DISCUSSION
The results of the present study elucidate:

1. the potential of the leprostatic drug dapsone (DDS) to promote hepatocarcinogenesis and its dose-response relationship,

2. the possible relationship, if any, between the promoting potential of the drug and its reported ability to induce methemoglobinemia (Israili, et al. 1971)

3. the potential of cimetidine and tolbutamide which inhibit cytochrome P450 enzymes (CYP 3A4 and CYP 2C9, respectively) involved in the metabolism of DDS, to modulate the effect of DDS in hepatocarcinogenesis promotion, and

4. the effects of some natural products viz., Picroliv (a hepatoprotective drug developed by CDRI, Lucknow; contains picrosides of *Picrorhiza kurroa*), betel (*Piper betel*) leaf and the Bengal gram (seeds of *Cicer arietinum*) on DDS promoted hepatocarcinogenesis.

The known hepatocarcinogenesis promoters 2-acetamidofluorene (AAF) and phenobarbitone (PB) were used as standards for comparison of DDS effects and for evaluating the effects of selected modulators.

All the above investigations were carried out using two established rodent models of two-stage hepatocarcinogenesis, the initiation-promotion model of Pitot, et al. (1978) and the medium-term model of Ito (1980), following the recommendations of the Food and Drug Administration, U.S. Department of Health and Human Services, U.S.A. (*Testing for Carcinogenicity of Pharmaceuticals*, Guidance # S1B; 63 FR 8983, Federal Register, 1998). The Pitot's and Ito's models are based on two distinct mechanistic consideration of
carcinogenesis process and therefore provided information that supplemented each other. Thus:

1. The sex of the animals used in the two models was different; female rats were used in the Pitot model and male rats were used in Ito model. Female rats were found to be more sensitive/susceptible in the Pitot protocol, whereas male rats were found more responsive in the Ito protocol. This provided information on sex dependant differences in the responses to the agents studied.

2. The duration of exposure to the promoters and promotion modulating agents was 12 weeks in Pitot model and 6 weeks in Ito model. This provided information on long-term and short-term effects of the agents studied.

3. In the Pitot model liver cell proliferation which is necessary for 'fixation' of the DNA damage produced by the initiator N-nitrosodiethylamine (DEN), was induced only once by surgical 2/3 hepatectomy 24hr before DEN (10mg/kg) administration. In the Ito model this was induced twice. The first stimulus for proliferation is provided by administration of a high dose (200mg/kg) of DEN that is both cytotoxic (chemically induced 'hepatectomy') and genotoxic (initiation), thus coupling both initiation and proliferation stimulus in a single step. The second stimulus is by surgical 2/3 hepatectomy one week after the start of exposure to promoter (with or without the modulator). This second proliferation stimulus was intended to provide a 'selection pressure' for initiated cells, but not non-initiated cells, to proliferate and form foci/nodules. The 'selection pressure' concept is based on the fact that initiated cells exhibit certain abnormalities in metabolic pathways such as suppressed Phase I and enhanced Phase II metabolism of drugs (Gravell, et al. 1975; Schulte-Hermann, et al.1981). Consequently, the initiated cells, in contrast to non-initiated normal cells, generate very low levels, if at all, of cytotoxic and cytostatic (mitoinhibitory) metabolites of drugs and are capable of effectively detoxifying the metabolites through
their enhanced Phase II pathway. Thus, when the animals exposed to a promoter such as AAF are subjected to hepatectomy, the mitoinhibitory AAF metabolites generated in normal cells prevent the cell to proliferate in response to proliferation stimulus, while the initiated cells, in which no mitoinhibitory metabolites are available, proliferate in response to hepatectomy to form foci and nodules. The use of both the models in the present study therefore, provided information as to whether the promotion or modulation potential of the agents was via the 'selection pressure' (Ito model) or due to a direct action on initiated or normal liver cells (Pitot model).

