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Antioxidant activity of Arthritin-A polyherbal formulation

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In complete freund's adjuvant induced arthritis in male albino rats, a significant increase in serum lipid peroxidase besides increase in paw swelling and a significant decrease in superoxide dismutase, glutathione peroxidase and total reduced glutathione levels were observed. Arthritin produced a marked reversal of these enzyme levels, besides a significant reduction in paw swelling. The results suggest that, the polyherbal formulation 'Arthritin' exerts its effects by modulating lipid peroxidation and enhancing anti-oxidant and detoxifying enzyme systems.

Keywords: Arthritin, Glutathione peroxidase, Lipid peroxidase, Superoxide dismutase

Oxygen free radical induced cellular damages have been implicated in many pathobiological conditions, viz. malignancy, ageing process and degenerating diseases, etc[1]. Increased generation of oxidative free radicals (or) impaired antioxidant defence mechanisms, have been implicated in the ageing process, neurodegenerative conditions, including Parkinsonism and Alzheimer's disease, chronic stress induced/perturbed homeostasis, including immuno depression, inflammation, diabetes mellitus, peptic ulcer and other diseased conditions[2].

Major natural defence system comprises oxidative free radical scavenging enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), Lipid peroxidase (LPO) and total reduced glutathione (TRG). Deficient functioning of these enzymes leads to accumulation of toxic oxidative free radicals and consequent degenerative changes.

Rheumatoid arthritis is a chronic progressive inflammatory disease affecting the synovium, cartilage and bone. The immuno complexes in the synovial fluid activate the complete system, attracting polymorpho nuclear leucocytes into the joint space[3]. Phagocytosis of the complex triggers the release of PMNL, lysosomal enzymes and free radicals into the joint space, which may cause damage[3]. There are many herbal formulations, available in the market for treating arthritis. One such formulation chosen for the study was Arthritin, a polyherbal formulation consisting of extracts of *Acacia arabica, Withania somnifera*[4]*, Juniperus communis, Asparagus racemosus, Tinospora cordifolia, Tribulus terrestris, Anethum sowa, Curcuma zerumber, Zingiber officinalis*.

The present study has been designed to investigate the *in vivo* antioxidant activity of Arthritin by estimating superoxide dismutase (SOD), glutathione peroxidase (GPX), Lipid peroxidation (LPO) and total reduced glutathione (TRG) in blood. Indomethacin, a standard antiinflammatory[5] drug was used for comparison.

Materials and Methods

Drug sample—Arthritin, an Ayurvedic formulation containing several important herbs[6] was obtained from M/s Herbal Galanicals Purasawalkam, Chennai 600 007. Indomethacin was from M/s. Goldstein Laboratories Pvt Ltd., Chennai and complete freund's adjuvant was obtained from M/s. Sigma Chemicals.

Animals—Adult male Swiss strain wistar albino rats weighing 150-200 g was obtained from Institute's Animal house and were housed in colony cages (5-6 per cage), at an ambient temperature of 25º ± 2º C and 45-55% RH with a 12:12hr L:D. The rats had free access to standard pellet chow and drinking water. Experiments were conducted between 09 and 14 hrs. The experimental protocol was subjected to the

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scrutiny of the institutional animal ethical committee and was cleared before starting. The animals were handled as per guidelines of animal care.

The animals were divided into five groups of six each. Group I served as normal control. Group II served as positive control whereas group III was kept as standard control and groups IV, V and VI were treated as drug (Arthritin) treated. Arthritis was induced in the rats of group II, III, IV, V and VI on 0 day by administering 0.1 ml of complete freund's adjuvant, intradermally in the right hind paw. Rats in group III and groups IV, V and VI were given 0.05 mg/kg, po, standard drug indomethacin and aqueous suspension of Arthritin, at 0.3, 0.5 and 1 g/kg, po, respectively from 11th to 18th day. Paw volume and body weight were measured on 0, 7th, 11th and 18th day. The paw volume was measured using plethysmograph[6]. On 19th day, blood was withdrawn from retro orbital plexus, centrifuged, and plasma was separated immediately.

Preparation of haemolysate—After separation of the plasma and the buffy coat, the red cells were washed four times with 0.9% saline and were centrifuged for 10 min at 300 rpm after each wash. The cell lysis was carried out by adding a known volume of ice cold distilled water to the washed cells. This was allowed to stand for 2 hr at 4°C and the resultant haemolysate was used to estimate SOD, GPX and GSH, as per the method of Quist[7].

