



ISSN: 2231-3354
Received on: 05-11-2011
Revised on: 16-11-2011
Accepted on: 05-12-2011

Effect of activit, a herbomineral formulation, on experimentally- induced gastric lesions in rats

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ABSTRACT

Activit, a herbomineral formulation, containing extracts derived from *Mucuna pruriens*, *Withania somnifera*, *Argyrea speciosa*, *Centella asiatica*, *Tribulus terrestris*, *Asparagus racemosus*, *Piper longum*, *Anacyclus pyrethrum*, *Nux vomica*, *Tinospora cordifolia* and Shring bhasma, was studied for its protective effect against gastric lesions induced by ethanol and pylorus-ligation. The formulation was tested at the doses of 125, 250, 500 and 1000 mg/kg body weight (p.o.) in rats for its effect on various gastric and antioxidant parameters. 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 Activit. The increase in the levels of endogenous antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH)] along with membrane bound enzymes (Ca^{2+} ATPase, Mg^{2+} ATPase and Na^+K^+ ATPase) and a decrease in lipid peroxidation (MDA) in both the ulcer models by Activit demonstrated its antioxidant effect. Thus it can be concluded that Activit possesses anti-ulcer activity, which can be attributed to its antioxidant mechanism of action.

Keywords: Activit, herbomineral formulation, anti-ulcer, ulcer-index, antioxidant, membrane bound enzymes.

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INTRODUCTION

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. There is an imbalance between the aggressive (i.e. acid, pepsin, free radicals, H. pylori) and the mucosal protective (i.e. mucus, bicarbonate, prostaglandins) factors in stomach. Thus, drug therapy of peptic ulcer has been commonly targeted at either counteracting the aggressive factors or stimulating the defensive ones (Piper and Stiel, 1986). Despite the progress in conventional chemistry and pharmacology in producing highly effective drugs, modern medicines possess several adverse effects like arrhythmias, impotence, gynaecomastia and haematopoietic changes (Anoop and Jegadeesan, 2003; Mahendran et al., 2002). However, screening plants for active drugs is important and may provide a useful source of new anti-ulcer compounds for developing pharmaceutical drugs or alternatively as simple dietary adjuncts to existing therapies (Borrelli and Izzo, 2001). Thus, the discovery of potential antiulcer agents from plant source is a developing area. Several plants have been screened for anti-ulcer activity and many formulations have been developed by combining extracts of these plants. 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

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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. Thus drugs with antioxidant properties may minimize tissue injury in human diseases (Barry, 1991).

Traditional medicine uses plants to treat gastrointestinal disorders, including peptic ulcers. Since decades many indigenous drugs have been known to possess anti-ulcer activity. The anti-ulcer properties of *Centella asiatica* (Cheng and Koo, 2000; Sairam et al., 2001; Abdulla et al., 2010), *Asparagus racemosus* (Bhatnagar and Sisodia, 2006; Bharati et al., 1996; Mangal et al., 2006), *Tinospora cordifolia* (Sarma et al., 1995), *Withania somnifera* (Bhatnagar et al., 2005) and *Piper longum* (Agrawal et al., 2000) have been proved in various animal models.

The antioxidant properties of *Mucuna pruriens* (Tripathi and Upadhyay, 2002), *Withania somnifera* (Bhattacharya et al., 2001; Mishra et al., 2000; Scartezzini and Speroni, 2000), *Centella asiatica* (VeerendraKumar and Gupta, 2002), *Asparagus racemosus* (Bhatnagar et al., 2005; Kamat et al., 2000), *Nux vomica* (Tripathi and Chaurasia, 2000), *Tinospora cordifolia* (Mathew and Kuttan, 1997) and shring bhasma (Shah and Vohora, 2002) 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 thus aimed to investigate the anti-ulcer effects of Activit (a herbomineral formulation containing the above ingredients) along with its effect on the anti-oxidant enzymes to justify whether the formulation possesses anti-ulcer action by means of its antioxidant effect.

MATERIALS AND METHODS

Composition

Activit, a herbomineral formulation contains extracts derived from *Mucuna pruriens* (L.) DC., *Withania somnifera* (L.) Dunal, *Argyrea speciosa* (L.) Sweet, *Centella asiatica* (L.) Urban, *Tribulus terrestris* (L.), *Asparagus racemosus* Willd., *Piper longum* (L.), *Anacyclus pyrethrum* (L.), *Nux vomica* (L.), *Tinospora cordifolia* Miers. and Shring bhasma.

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. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Experimental Procedure

The animals were divided into five 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 Activit orally at the doses of 125, 250, 500 and 1000 mg/kg body weight, respectively.