5.1 PROMOTION OF DEN-INITIATED HEPATOCARCINOGENESIS BY DAPSONE (DDS)

5.1.1 Comparative Study on the Promotion Efficacy of Dapsone (DDS) and the Standard Promoters 2-Acetamidoflourene (AAF) and Phenobarbitone (PB) in the Ito Model of Hepatocarcinogenesis

In the present study oral administration of DDS once daily for 6 weeks at 50mg/kg dose increased the focal volume by 103% over the DEN control. This clearly demonstrated that DDS is a promoter of hepatocarcinogenesis. However, it is not as potent as AAF and PB, which increased the foci volume by 986% and 680% respectively. The oral LD$_{50}$ of DDS in male rats is 630mg/kg and the maximum tolerated dose (MTD) is 150mg/kg (Wu and DuBois, 1970). Long-term (>2years' duration) carcinogenicity studies in rats conducted by National Cancer Institute, USA, employed DDS doses of 600 and 1200mg/kg diet (National Cancer Institute, 1977) which produced mesenchymal tumors (viz., splenic sarcomas, splenic fibromas and peritoneal fibromas) in a dose related manner. When calculated on the basis of average food consumed by a rat of ~300g body wt. per day, the doses used in the above studies are approximately 57 and 114mg/kg body weight, respectively. The dose used in the present study (50mg/kg body weight) is thus less than the
lowest dose used in the long-term study and is \( \frac{1}{3} \) of MTD or approximately \( \frac{1}{12} \) of \( \text{LD}_{50} \) of rat. The present study also revealed that the carcinogenic effect of DDS could be elicited even by a lower dose (50mg/kg) and shorter exposure times (6 weeks only) in the Ito's assay model.

DDS is structurally related to several mutagenic and hepatocarcinogenic arylamine compounds such as benzidine, 4,4' - oxydianiline, 4, 4' - methylene dianiline, 2, 7- fluorenediamine and azodyes (Grisword, et al. 1968). However, several studies have convincingly established that dapsone or its metabolites are non-mutagenic (Peters, et al. 1983). In the present study also the number of foci/liver, which is an index of initiation (mutagenic/genotoxic) potential, found in DDS promoted group was not increased and was comparable to that of the PB promoted group. This further confirmed the non-genotoxic nature of DDS. Interestingly, the number of foci in DDS group was significantly more than that in the AAF promoted group, making it appear as though AAF somehow failed to promote all the DEN initiated cells to form foci/nodules. This was not unusual because the intense selection pressure characteristic of AAF (Solt and Farber, 1976) results in rapidly growing and space-filling foci. In this process the growing foci in the core of the liver eventually are packed closely and even merge with each other, while those in the periphery protrude out presenting a 'nodular' surface to the liver. At a low magnification at which the liver sections were analyzed by Pitot's program, a 'nodule' that is actually composed of two or more individual compressed nodules presents an almost circular or ellipsoid profile contour and consequently the program assigns a single centroid to this nodule for computation. This can of course be remedied by manually editing the image but such editing will give rise to errors in analysis, as the separated profiles no longer have a circular or ellipsoid contour. Another feature of AAF promotion, which contributes to this problem, is the conversion of nodules into 'spongiotic' and 'cystic' structures, (Fig. 18) which are not included in the count.

PB is a relatively less intense promoter of hepatocarcinogenesis and the mechanism of PB induced selection pressure is considered different from that
of AAF (Lans, et al. 1983). PB promotion results in foci and nodules that usually do not merge with each other and present a circular or ellipsoid profiles in sections. Thus we compared the number of foci/liver that were detectable by DDS promoted growth with those promoted by PB. There was no significant difference between PB and DDS promoted number of foci indicating that all the foci clonally derived from DEN initiated cells could be resolved after both DDS and PB promotion.

It is interesting to note that DDS has been reported to cause hepatitis in humans (Stone and Goodwin, 1979) indicating that liver is one of the target organs of DDS induced hepatitis. The results of the present study indicate that in rodents DDS can act as a promoter of genotoxin-initiated liver carcinogenesis. However, since rodent liver is known to be a predominant target organ not only for liver carcinogens but also for carcinogens affecting extrahepatic organs/tissues (Ito, et al. 1989), DDS carcinogenicity in rodents need not be solely liver specific.

