9 DISCUSSION
DISCUSSION

Alcoholic extract (WC-1), total alkaloids(WC-2) and aqueous extract (WC-3) of the fruits of Withania coagulans produced signs of mild CNS depression in rats but the animals never passed to hypnotic stage. This effect was observed by the docile nature of the animals, reduced fright response in animals exposed to auditory stimulus and prolongation of hypnosis due to pentobarbitone. However, the effect did not appear to be due to any generalised depression of CNS because these showed no analgesic effect and did not protect the animals against metrazol and maximal electroshock induced seizures. On the other hand, these aggravated the metrazol and methylamphetamine toxicity and stimulated respiration. Reserpine has been shown to exacerbate metrazol seizures (Lewis, 1958) but reduce the mortality in amphetamine treated aggregated mice (Burn and Hobbs, 1958). The extracts resemble reserpine in aggravating the metrazol toxicity but differ from it in reducing amphetamine toxicity.

Fruits of W. coagulans have been found to contain a number of alkaloids. It is possible that the variety of actions of the total alkaloids on CNS are due to different alkaloids.
VO-1 and WO-2 potentiated the pentobarbitone induced hypnosis significantly. The hypnotic potentiation action of the many drugs is related to hypothermic action of the drugs (Swinyard et al, 1959; Malhotra et al, 1965 b), but in the present case, this does not seem to be the sole mechanism as only insignificant hypothermia has been produced with VO-1 or WO-2. A comparison of the protective dose ($PD_{50}$) against fright response in animals exposed to auditory stimulus showed that the total alkaloids are about 9 times more active than total alcoholic extract. Moreover, pentobarbitone sleeping time increased from the control mean value of 60 min. to 89 min. and 98 min. with 1 gm/kg of VO-1 and 200 mg/kg of WO-2 respectively. It again shows that the alkaloids are 5 times more active than alcoholic extract.

VO-1 and WO-2 reduced the convulsions produced by auditory stimulus but not of metrazol or electroshock seizures. This may be due to the fact that the mechanism of the convulsions in two cases is different (Corn et al, 1955).

WO-4 at 100 mg/kg dose i.p. did not produce any effect on CNS but 30 mg/kg i.v. dose produced some
taming effect. All the extracts tested seem to be non-toxic since the alcoholic extract up to a dose of 10 gm/kg, alkaloids up to a dose of 6.4 gm/kg p.o. and 2.5 gm/kg i.p. in rats and the withanolide up to a dose of 625 mg/kg i.p. in mice did not show any mortality within 48 hrs. Moreover, these are non-toxic to liver. On the contrary W0-3 and W0-4 have been found to possess significant protective effect against CCl₄ induced acute hepatotoxicity.

WC-1(1 gm/kg) and WC-2(200 mg/kg) exhibited significant anti-inflammatory activity in subacute inflammation induced with formalin and also reduced granulation tissue formation. Acute inflammation produced with egg albumin too was reduced by these extracts. However, these extracts have not been found to be effective in acute model of inflammation induced with formalin.

The withanolide (W0-4) has been found to possess significant anti-inflammatory property in subacute conditions at dose level of 10 mg/kg when tested against formalin induced oedema and granulation tissue formation. On weight basis it has been found to be about 5 times more active than phenylbutazone and almost equipotent to that of hydrocortisone.
Since the withanolide has been found to be almost as active as hydrocortisone and its concentration in fruits is about 30 mg%; the anti-inflammatory effect of the fruits can be mainly attributed to this withanolide though the alkaloids and some other constituents have additive effect.

The withanolide has been found to be free from any apparent effect on CNS. In view of the significant anti-inflammatory effect of the compound and wide margin of safety, the withanolide appears to be a promising anti-inflammatory agent of this group. The present study also substantiates the fact that the C-28 steroidal lactones have significant anti-inflammatory and anti-arthritic activity (Pugner, 1973; Sethi, 1976).

A major problem encountered in the clinical use of presently available corticosteroids is that of increased susceptibility to bacterial infection by lowering the host resistance to microbial infection (Adlam et al, 1983; Dale and Petersdorf, 1973; Robinson et al, 1974; Jasani, 1979). In addition, the corticosteroids have undesirable metabolic and endocrine effects. Many of the withanolides have been observed to possess anti-
bacterial as well as anti-inflammatory activity. Thus the withanolides, if found otherwise free from toxic effects, would have a significant advantage over corticosteroids as an anti-inflammatory agents.

Formalin induced arthritis and cotton pellet granuloma methods are common animal models for assessing the anti-inflammatory activity of drugs. As the total alkaloids, alcoholic extract and the withanolide have been found to have significant anti-inflammatory effect when tested by these models, the use of *withania coagulans* in chronic inflammatory conditions is justified by this study.

