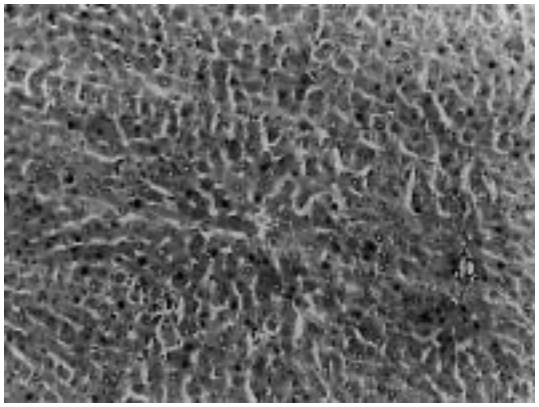

Figure 4. Erythromycin estolate treated animals liver: H&E × 10. Dilated sinusoids.

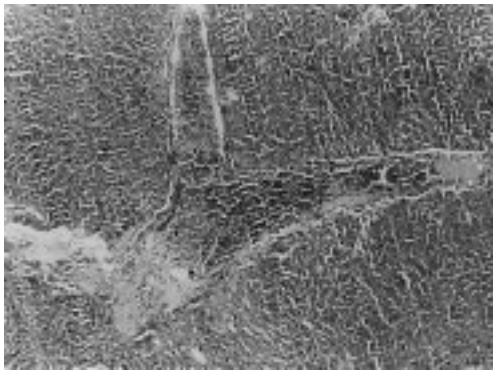

Figure 5. Erythromycin estolate treated animals liver: H&E × 4. Dilated blood vessels.

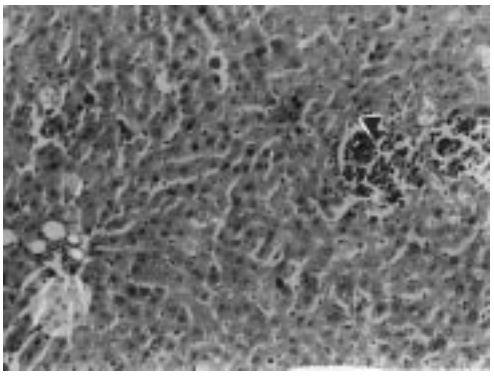

Figure 6. Erythromycin estolate treated animals liver: H&E × 10. Mixed inflammatory cell infiltrate in Zone II (▼).

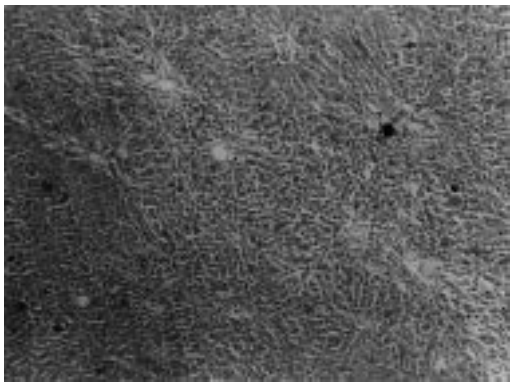

Figure 7. Erythromycin estolate + Livex treated animals liver: H&E × 4. Near normal appearance with focal inflammatory cell infiltration in the liver parenchyma and few dilated sinusoids.

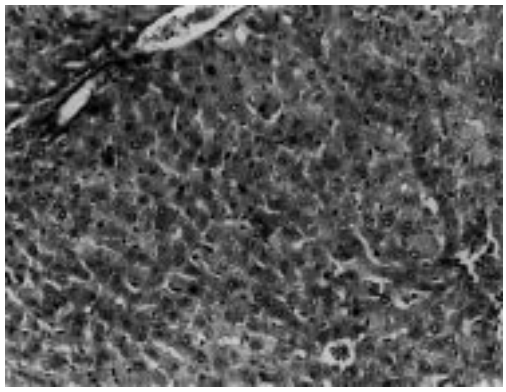

Figure 8. Erythromycin estolate + Livex treated animals liver: H&E × 10. Near normal appearance with focal inflammatory cell infiltration in the liver parenchyma and few dilated sinusoids.

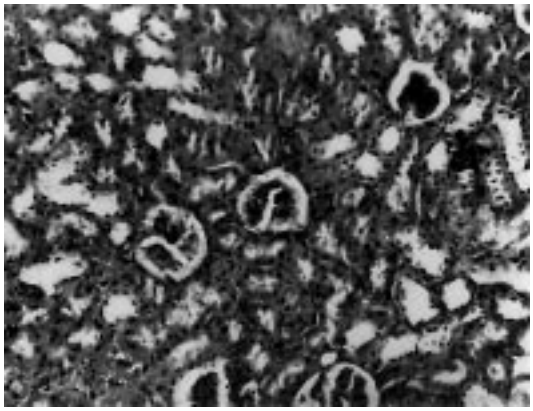

Figure 9. Control animals kidney: H&E × 10.