LPO estimation—LPO estimation was estimated in plasma as per Okhawa *et al.*[8] in terms of thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) was taken to represent the TBARS and the pink colour formed was measured at 532nm. The MDA content was expressed in nmoles/dl plasma.

TRG estimation—TRG in haemolysate was estimated in presence of Phosphate buffer, which reacts with DTNB reagent to give a yellow colour and the intensity of the colour was measured as per Moron *et al* [9].

Data obtained were analysed statistically using Student's *t* test. $P< 0.05$ was considered as significant.

Histopathological examination—Histopathological studies were done in hind limb joints of the animals. The joint bone tissues were fixed in formalin, decalcified and embedded in paraffin blocks. Sections (10-12 μm thick) were stained with haemotoxylin and eosin and were examined under microscope and photographed[10] .

Results

The body weight was not significantly altered in any of the groups. A considerable reduction in paw swelling was observed in groups III, V and VI compared to group II reflecting the immunological protection rendered by Indomethacin and Arthritin respectively (Fig. 1).

Arthritin showed inhibition of LPO in groups III, IV, V and VI. It showed a significant reduction in groups V and VI, when compared to standard drug treated group. The elevated level in Group II, indicates that the tissues were subjected to increased oxidative stress, whereas a statistically significant reduction of LPO was observed in Arthritin and standard drug treated groups.

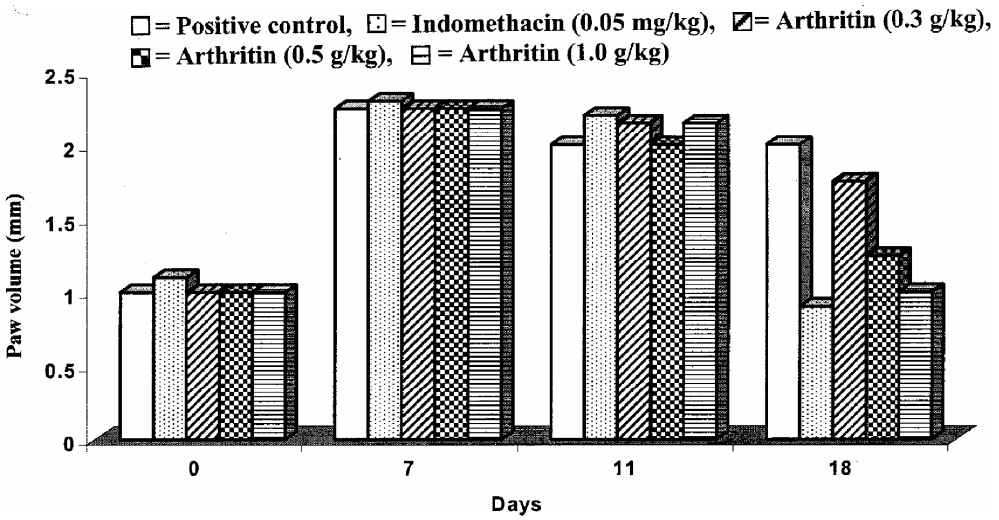

Fig. 1—Effect of Arthritin and indomethacin on paw volume changes of arthritic rats

Glutathione content was lowered in the disease induced group, whereas the same was significantly elevated in standard and Arthritin treated groups.

A reduced activity of superoxide dismutase was observed in the disease induced group compared to standard and Arthritin treated animals.

A significant increase in GPX was observed in the standard and drug treated groups compared to the disease induced group. The biochemical parameters are presented in Table 1.

Histopathological studies revealed marked infiltration of leukocytes and eosinophilic exudate in the synovial membrane on 18th day of arthritis induced group. Regeneration of synocytes and disappearance of inflammatory exudate were observed in standard drug treated group. Arthritin treated groups also showed normal synovium (Fig. 2).

Discussion

Free radical oxidative stress has been implicated in the pathogenesis of variety of diseases, resulting usually from defective natural antioxidants, hence therapy should include either enzymes or agents natural antioxidant enzymes or agents, which are capable of augmenting the function of these oxidative free radical scavenging enzymes[4]. By virtue of their proposed properties and clinical use in Ayurveda, polyherbal formulations may provide potential, therapeutic intervention against oxidative threats, both in health and diseased conditions[5]. Phyto constituents such as phenols, flavonoids, terpenoids, alkaloids, glycosides present in the various constituents of the polyherbal formulation, act as natural free radical scavengers[11]. Neurodegenerative diseases like ageing, rheumatoid arthritis and metabolic diseases like diabetis, hypertension etc are considered to be free radical mediated diseases[12].

