RESPONSE OF ALPHA-1 GLOBULINS OF SERUM DURING INFLAMMATION

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INFLAMMATION induced by various agents is reflected in the production of modulator proteins by the liver[1]. These proteins, referred to as Acute Phase Reactants[2] (APR), are circulated in the blood stream. Their early onset (< 5 hr after injury), inhibitory nature of other proteases liberated during tissue injury and quantity of their acute phase response in relation to the degree of inflammation suggest that, their physiological function is specifically related to the inflammatory process[3,4]. Darcy's protein[5], α-1 acid glycoprotein[6] and the recently reported α-1 Major Acute Phase protein (α-1 MAP)[4] respond to inflammatory stimuli, as a result, levels of APR in the serum increase 10–20 fold. It may be surprising, that treatment with non-steroidal anti-inflammatory drugs has none or only weak influence on most of the APR, contrary to steroidal anti-inflammatory drugs[7]. However, no information is available, whether an Indian Medicine has any role to play on APR. Hence the present work on Ashwagandha was undertaken.

Ashwagandha, a drug from *Withania somnifer* Dun., was investigated in inflammed rats. Inflammation was induced by subplantar injection of 0·1 ml of 3% formalin. Ashwagandha in therapeutic doses (100 mg/100 g bw rat)[8] was administered to a group of rats an hour before the induction of inflammation. Blood was collected from the control and treated rats after 6 hr. The degree of inflammation was also monitored, plethysmographically. Serum proteins were analysed by crossed immuno-electrophoresis. The method of Clarke and Freeman[9] was followed with some modifications. 2 μl of undiluted serum sample (pooled) were used for CIE and immunoprecipitation peaks were stained for proteins with Coomassie Brilliant Blue. Quantitation of the precipitation peaks was done using an electronic area meter (LAMDA INST CO. USA).

Figure 1A showed the CIE profile of normal serum proteins. About 29 to 30 individual peaks were resolved in the immunoelectrophoretograms as reported by earlier workers[10]. Peaks 1 and 2 in the α-1 region were increased 390% and 200% respectively during inflammation. Drug treatment significantly reduced both the peaks. Peak 1 was reduced by 180% and Peak 2 showed 116% reduction in serum level. Ashwagandha showed 46% anti-inflammatory action on paw volume.

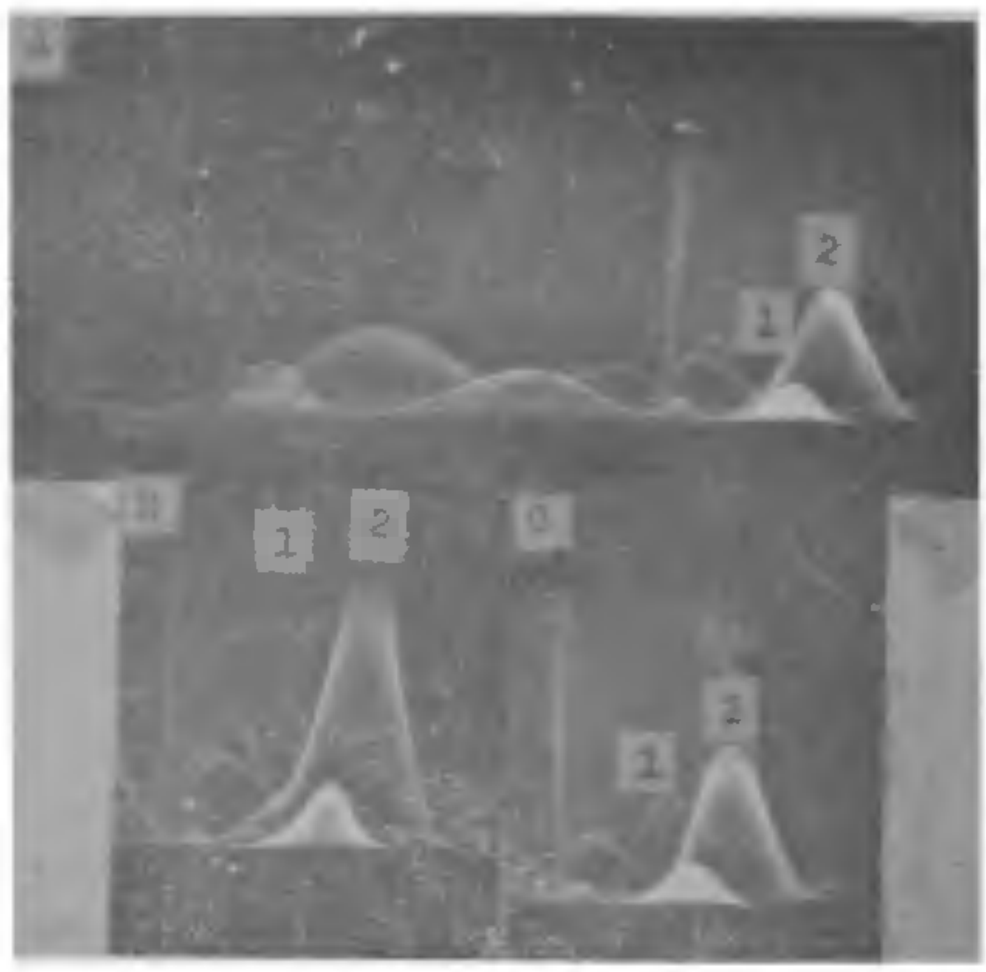

FIG. 1. CIE profile of pooled serum obtained from A—normal rats; B—inflammed rats and C—inflammed rats treated with Ashwagandha.