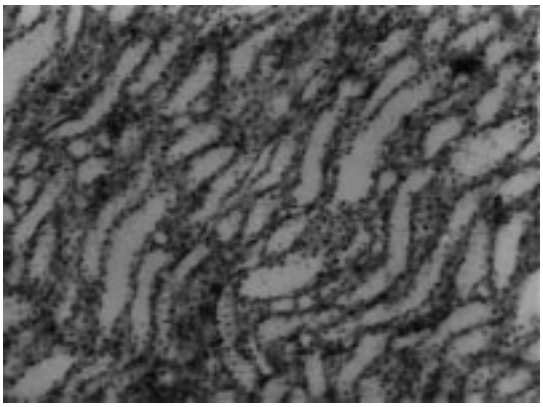

Figure 10. Erythromycin estolate treated animals kidney: H&E × 10. Cloudy swelling of the tubules.

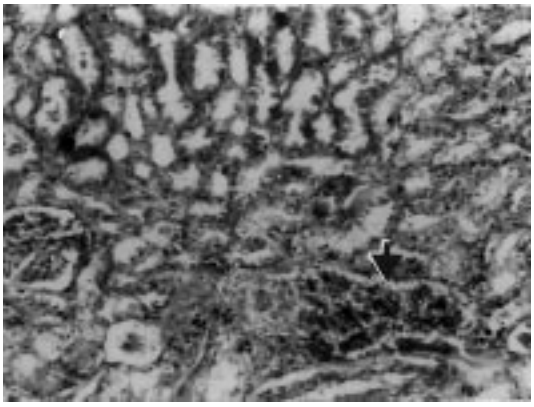

Figure 11. Erythromycin estolate treated animals kidney: H&E × 10. Cloudy swelling of the tubules and congested vessels. (↓).

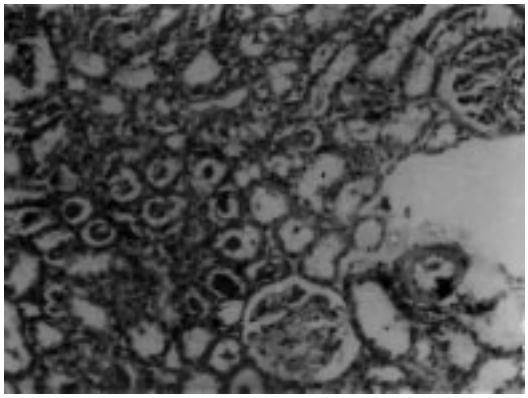

Figure 12. Erythromycin estolate + Livex treated animals kidney: H&E × 10. Tubules show casts.

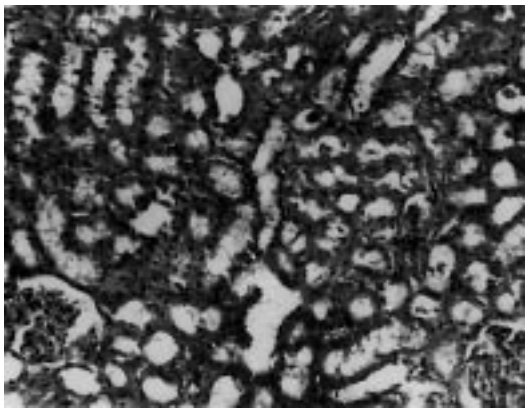

Figure 13. Erythromycin estolate + Livex treated animals kidney: H&E × 10. Near normal appearance of the kidney.

($p < 0.01$) of rats treated with erythromycin estolate (Table 3.) The activities of antiperoxidative enzymes namely, SOD, CAT and GSH-PX were significantly reduced in liver and kidney ($p < 0.001$, $p < 0.01$ respectively) of erythromycin estolate treated animals, (Tables 4 and 5). The content of GSH in liver and kidney were significantly ($p < 0.001$, $p < 0.01$) decreased while the level of serum ceruloplasmin was significantly increased ($p < 0.001$) in rats treated with erythromycin estolate (Table 6). The treatment with Livex and erythromycin estolate afforded significant protection against erythromycin estolate induced alterations in the level of serum enzymes, in the level of tissue lipid peroxides and antioxidant enzymes (Tables 2–6).