In arthritic condition the granulocytes and macrophages accumulate in the affected area and produce large amounts of superoxide and hydrogen peroxide radicals[3] and the estimation of these active species in the disease induced and the drug treated animals help in assessing the free radical scavenging property and indirectly the antiarthritic potential of the formulation.

Paw swelling is one of the major factor in evaluating the degree of inflammation and therapeutic efficacy of the drug. The arthritic rats showed soft tissue swelling around the ankle joints during the acute phase of arthritis. A significant reduction in paw swelling was observed in both standard and drug treated groups showing the anti inflammatory potential of Arthritin.

Extent of LPO is measured through malondialdehyde activity (MDA), a pro oxidant factor which determines the oxidative damage. In the present study, MDA contents of arthritis induced animals (Group II) were significantly increased compared with the normal group. This indicates that the tissues were subjected to increased oxidative stress while a statistically significant ($P < 0.05$) reduction of lipid peroxidation activity was observed in standard drug and Arthritin treated groups. The reduction of MDA caused by Arthritin could be due to the presence of potent anti-oxidants such as *Withania somnifera*[11], *Alpinea galanga*, *Asparagus racemosus*[13], *Curcuma zerumber* in the formulation.

Glutathione is an intracellular thiol rich tripeptide which plays a major role in the protection of cells and

Table. 1—Effect of Arthritin on lipid peroxidation, glutathione peroxidase, total reduced glutathione and superoxide dismutase of control and experimental animals

[Values are mean ± SD of 6 animals in each group]

Treatment	Control	Arthritis induced	Indomethacin 0.05mg/kg;po	Arthritin (g/kg;po)		
				0.3	0.5	1
LPO (nmole/dl plasma of MDA)	0.34± 0.08	0.73± 0.46^{b}	0.411± 0.027^{a}	0.69 ± 0.36^{a}	0.61± 0.16^{a}	0.48± 0.19^{a}
GSH (nmole of GSH oxidised / min/ml haemolysate)	19.33± 8.52	8.78± 5.49^{b}	10.52± 0.25^{b}	9.68±5.89^{a}	11.00± 5.75^{a}	16.08± 3.91^{b}
SOD(μ/ml haemolysate)	27.49± 7.64	18.03± 6.23^{c}	22.4 ± 6.8^{b}	13.56± 8.82 ^{a}	14.67± 9.83^{a}	27.00± 12.07^{a}
GPX(mg of GSH/ml haemolysate)	10.82± 1.76	8.805 ±0.52^{b}	10.11±0.14^{b}	8.98±3.58^{a}	9.26± 2.75^{a}	10.27± 3.09^{b}