In the present investigations, WO-1, WO-2 and WO-4 have been used in doses of 1 gm/kg, 200 mg/kg and 10 mg/kg body wt. respectively. Comparison of the anti-inflammatory effect of various preparations against formalin induced arthritis and cotton pellet technique proved that WO-2 and WO-4 are more active than WO-1.

From these results it can be concluded that total alkaloids are 5 times more active than alcoholic extract and the withanolide is more than 100 times active than the alcoholic extract.
So far no attempt has been made to elucidate the mechanism of anti-inflammatory effect of the withanolides. However, it may be assumed that the withanolides being steroidal in nature these may be acting like other steroidal anti-inflammatory agents viz. by (i) producing a factor that stimulates migration of polymorphonuclear leucocytes (Stevenson, 1973); (ii) their inhibitory effect on fibroblast (Gray et al, 1971); (iii) inhibiting the activator of plasmogen (Vassalli et al, 1976); (iv) decrease of formation of prostaglandin and related substances like endoperoxides and thromboxanes (Gryglewski et al, 1975; Hong and Levine, 1976; Blackwell et al, 1978).

Various extracts especially WC-1a, WC-2 and WO-3 caused acute fall in blood pressure returning to normal followed by prolonged moderate fall in blood pressure and stimulation of respiration (p<.05). WC-2a produced acute fall in blood pressure and WC-2b produced moderate prolonged fall in blood pressure. The hypotensive response especially the initial fall of blood pressure seems to be mainly due to direct depressant effect on the myocardium as the hypotensive effect was not blocked by atropine, propranolol, mepyramine, indomethacin and hexamethonium bromide. The depressant effect on the
frog heart, rabbit Langendorff preparation and ECG studies further support this contention. However, partial central effect for hypotensive response cannot be ruled out. These extracts did not produce any vasodilatation in rat hind limb preparation.

The stimulation of respiration seems to be partly secondary to hypotensive effect and partly due to some respiratory stimulant component because the drug given by intragastric route caused stimulation of respiration without causing concomitant fall of blood pressure. The hypotensive effect and stimulation of respiration with water soluble part of the alcoholic extract of the fruits has been reported by Siddique et al (1963) also. The present study confirms their findings. Further, total alkaloids have cardiovascular effect similar to that of alcoholic extract. Thus the alkaloids are the main active principles responsible for hypotensive response.

Phytochemical together with pharmacological studies further reveal that the water soluble alkaloids are mainly responsible for the immediate transient fall of blood pressure due to direct depression of the
myocardium. The water insoluble alkaloids and other constituents are responsible for sustained hypotensive effect.

In the present study the Withanolide (WO-4) also caused moderate fall in blood pressure (34±2.1 mmHg) and decreased in mean arterial pressure in dogs when administered in alcoholic solution at 5 mg/kg. The hypotensive effect was blocked by atropine (2 mg/kg) but not by propranolol or mepyramine. The hypotensive response was less marked with the suspension of withanolide at the same dose. The insolubility of the drug might be responsible for this difference since the insoluble cardiac aglycones are invariably less potent than the soluble ones (Fieser and Fieser, 1959). From the results it is evident that the hypotensive effect is cholinergic in nature and the possible site of action may be the myocardium. The ECG studies on dogs and the negative inotropic and chronotropic response on the rabbit Langendorff preparation further support this contention.

Positive inotropic and chronotropic effect observed in frog heart with the withanolide could be due to species differences as many other lactones and
glycosides exert different responses in amphibians and mammals (Chen et al., 1936; Chen et al., 1940; Krayer et al., 1942; Krayer, 1943; Krayer et al., 1943; Mendez, 1944; Fieser and Fieser, 1959; Tanz and Kerby, 1962).

The withanolides have close structural similarity to aglycones of the cardiac glycosides in possessing hydroxyl group at C14 and a 6-membered unsaturated lactone attached to a steroidal ring at C-20 instead of C-17 as given in Fig. 34.

As the pharmacological activity of the glycosides resides in aglycone (Chen and Henderson, 1954; Fieser and Fieser, 1959; Tanz and Kerby, 1962), the withanolide was anticipated to have cardiotonic effect like the aglycones but in the present study it was found to have negative inotropic and chronotropic effects. Even the hypotensive response is contrary to the effects of cardiac aglycones which cause an increase in arterial blood pressure in man.