Histopathology

Histopathological studies demonstrated congestion of vessels, dilation of blood vessels, infiltration of mixed inflammatory cells in zone II and sinusoidal dilation in liver (Figs 3–6 and Table 7) and congestion of vessels and cloudy swelling of the tubules in kidney (Figs 10 and 11 and Table 8) induced by erythromycin estolate and these changes were very much reduced in rats treated with erythromycin estolate and Livex (Figs 7, 8, 12 and 13 and Tables 7 and 8).

DISCUSSION

An elevation in the levels of some serum enzymes is generally regarded as one of the most sensitive markers of hepatic damage. The data reported here revealed an increase in AST and ALT levels in the serum of erythromycin estolate treated rats, suggesting a release of the enzymes following hepatocellular damage with

erythromycin estolate. However, the administration of Livex along with erythromycin estolate caused reversal in the leakage of these enzymes thus exhibiting protection which may be a consequence of the reduction in erythromycin estolate induced cell membrane disturbances. This finding coincides with an earlier study (Gilani and Janbaz, 1994), which reported that the crude extracts of *Cichorium intybus* (one of the ingredients of Livex) seeds reduces the level of serum AST and ALT in acetaminophen and CCl_4-induced hepatotoxicity.

GSH, being the most important biomolecule against chemically induced toxicity, can participate in the elimination of reactive intermediates by reduction of hydroperoxides in the presence of GSH—Px. GSH also functions as a free radical scavenger and in the repair of radical caused biological damage (Meister, 1984). SOD and CAT are other enzymatic defence factors against a variety of toxins. In the present study, the reduction obtained in the activities of GSH-PX, SOD, CAT and GSH status in erythromycin estolate intoxicated rats may potentiate necrosis and thus represent a pathogenic mechanism of erythromycin estolate induced injury. As the alterations produced in the antioxidant activities indicate involvement of deleterious oxidative changes, increased activities of the components of this defence system would therefore be important in protecting against toxicity.

The over expression of these antioxidant enzymes in rats treated with Livex and erythromycin estolate implies that this potential antioxidant defence is reactivated by the plant principles of Livex with a resulting increase in the capacity of detoxification through enhanced scavenging of oxyradicals. It was well correlated with the protective actions of chlorogenic acid (an active fraction from *Anthocephalous cadamba*) which showed antiperoxidative changes against CCl_4 induced lipid peroxidation (Kapil *et al.*, 1995).

Ceruloplasmin is an important enzyme which oxidizes iron from the ferrous to ferric state and it has been demonstrated that iron catalysed lipid peroxidation requires both Fe (ii) and Fe (iii) and the maximum rate occurs when the ratio is approximately one (Bucher *et al.*, 1983). The level of serum ceruloplasmin was found to be increased in rats treated with erythromycin estolate. The increase in ceruloplasmin is an indication of increased antioxidant defence to compensate the loss of other antioxidant enzymes. Administration of Livex along with erythromycin estolate was seen to restore the level of serum ceruloplasmin to near normal.

The induction of lipid peroxidation due to erythromycin estolate treatment may be a manifestation of interaction of nitrosoalkane and reactive nitrosoradicals of erythromycin estolate metabolism with the membrane fatty acids (Pessayre *et al.*, 1985). Since erythromycin estolate produces toxicity via toxic intermediates (Pessayre *et al.*, 1985), it is likely that Livex may be acting by altering the detoxification of erythromycin estolate leading to reduced generation of toxic metabolites. It may be responsible for the restoration of the antioxidant defence system in Livex administered rats.

Erythromycin estolate causes congestion of portal vessels, dilation of blood vessels, infiltration of mixed inflammatory cells in zone II, dilation of sinusoids in the liver and also causes congestion of the vessels and cloudy swelling of the tubules in kidney when taken in substantial over dosage (Figs 3–6, 10 and 11). It is provoked by the increased production of a highly reactive intermediate of erythromycin estolate—nitrosoalkane derivatives, which is normally detoxified by endogenous GSH but in excess it may deplete GSH stores, allowing the reactive intermediate to react with and destroy the hepatic, renal and other cells (Pessayre *et al.*, 1985). All these changes were very much reduced in rats treated with erythromycin estolate and Livex (Figs 7, 8, 12 and 13).

In conclusion, the present investigation suggests that Livex, a compound formulation of nine herbal plants, exerts significant protection against erythromycin estolate induced toxicity which may be attributed to its protective action on lipid peroxidation and to the enhancing effect on cellular antioxidant defence contributing to the protection against oxidative injury by erythromycin estolate.

Further experiments are necessary to isolate the active principles of the medicinal plants of Livex and to establish their chemical nature responsible for their antiperoxidative property.

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