Study of anti-ulcer and antioxidant 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, Activit was administered to the animals. During the course of experiment food was withdrawn. After the pretreatment period of 1 hour, 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 in diameter

Score 25: a perforated ulcer

The stomach was then weighed and homogenized in chilled Tris buffer (10mM, pH 7.4) at a concentration of 10% w/v and centrifuged. The supernatant was used for the assays of lipid peroxidation (MDA), 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 ($\text{Na}^+\text{K}^+\text{ATPase}$, $\text{Ca}^{2+}\text{ATPase}$ and $\text{Mg}^{2+}\text{ATPase}$) and proteins.

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

Activit was administered orally to the rats for a period of 10 days. On the 10th day, 1 h after the final dose of Activit, 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). The stomach was then weighed and processed for antioxidant estimations as mentioned in previous section.

Biochemical Estimations

Superoxide dismutase (SOD) was determined by the method of Misra 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 product (malondialdehyde) was estimated by the method of Slater and Sawyer (1971). Membrane bound enzymes namely $\text{Na}^+\text{K}^+\text{ATPase}$, $\text{Ca}^{2+}\text{ATPase}$ and $\text{Mg}^{2+}\text{ATPase}$ 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).

Statistical analysis

Results were expressed in terms of mean \pm SEM. Difference between the groups was statistically determined by analysis of variance followed by Tukey-Kramer Multiple Comparisons test, with the level of significance set at $p < 0.05$.

RESULTS

Anti-ulcer and antioxidant activity in pylorus ligation-induced ulcer method

In the pylorus-ligation model, 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. Activit was found to produce a reduction in ulcer index at all the four doses, the percentage reduction being 1.08%, 11.05%, 30.13% and 42.53%, respectively. All the ulcers were of score 1, 2, 3, and 4 and no perforated ulcers (ulcer score 25) were observed (Table 1).

Table 1: Effect of Activit 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	127.0 ± 3.63
Activit (125mg/kg)	$91.75 \pm 4.66^{\text{NS}}$ (1.08%)	$6.80 \pm 0.51^{\text{***}}$	$2.20 \pm 0.19^{\text{NS}}$	$119.5 \pm 4.17^{\text{NS}}$
Activit (250mg/kg)	$82.50 \pm 8.45^{\text{NS}}$ (11.05%)	$5.05 \pm 0.38^{\text{***}}$	$3.58 \pm 0.22^{\text{***}}$	$110.0 \pm 5.37^{\text{NS}}$
Activit (500mg/kg)	$64.80 \pm 4.17^*$ (30.13%)	$4.08 \pm 0.19^{\text{***}}$	$5.55 \pm 0.32^{\text{***}}$	$99.25 \pm 5.12^{**}$
Activit (1000mg/kg)	$53.30 \pm 1.89^{\text{***}}$ (42.53%)	$2.98 \pm 0.14^{\text{***}}$	$6.88 \pm 0.38^{\text{***}}$	$93.25 \pm 5.89^{**}$
F value	10.714	64.637	81.860	8.050
P value	0.0003	<0.0001	<0.0001	0.0011

Values are expressed as mean \pm SEM. Activit 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.

In the pylorus-ligation model, Activit was found to significantly ($p < 0.001$) decrease the acid volume at all the four doses; and also decreased ($p < 0.01$) the total acidity of gastric fluid at the doses of 500mg/kg and 1000mg/kg. Activit increased the pH significantly ($p < 0.001$) at the doses of 250, 500 and 1000 mg/kg (Table 1).

In the stomach of pylorus-ligated control group there was a significant ($p < 0.001$) increase in the level of lipid peroxidation and decrease in the activities of SOD, catalase, $\text{Na}^+\text{K}^+\text{ATPase}$, $\text{Ca}^{2+}\text{ATPase}$, $\text{Mg}^{2+}\text{ATPase}$ and reduced glutathione level. As evident from the data presented in Table 2, Activit significantly reduced the MDA level and increased the levels of the various endogenous antioxidant enzymes (SOD and catalase), membrane bound enzymes and reduced glutathione to levels of that of normal rats.

Anti-ulcer and antioxidant activity in ethanol-induced ulcer method

In the present study, 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 Activit by 83.00%, 91.85%, 94.76% and 99.48%, respectively (Table 3).

In the ethanol-treated control group the levels of SOD, catalase, reduced glutathione and membrane bound ATPases in the stomach tissue were significantly ($p < 0.001$) reduced with an increase ($p < 0.001$) in lipid peroxidation. Activit significantly reduced the MDA level and increased the endogenous enzymes, reduced glutathione and membrane bound ATPases; thus restoring it back to normal levels (Table 3).

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 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.