P values : $^{a}< 0.05$ $^{b}< 0.01$ $^{c}<0.001$

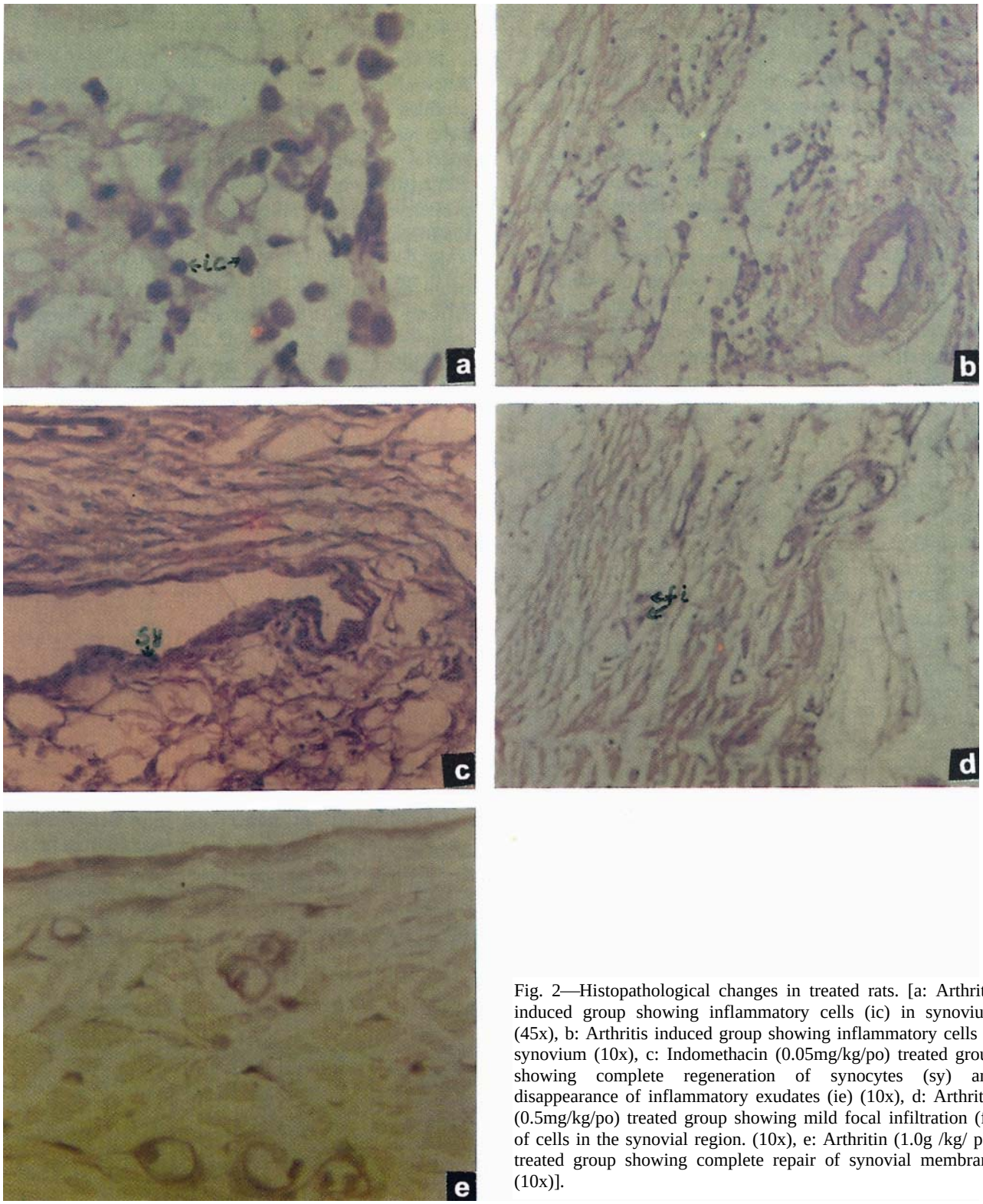

Fig. 2—Histopathological changes in treated rats. [a: Arthritis induced group showing inflammatory cells (ic) in synovium (45x), b: Arthritis induced group showing inflammatory cells in synovium (10x), c: Indomethacin (0.05mg/kg/po) treated group showing complete regeneration of synocytes (sy) and disappearance of inflammatory exudates (ie) (10x), d: Arthritin (0.5mg/kg/po) treated group showing mild focal infiltration (fi) of cells in the synovial region. (10x), e: Arthritin (1.0g /kg/ po) treated group showing complete repair of synovial membrane (10x)].

tissue structures[14] . Low concentration of glutathione has been implicated in rheumatoid arthritis[15]. In the present study glutathione content was found to be lower in the disease induced group. While the same was elevated in standard and Arthritin treated groups thereby indicating the free radical scavenging activity.

Superoxide dismutase, an enzymatic antioxidant catalytically scavenges the super oxide radical and this provides the first line of defense against free radical damage[15]. A reduced activity of SOD was observed in the arthritis induced group compared to the standard and drug treated groups.

This may be due to the presence of ingredients such as *Withania somnifera*[11], *Asparagus racemosus*, *Tribulus terrestris*[13] and *Zingiber officianalis* in Arthritin, causing elevation of SOD and GPX levels.

Glutathione peroxide catalyses the reduction of hydrogen peroxide in presence of glutathione to form water and oxidized glutathione. A reduced glutathione peroxidase activity was observed in disease induced group compared to normal, standard and drug treated groups, indicating the reduction of hydrogen peroxides formed during free radical damage.

Superoxide is inactivated by SOD, the only enzyme known to use a free radical as a substrate. However, the free radical scavenging activity of SOD is effective only when there is an increase in GPX activity. Since SOD generates hydrogen peroxide as a metabolite, which is more toxic than oxidative radicals and has been removed by GPX. Thus concomitant increase in GPX activity is essential for a beneficial effect in SOD activity[16].

The repair of synovium showed by the Arthritin treated groups further confirmed the antiarthritic potential of Arthritin. Therefore from the results of the present study, it may be concluded that 'Arthritin' possesses a significant anti-inflammatory activity and free radical scavenging activity and is responsible for antiarthritic activity. Further clinical studies are needed to establish the efficacy in arthritic patients.

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