Although the effects of the withanolide are not similar to those of cardiac glycosides, the possibility of cardiotonic response with other withanolides cannot be ruled out at present since the screening of a large number of cardiac glycosides and aglycones has revealed that the position of lactone ring attached to ring D and other moieties present in the steroid ring effect the potency of cardiac glycosides considerably (Chen and Henderson, 1954; Fieser and Fieser, 1959; Tanz and Kerby, 1961; Imai et al, 1965; Haustein et al, 1973; Haustein, 1974 a; Haustein and Hauptmann, 1974b; Haustein, 1980a; Haustein and Glusa, 1980b). Structure of various rings and spatial arrangement in the steroid ring system also effect the potency of the glycosides (Sayers and Solomon, 1960; Tamm, 1963). Another reason for the absence of positive cardiotonic response may be due to the fact that lactone ring at C-20 does not give the same type of response as when at C-17.

The withanolide produced stimulation of respiration which seems to be central since the stimulation
of respiration occurred even with the suspension of withanolide which did not cause a significant fall in blood pressure.

The W0-3 and W0-4 were found to be significantly protective against carbon tetrachloride induced acute hepatic damage in albino rats. The withanolide significantly reduced the pentobarbitone induced sleeping time and serum levels of GOT and GPT. Results were further confirmed by histopathological examination of liver tissues. The results were comparable with the equivalent dose of hydrocortisone. These studies substantiate the use of withania coagulans, fruits in chronic liver complications (Kirtikar and Basu, 1933).

Though some of the withanolides especially withaferin A, withanolide D and E have been known to have antitumor (Shohat et al, 1970; Shohat and Joshua, 1971; Shohat and Joshua, 1971; Shohat et al, 1978; Chowdhury and Neology, 1975) and antibacterial (Kurup, 1956; Ben-Efrain, 1962) activity, the withanolide under present investigation (WO-4) did not show any antitumor activity when tested against P388 lymphocytic leukemia. It was found to have only mild antibacterial activity when
tested against standard strains of *E. coli* and *Staph. aureus*. The present study and reports by Sethi et al (1973) and Chatterjee et al (1980) show that antibacterial activity of withanolides is a function of their structural constitution.

**WO-1**, **WO-2** and **WO-3** were found to have varying degree of relaxant effects on the various smooth muscles in different species of laboratory animals. The extracts produced much better relaxation of isolated rat's ileum as compared to that of rabbit or guinea pig. These antagonised the spasm induced by acetylcholine, histamine and barium chloride on the various smooth muscles. In general, the pattern of relaxant activity of the various extracts is similar to that of papaverine with the differences that these are much weaker relaxants as compared to papaverine. The mechanism of action of various extracts on smooth muscles is papaverine like direct non-specific musculotropic action. **WO-2b** caused the minimum relaxant effect whereas **WO-2a** was the most active in this respect.

Most of the steroidal compounds viz. cardiac glycosides, glucocorticoids, estrogens, progestins and androgens are metabolised primarily in the liver and excreted as glucuronides or sulphate. It is most likely that **3β**-
hydroxy-2,3, dihydrowithanolide and other withanolides being steroidal in nature are metabolised in the liver and excreted in urine like other steroidal compounds.

In general, the alcoholic extract and total alkaloids of *Withania coagulans* have been found to have qualitatively similar effects as those of alcoholic extract and total alkaloids of *Withania ashwagandha* Kaul both of which cause mild CNS depression, moderate prolonged hypotension, bradycardia and respiratory stimulating activity, anti-inflammatory and smooth muscle relaxant effect of musculotropic type (Malhotra et al, 1960a and b; Malhotra et al, 1961; Malhotra et al, 1965a and b). Moreover the active principles responsible for the actions on CNS, CVS and inflammation in both cases are alkaloids. In our present study withanolide has also been found to be responsible for anti-inflammatory effect. This may be so in *W. ashwagandha* also as some withanolides have recently been isolated from this source too (Subramanian and Sethi, 1971a).

Various crude extracts of *W. somnifera* roots have been found to have sedative and antiarthritic effect. Some of the withanolides isolated from *W. somnifera* have
also been found to have anti-inflammatory, immunosuppressive and antibacterial actions. Therefore, in general, genus *Withania* appears to have CNS depressant, anti-inflammatory and hypotensive properties.

The use of *W. coagulans* fruits in Ayurvedic and Unani systems of medicine as a sedative and their use in skin diseases, piles, eye affections and chronic liver complications appear to be substantiated by the present pharmacological investigations. However, their use as diuretic could not be rationalised. Present phytochemical together with pharmacological studies have revealed that the alkaloids are mainly responsible for effects on CNS and CVS whereas withanolide have anti-inflammatory and protective effect on CCl₄ induced hepatotoxicity though the alkaloids and other minor constituents have additive effects. Aqueous extract of the fruits used in Ayurvedic system contains all the active constituents viz. alkaloids, withanolides and enzymes responsible for various effects but it deteriorates on storage.

In view of the interesting and varied biological activity of the extracts especially of the withanolide, further assessment of metabolic and endocrine effects of the withanolide is warranted. It is also desirable to isolate other active principles of the fruits and screen them pharmacologically.