2 μl of pooled serum from each group was used for immunoelectrophoresis in Agarose gels (5 × 8 cm), for 2 hr at 12 V^{-cm} in Tris-Glycine buffer (38 mM, pH 8 6). Second dimension electrophoresis was done at right angles to the first direction into a gel containing total antibodies to rat acute phase serum. Immunoprecipitation and separation was allowed for 22 hr at 3 V^{-cm} in the same electrophoretic buffer.

Peaks 1 and 2 in α–1 region by their electrophoretic mobility, glycoprotein staining and response to inflammation may correspond to the above mentioned α–1 acid glycoprotein and α–1 major acute phase protein. Only the α–2 GP level in the serum is considered as a sensitive index of inflammation because of its maximum response during inflammation[10]. The present report is quite interesting in the sense, that Ashwagandha, an Indian Medicine, influences most of the APR in a very short duration of treatment during inflammation which is first of its kind to our knowledge. Since the degree of inflammation is also reduced by Ashwagandha treatment, the possibility, of course, remains that Ashwagandha possessing several potent Withanoloids[11] (steroidal lactones) may act in the regulation of modulator protein synthesis. Further investigations are in progress towards the regulation of protein synthesis by active component/s of the drug.

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PHENOXY ANTIMONY(V) TETRACHLORIDE

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THE poor acceptor properties of metal alkoxides are mainly due to their polymeric nature[1]. Incorporation of an electron withdrawing group in place of alkoxide reduces the degree of polymerisation[2,3] and enhances their acceptor properties[1]. Surprisingly, as compared to the metal alkoxides[5-8], very little information is available about the metal phenoxides. Titanium(IV) phenoxide has been found to behave as solvo acid in fused phenol[9]. Lewis acid character of phenoxides of titanium(IV)[10], niobium(V) and tantalum(V)[11] has already been established.

Ethoxyantimony(V) tetrachloride is dimeric[12] and its acceptor properties have been established by the formation of addition compounds with a number of donor molecules[13]. A similar study in the case of phenoxides of antimony is lacking. Accordingly phenoxyantimony(V) tetrachloride has been prepared for the first time and its acceptor properties have been established by isolating a number of addition compounds with bases.

When equimolar solutions of antimony(V) chloride and phenol in carbon tetrachloride were mixed at $-10^{\circ}C$, a moisture sensitive yellow crystalline compound of composition $SbCl_4(OC_6H_5)$ separates out which turns brown when brought to room temperature. Compound of similar composition was obtained when $SbCl_4(OEt)$ and pherol were mixed in equimolar ratio in carbon tetrachloride suggesting low reactivity of Sb-Cl bond as compared to Sb-O bond. Molar conductance values of 10^{-3} molar solution in nitrobenzene is low (Table I) which suggest it to be a non-electrolyte but the molar conductance value of millimolar solutions in acetonitrile is 26·4 $ohm^{-1}\ cm^2\ mole^{-1}$ which is very high value for a non-electrolyte. By analogy with the ion $SbCl_4(CH_3CN)^+ \cdot SbCl_6^-$ in acetonitrile[15], possible ions of $SbCl_4(OC_6H_5)$ in acetonitrile may be postulated as

$$2SbCl_4(OC_6H_5) \xrightarrow{CH_3CN} [SbCl_2(OC_6H_5)_2 \cdot CH_3CN]^+ + SbCl^{6-}.$$

Other possible ions such as $[SbCl_3(OC_6H_5) \cdot CH_3CN]^+$ $[SbCl_5(OC_6H_5)]^-$ etc. cannot be ruled out.

Molecular weight determinations of very dilute solution in nitrobenzene give an average value of 690 indicating it to be dimer in this solvent. I.r. spectrum of the compound shows intense bands at 638 and 336 cm^{-1} due to terminal ν(Sb–Cl) stretching modes in octahedral environment[16]. No band has been observed below 300 cm^{-1} where bridging Sb⟨Cl⟩Sb stretching modes are expected to lie and thus excludes the possibility of dimerisation through chlorine bridging. Terminal antimony-oxygen stretching modes are expected to lie between 540-590 cm^{-1} but no band has been observed in this region: Instead a sharp band at 520 cm^{-1} with a shoulder at 495 cm^{-1} has been observed which has been assigned to Sb⟨O⟩Sb stretching modes[12,17] suggesting that dimerisation of $SbCl_4(OC_6H_5)$ takes place through the phenoxy group. Similar observations[18] have been made in the case of the compound $NbCl_4(OC_6H_5)$ wherein polymerisation takes place through the phenoxy group. A possible dimeric structure for the compound $SbCl_4(OC_6H_5)$ where each antimony atom has an octahedral environment may be proposed as