In the present study, DDS produced splenomegaly and splenic hemosiderosis, which are known toxic effects of DDS (Graham, 1975; Ciccoli, et al. 1999). N-hydroxylated and N-acetylated metabolites of DDS have been implicated in the development of methaemoglobinemia and hemolytic anemia (Cucinell, et al., 1972). Whether any or all the known DDS metabolites such as N-acetyldapsone, N-diacetyldapsone and N-hydroxylaminedapsone, which are implicated in the induction of methemoglobinemia, splenomegaly and hemosiderosis are also involved in the promotion of liver carcinogenesis is not known. It is likely that some of these toxic metabolites, though not mutagenic, (Peters, et al. 1978) may induce cell death or inflammatory reactions in liver and the resultant compensatory cell proliferation may enhance the growth of foci. This derives support from several reports that inflammation is an important hallmark of carcinogenesis and anti-inflammatory agents such as piroxicam inhibit breast, colon and liver tumorigenesis (Kelloff, et al. 1990). It is also known that DSS causes hypersensitivity reactions in humans (Joseph, 1985).
N-hydroxylated metabolites of compounds are known to be more toxic. Aminofluorene, an aromatic amine like DDS, is N-hydroxylated yielding the metabolite that is responsible for its genotoxic effects (Miller, et al. 1960). By analogy with aminofluorene it is possible that DDS may be transformed in part to DDS-N-acetyl-N-O-β-glucuronide or a nitroxide radical, and these metabolites may be toxic (Stier, et al. 1972). However, it has been reported that N-hydroxyl dapsone a metabolite of DDS is not mutagenic in the Ames assay (Peters, et al. 1978).

Thus DDS promotes DEN initiated hepatocarcinogenesis. The possible relationship between DDS metabolism and tumor promotion is presented in subsequent parts of the work.

5.1.2 Dose Response Study of DDS in Pitot Model of Hepatocarcinogenesis

The relationship between the dose of DDS and its promotion potential was investigated in the study the Pitot’s model, since the model permits a relatively long-term exposure (12 weeks) to the agent. Long-term exposures are considered necessary to detect any adaptive phenomenon or toxic manifestations in response to higher doses of the agent. In this study, a dose dependent increase in the foci volume was observed only at 10 and 25mg/kg dose levels; at higher dose of 50mg/kg, no further increase in the focal volume was observed, but instead there was a slight reduction in focal volume. Promoters are generally known to exhibit a dose response relationship (Ashendel, 1985; Verma and Boutwell, 1980). However, several exceptions to this have been reported. An example is the hypolipidemic liver tumor promoter Wy-14643 that shows a lack of dose response at higher doses (Glauert, et al. 1986). Dose dependent methemoglobinemia (MHB) and the degree of hemosiderosis were also observed in the DDS promoted groups. At 25mg/kg dose DDS showed greater foci volume than at 50mg/kg. Thus the relation between MHB/hemosiderosis and the foci volume is linear only at the lower doses but not at higher doses. As mentioned earlier, N-hydroxy and N-hydroxy
Acetylated metabolites of DDS are responsible for methemoglobinemia and the hemosiderosis in the spleen (Chang, et al. 1996). Methemoglobin and the degree of hemosiderosis were linear, but this dose dependent linearity was not observed in the increase in the foci volume at the higher dose. Although the reason for this dissociation of dose response between methemoglobinaemia/hemosiderosis and foci volume at 50mg/kg is not readily apparent, several possibilities exist including the influence of sex of the animal (females were used in this study). DDS-hydroxylamine oxidizes the Hb in the red cells. The red cells so damaged are sequestered in and removed from the circulation by splenic histiocytes. In the histiocytes, Hb is converted and stored as hemosiderin. This sequestration and elimination of defunct red cells by spleen may effectively reduce the MHb levels to non-toxic levels. The result is low MHb levels and siderotic spleens. It is possible that at higher doses, the saturation of metabolic pathways may result in a reduced production of toxic metabolites selectively responsible for promotion. Alternatively, depletion of liver glutathione as observed in the present study, at DDS 50mg/kg indicates that the GSH is utilized for the regeneration of DDS from DDS N-hydroxylamine. The consequent decreased availability of the toxic DDS-hydroxylamine might reduce the promotion pressure on the DEN-initiated foci. Indeed the foci volume in DDS50mg/kg dose group was not significantly different from that seen in the DDS25mg/kg dose group, in which very slight depletion of GSH occurred.