The present study was undertaken to evaluate the anti-ulcerogenic effect of Activit, a herbomineral formulation consisting of plants that are mentioned in Indian system of medicine (Ayurveda) for their remedial properties. The anti-ulcer effect of Activit was tested against gastric lesions induced by pylorus-ligation and ethanol, the experimental models related to lesion pathogenesis with production of reactive species.

It is well known that pylorus-ligation causes gastric hypersecretion (Shay et al., 1945; Brodie et al., 1962; Ishii, 1969) due to poorly understood mechanisms. The activation of the vagus-vagal reflex by stimulation of pressure receptors in the antral gastric mucosa in the hypersecretion model of pylorus ligation is believed to increase gastric tonus and secretion. The stomach digestive effect of accumulated gastric juice in the induction of gastric ulcers is well documented in the pylorus-ligation model (Brodie, 1966). The gastric distension produced by accumulated secretion seems to influence the secretion of gastric acid in this model, possibly by increasing the release of gastrin hormone, and consequently further increasing acid secretion (Nagy et al., 1968). In the pylorus-ligation ulcer model, Activit was found to produce a

Table 2: Effect of Activit on the biochemical parameters in stomach of pylorus ligated rats.

GROUPS	Lipid Peroxidation (nmoles of MDA/mg protein)	Reduced Glutathione (μ g of GSH/mg protein)	Superoxide Dismutase (Units/mg protein)	Catalase (μ moles of H ₂ O ₂ consumed/min/mg protein)	Na ⁺ K ⁺ ATPase (μ moles of inorganic phosphorus liberated/min/mg protein)	Ca ²⁺ ATPase (μ moles of inorganic phosphorus liberated/min/mg protein)	Mg ²⁺ ATPase (μ moles 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 ^{***}	0.58 ± 0.14 ^{***}	2.37 ± 0.26 ^{***}	5.93 ± 0.60 ^{***}	1.49 ± 0.10 ^{***}	1.62 ± 0.17 ^{***}	1.54 ± 0.13 ^{***}
Activit (125mg/kg)	9.66 ± 0.55 ^{NS}	1.58 ± 0.30 [*]	2.29 ± 0.12 ^{NS}	5.97 ± 0.15 ^{NS}	2.18 ± 0.13 ^{NS}	2.12 ± 0.14 ^{NS}	2.02 ± 0.14 ^{NS}
Activit (250mg/kg)	7.30 ± 0.42 ^{**}	2.35 ± 0.22 ^{***}	2.30 ± 0.13 ^{NS}	6.03 ± 0.26 ^{NS}	2.80 ± 0.17 ^{***}	2.61 ± 0.36 ^{NS}	2.50 ± 0.15 ^{***}
Activit (500mg/kg)	5.84 ± 0.38 ^{***}	2.63 ± 0.25 ^{***}	3.13 ± 0.09 ^{NS}	6.51 ± 0.29 ^{NS}	3.06 ± 0.13 ^{***}	2.91 ± 0.13 ^{**}	3.14 ± 0.06 ^{***}
Activit (1000mg/kg)	4.90 ± 0.36 ^{***}	3.07 ± 0.19 ^{***}	4.40 ± 0.32 ^{***}	7.46 ± 0.21 [*]	4.26 ± 0.20 ^{***}	3.33 ± 0.13 ^{***}	3.31 ± 0.06 ^{***}
F value	42.061	22.436	36.541	8.792	79.436	11.444	34.538
P value	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001

Values are expressed as mean ± SEM. Control group was compared with normal group. Activit groups were compared with control group. *p<0.05; **p<0.01; ***p<0.001; NS = Non Significant.

Table 3: Effect of Activit on the ulcer index and biochemical parameters in stomach of ethanol-treated rats.