The absence of any dose related effect on the numerical density of foci is not unusual, since the promoting effect of DDS on foci is expected to increase only the total volume of foci but not the number. The number of foci/liver is a direct indicator of the number of hepatocytes initiated by a given dose of DEN, since it is generally agreed that foci are clonal in origin i.e. each initiated hepatocyte proliferates to give rise to a focus (clonal expansion) (Pitot, et al. 1987). This finding further substantiates that DDS has no initiating effects on hepatocarcinogenesis. Thus DDS only promotes the growth of the existing initiated cells (thereby increasing the volume of foci) in a dose dependent
manner up to 25mg/kg but has no effect at any of the tested dose levels on the number of foci. DDS at 50mg/kg dose had a foci volume 158% more than the DEN-VEHICLE controls.

Thus, there may be a correlation between the extent of DDS metabolism and the promotion potential of DDS at the lower doses. As mentioned above, the N-hydroxylated metabolites of chemicals are more toxic, and in the case of aminofluorene are carcinogenic. The metabolites of DDS may thus be suspected to be the ultimate promoters for carcinogenesis. Separate studies to evaluate the promotion potential of DDS metabolites are needed to identify the metabolite(s) selectively responsible tumor promotion.

5.2 MODULATION OF DDS PROMOTED HEPATOCARCINOGENESIS

5.2.1 Modulation by Cytochrome P<sub>450</sub> Inhibitors

5.2.1.1 Cimetidine (CMT; a CYP 3A4 isoform inhibitor) and Tolbutamide (TLB; a CYP 2C9 isoform inhibitor) on DDS Promoted Hepatocarcinogenesis in Pitot Model

The relatively lower gain in body weight seen in DEN-DDS-TLB group rats was probably related to the known hypoglycemic property of tolbutamide (TLB) via enhanced insulin secretion. In the absence of exogenous supplementation with sufficient amounts of carbohydrates, the hypoglycemia induced by tolbutamide may adversely affect the energy metabolism in peripheral tissues such as muscles and reduce the lipid depots in adipose tissues. The net effect is the impaired weight gain. Both TLB and CMT were capable of limiting the promotion of the DEN induced pre-neoplastic lesions by DDS as indicated by the reduced foci volume in the DEN-DDS-TLB and DEN-DDS-CMT groups compared to the DEN-DDS group.

TLB inhibited the growth of DDS promoted foci by 68%. TLB, a specific CYP2C9 inhibitor has been shown to reduce DDS-NHY formation by about 41% in vitro (Winter, et al. 2000). The present study is the first to
investigate the \textit{in vivo} efficacy of TLB in not only methemoglobinemia but also liver carcinogenesis promotion induced by DDS. Although TLB did not significantly inhibit the MHb formation, the degree of hemosiderosis was reduced. Being a specific CYP2C9 inhibitor, TLB is not expected to affect other CYP isozymes (CYP3A4 and CYP2E1) are also involved in the metabolism of DDS (Fleming, et al. 1992), and therefore other metabolites may responsible for the hemotoxic effects. In addition the dose of TLB (50mg/kg) used in the study may be relatively small for reducing the metabolism of a large dose (50mg/kg) of DDS. The study nevertheless demonstrates that TLB has the ability to reduce the liver foci promotion effect of DDS and further studies will reveal the most effective and safe dose of TLB for this purpose.

Metabolites of DDS may cause other manifestations of toxicity of sulfones such as Heinz body formation and hemolysis (Hjelm and deVerdier, 1965). Since, the methemoglobinemia was not severe in the DEN-DDS-TLB group, the splenic siderosis was minimal even in the presence of detectable methemoglobinemia. It therefore follows that the relationship between methemoglobinemia and siderosis may depend on the severity of methemoglobinemia. Thus the metabolites produced were probably at threshold concentrations so that the iron overload on the spleen was prevented. Coleman, et al (1991) reported 50% reduction in methemoglobin levels in rats treated with 50mg/kg CMT one hour before the administration of 10mg/kg DDS. CMT has also been reported to reduce DDS induced methemoglobin by 26% in humans (Coleman, et al. 1992). In the present study CMT (a CYP3A4 inhibitor) prevented the growth of DDS promoted foci by 71% but did not significantly inhibit the methaemoglobinaemia caused by DDS, although the degree of hemosiderosis was considerably lowered.

The absence of significant lowering of MHb levels may be due to the higher dose (50mg/kg) of DDS used in the present study as compared to the very low dose (10mg/kg) used by Coleman, et al. (1992). Further, the effect may also be sex dependent as the above authors used male Wistar rats and the
present study used female CF rats. It is known that the extent of MHb induced in female rats is less than that in male rats (Tingle, et al. 1997).

Taken together, the results of the present study clearly demonstrate for the first time that both CMT and TLB have the potential to interfere with and reduce DDS induced promotion of liver carcinogenesis in rat. A large number of metabolites of DDS are possible such as O- and N-glucuronides, sulfates, acetyl, N-hydroxy, azoxy and nitroso derivatives and their combinations were identified, and considerable effort is needed to learn which metabolites are specifically responsible for a particular side effect (Israili, et al.1973). As TLB and CMT inhibit two different isofoms of CYP450 enzymes, involved in DDS metabolism, the results of the present study also indicate that those metabolites of DDS that are produced by these two isoforms may play a major role in DDS induced promotion.

In addition to inhibiting DDS induced promotion, CMT & TLB also reduced the number of foci/liver significantly. However, this reduction is less (39%) in CMT treated group than in TLB (62%) treated group. The decrease in the numerical density of foci in DEN-DDS-CMT and DEN-DDS-TLB groups is evidently not due to any inhibitory effect of these agents on the initiation stage, since the agents were administered long after the initiation by DEN. For CMT, there are reports that it is genotoxic but not carcinogenic (Martelli, et al. 1986; Martelli, 1997). This non-carcinogenic type genotoxicity might have induced foci cell death by apoptosis or necrosis, Consequently reducing the number of foci. Similarly TLB might have impaired the survival of some susceptible foci by its known potent hypoglycemic effects but the mechanism by which TLB could induce this effect is not known. It is also likely that the potent anti-promotion effects of CMT and TLB resulted in the failure of a large number of small foci to grow to a size that is above the truncation value set for the numerical density analysis in the Pitot’s program.
5.2.1.2 Cimetidine (CMT; a CYP 3A4 inhibitor) and Tolbutamide (TLB; a CYP 2C9 inhibitor) on DDS Promoted Hepatocarcinogenesis in Ito Model

In the Ito model DDS increased the foci volume by 103% over the DEN-VEHICLE demonstrating that DDS is a promoter of DEN initiated hepatocarcinogenesis (sec. 1.1 above). As discussed above, 12 weeks administration of both TLB and CMT during the DDS induced promotion in female rats inhibits the DDS promoted hepatocarcinogenesis and reduces hemosiderin formation in spleen, thus demonstrating a likely relation between the promotion potential of DDS and the extent of DDS metabolism. In the present study the short-term efficacy of CMT and TLB on DDS promoted hepatocarcinogenesis was evaluated in male rats using the Ito’s model.

Administration of TLB did not have any effect on DDS induced methaemoglobin formation or the deposition of hemosiderin. In vitro TLB has been reported to inhibit CYP2C9 mediated N-hydroxylation of DDS by 41% (Winter, et al. 2000), but there are no reports in literature as to whether TLB inhibits DDS induced methemoglobinemia or hemosiderosis in vivo. Thus the exact in vivo efficacy of TLB with respect to inhibition of DDS N-hydroxylation and its role in DDS induced methemoglobinemia or hemosiderosis is unclear. In the present short term in vivo studies using the Ito model, TLB inhibited the DDS promoted hepatocarcinogenesis by 79%. In view of the current understanding of TLB inhibiting DDS N-hydroxylation, DDS-N-hydroxylamine may be the predominant metabolite of DDS responsible for the promotion potential of DDS.