GROUPS	Ulcer Index	Lipid Peroxidation (nmoles of MDA/mg protein)	Reduced Glutathione (μ g of GSH/mg protein)	Superoxide Dismutase (Units/mg protein)	Catalase (μ moles of H ₂ O ₂ consumed/min/mg protein)	Na ⁺ K ⁺ ATPase (μ moles of inorganic phosphorus liberated/min/mg protein)	Ca ²⁺ ATPase (μ moles of inorganic phosphorus liberated/min/mg protein)	Mg ²⁺ ATPase (μ moles 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 ^{***}	0.79 ± 0.17 ^{***}	2.23 ± 0.14 ^{***}	5.12 ± 0.21 ^{***}	2.03 ± 0.11 ^{***}	1.50 ± 0.21 ^{***}	1.49 ± 0.13 ^{***}
Activit (125mg/kg)	48.95 ± 4.39 ^{***} (83.00%)	6.46 ± 0.26 ^{NS}	0.91 ± 0.10 ^{NS}	2.29 ± 0.15 ^{NS}	5.13 ± 0.15 ^{NS}	2.05 ± 0.06 ^{NS}	1.43 ± 0.15 ^{NS}	1.78 ± 0.15 ^{NS}
Activit (250mg/kg)	23.48 ± 1.72 ^{***} (91.85%)	5.90 ± 0.12 ^{NS}	2.20 ± 0.15 ^{***}	3.16 ± 0.23 [*]	5.98 ± 0.13 [*]	2.34 ± 0.13 ^{NS}	1.79 ± 0.20 ^{NS}	2.33 ± 0.09 ^{**}
Activit (500mg/kg)	15.10 ± 2.55 ^{***} (94.76%)	4.16 ± 0.17 ^{***}	2.77 ± 0.18 ^{***}	3.84 ± 0.14 ^{***}	6.81 ± 0.16 ^{***}	4.18 ± 0.17 ^{***}	1.86 ± 0.13 ^{NS}	3.06 ± 0.11 ^{***}
Activit (1000mg/kg)	1.50 ± 0.65 ^{***} (99.48%)	3.96 ± 0.16 ^{***}	3.14 ± 0.15 ^{***}	4.98 ± 0.15 ^{***}	7.43 ± 0.18 ^{***}	5.27 ± 0.03 ^{***}	2.78 ± 0.18 ^{**}	3.22 ± 0.11 ^{***}
F value	208.56	17.128	53.734	49.095	50.473	155.14	18.813	37.081
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Values are expressed as Mean ± SEM. Control group was compared with normal group. Activit 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.

reduction in ulcer index at all the four doses, significant effects observed at 500 and 1000mg/kg. Activit was also found to increase the pH and decrease the acid volume and total acidity of gastric fluid in pylorus-ligation model, thus proving its anti-ulcer activity. These effects of Activit treatment on the parameters that influence the initiation and induction of ulceration may be considered as highly desirable property of an anti-ulcerogenic agent.

Ethanol is a necrotizing agent that produces gastric ulceration by causing direct damage to the mucosa independent of gastric acid secretion (Takase et al., 1994). Studies focusing on the pathogenesis of ethanol-induced injury have suggested that several factors are implicated in such processes: products of arachidonate metabolism e.g. leukotriene (Peskar et al., 1986), mast cell secretory products (Oates and Hakkinen, 1988) and reactive oxygen species (Pihan et al., 1987; Mizui et al., 1987). In the ethanol-induced ulcer model, administration of ethanol produced significant ulcers in the control group, which were significantly reduced in all the Activit treated groups.

Thus, it was found that Activit prevented the mucosal lesions induced by both, pylorus-ligation and ethanol administration.

Oxidative stress plays an important role in the pathogenesis of various diseases including gastric ulcer, with antioxidants being reported to play a significant role in the protection of gastric mucosa against various necrotic agents (Trivedi and Rawal, 2001). 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*. As compared to normal rats, pylorus-ligation and ethanol administration was found to increase lipid peroxidation and decrease SOD, catalase and reduced glutathione in the control groups, thus leading to oxidative stress. Thus, results obtained in the present study also indicate alterations in the antioxidant status of pylorus-ligated and ethanol-treated rats.

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, 1995). Administration of Activit in both the models 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 control groups reported an increase in lipid peroxidation. The study has revealed a significant decrease in lipid peroxidation by Activit in both the experimental models, which suggests its protective effect.

Na⁺K⁺ATPase, Ca²⁺ATPase and Mg²⁺ATPase are membrane bound enzymes. The inactivation or decrease of ATPases on pylorus-ligation and ethanol administration could be due to enhanced lipid peroxidation by free radicals (Gubdjanson et al., 1983). An enhancement in the membrane bound ATPases in both the ulcer models was observed in Activit treated groups.

CONCLUSIONS

Overall data clearly indicates that Activit 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. The study thus provides a basis for the clinical use of Activit in several clinical conditions involving oxidative stress, including ulcers. This was also proved in our earlier study (Bafna and Balaraman, 2004) where Activit due to its antioxidant activity exerted a protective effect in Isoprenaline-induced cardiac damage and Cisplatin-induced renal damage, the animal models reflecting free radical-induced tissue damage.

ACKNOWLEDGEMENT

The authors are thankful to The Head, Pharmacy Dept., M. S. University of Baroda, Vadodara (Gujarat) for providing all the facilities required to carry out this research work. The authors also extend their thanks to Ayur Herbals Pvt. Ltd., Baroda for providing the formulation, Activit; and UGC for providing financial assistance for successfully carrying out this work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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