Tingle, et al. (1991) demonstrated 75% reduction in the formation of the N-hydroxyl amine of DDS and 90% reduction in methemoglobin formation in parallel experiments, when human liver microsomes pre-incubated with CMT were treated with DDS. In the present study, CMT inhibited the DDS promoted increase in foci volume by 78% and also effectively inhibited any rise in methaemoglobin and the degree of hemosiderin deposition by DDS in the
spleen. This clearly demonstrates the involvement of DDS metabolites especially DDS N-hydroxylamine in promoting DEN initiated hepatocarcinogenesis by DDS.

In the long-term model of Pitot, CMT or TLB did not reduce the DDS induced methemoglobin formation, but both CMT and TLB efficiently reduced hemosiderosis. In the short-term Ito's model study, CMT has prevented any rise in the methemoglobin and also the extent of hemosiderin deposition. In the Pitot model study, there was gradual reduction in the MHb levels in all the groups on chronic long-term administration. Thus the MHb levels reach the non-toxic levels and siderosis also seems to be reduced on treatment with CMT and TLB. In the Ito model study, CMT alone efficiently inhibited MHb formation and hemosiderosis but TLB did not prevent. The reason for this is not readily apparent but appears to be sex related. Male rats (used in the this study) have been found to be more susceptible to the formation of methemoglobin (Tingle, et al. 1997), thus higher values of methemoglobin were in the Ito model, and lower values in the Pitot's model study. The absence of significant lowering of MHb levels may be due to the higher dose (50mg/kg) of DDS used in the present study as compared to the very low dose (10mg/kg) used by Coleman, et al. (1992).

Depletion of liver glutathione in DEN-DDS-CMT group indicates that the GSH is probably utilized for the regeneration of DDS from DDS N-hydroxylamine. The consequent decreased availability of the toxic DDS-hydroxylamine might reduce the promotion pressure on the DEN-initiated foci. Indeed the foci volume in DEN-DDS-CMT group was significantly lower different from that seen in the DEN-DDS group, in which depletion of GSH occurred. Moreover, the status of the liver is different in both the Ito's and Pitot's models. In the Ito's model, a high dose of DEN (200mg/kg) is administered, which is necrogenic to the liver and also genotoxic.

CMT and TLB treatments also effectively reduced the number of foci/liver. The decrease in the numerical density of foci in DEN-DDS-CMT and DEN-DDS-TLB groups is evidently not due to any inhibitory effect of
these agents on the initiation stage, since the agents were administered long after the initiation by DEN. For CMT, there are reports that it is genotoxic but not carcinogenic (Martelli, et al. 1986; Martelli, 1997). This non-carcinogenic type genotoxicity might have induced foci cell death by apoptosis or necrosis, consequently reducing the number of foci. Similarly TLB might have impaired the survival of some susceptible foci by its known potent hypoglycemic effects but the mechanism by which TLB could induce this effect is not known. It is also likely that the potent anti-promotion effects of CMT and TLB resulted in the failure of a large number of small foci to grow to a size that is above the truncation value set for the numerical density analysis in the Pitot's program.

5.2.1.3 Effect of TLB (a CYP 2C9 Inhibitor) on PB Promoted Hepatocarcinogenesis in Ito Model

PB is a potent promoter of hepatocarcinogenesis and is used as a standard promoter in various medium term hepatocarcinogenicity assays (Peraino, et al. 1973; Cater, et al. 1985; Watanabe and Williams, 1978; Kolaja, 1996). It is a potent inducer of liver microsomal enzymes and is known to enhance the metabolism of a number of drugs that are metabolized by the liver microsomal enzyme system (Matsushima, et al. 1977). The exact mechanism of tumor promotion by PB is still unknown. Inhibition of gap junction intercellular communication and increase in the levels of oxygen free radicals have been observed on treatment with PB and are suspected to be the mechanisms of tumor promotion by PB (Shoda, et al, 1999; Umemura, et al. 1999). Chronic oxidative injury may lead to a non-lethal modification of normal cellular growth control mechanisms. Cellular oxidative stress can modify intercellular communication, protein kinase activity, membrane structure and function and gene expression and result in modulation of cell growth (Klaunig, et al. 1998). PB is also reported to increase DNA synthesis in hepatocyte cultures and in vivo (Hasmall and Roberts, 2000; Lake, et al. 1998). It has also been reported that the peak of DNA synthesis induction is almost 40hr after treatment of the culture with PB (Plant, et al. 1998).
In the present study, co-administration of TLB along with PB caused 90% reduction in preneoplastic foci volume promoted by PB and also inhibited the increase in the liver weight by PB. CYP2C9 plays a major role in the metabolism of PB while CYP2C19 and 2E1 also are involved to a small extent and 4-hydroxyphenobarbitone is the major metabolite of PB (Namara, 2001; Hadana, et al. 2001). In the present study TLB was administered one hour before the administration of PB, the same regimen was then repeated after 24 hours, thus a cyclic treatment followed. TLB is known to inhibit CYP2C8/9 at clinical concentrations (Unadkat, 2000) and PB is known to induce CYP2C1, 2C9 enhancing its own metabolism. Till date there are no reports on the tumor promotion effects of the metabolites of PB. The inhibition of CYP2C9 by TLB may increase the levels of PB, although PB induces CYP2C9, the presence of TLB will inhibit the metabolism of PB; thus the cycle might have continued in the present study. The net effect of this cycle would be increased levels of unmetabolized PB. Miyazaki, et al. (1998) reported that PB at higher concentrations (3-4mmol/L) inhibits DNA synthesis in primary culture normal rat hepatocytes where as lower concentrations (0.5-2mmol/L) stimulate DNA synthesis. Thus it is possible that the decrease in the focal volume and number of foci/liver was due to the inhibition of DNA synthesis by accumulating PB concentrations in TLB treated rats. Indeed, the decreased liver weights in TLB treated group further indicates the mitoinhibition produced by this pharmacokinetic interaction between PB and TLB. An analogous situation of a slightly different type is seen with combined administration of PB and 2-AAF. Co-administration of 2-AAF with PB delayed the cancer development by several months (Rissler, et al., 1997), since AAF and PB have the opposing mechanisms for tumor promotion. Metabolites of AAF are mito-inhibitory whereas PB is mitogenic as well as a cytP<sub>450</sub> enzyme inducer. Thus the enhanced metabolism of AAF may lead to increase in the metabolites that exert a mito-inhibitory effect on the initiated cells. In a similar way, TLB administration leading to increased levels of PB may be responsible for inhibiting the PB induced tumor promotion by PB.
5.2.2 MODULATION BY NATURAL PRODUCTS

5.2.2.1 Effect of Picroliv (PLV) on DDS Promoted Hepatocarcinogenesis in Pitot Model

In the present study, Picroliv (PLV) suppressed the DDS induced increase in focal volume by 66% suggesting that PLV is a potent anti-promoter of DDS promoted carcinogenesis. PLV is a known hepatoprotective and antioxidant (Picrorhiza kurroa, monograph). It contains a number of glycosides showing anti-oxidant and anti-cancer properties. It has recently been reported (when the present study was in progress) that PLV may protect against DEN induced hepatocarcinogenesis in the rat (Rajeshkumar and Kuttan, 2000). However the parameters of assessment employed by these authors did not include pre-neoplastic foci/nodule demonstration or their quantitation. In the present study we have investigated the effects of chronic PLV treatment on hepatocarcinogenesis promotion by DDS. PLV effectively inhibited the DDS promoted focal volume by 66%. PLV administration also caused depletion of liver GSH levels. The depletion of liver GSH levels in the DEN-DDS-PLV group indicated that GSH is probably utilized for the regeneration of DDS from DDS-hydroxyamine. The consequent decreased availability of the toxic DDS-hydroxyamine might reduce the promotion pressure on the DEN-initiated foci. Indeed the DEN-DDS-PLV group had a lower foci volume than the DEN-DDS group. Alternatively PLV may have provided protection to RBC’s from the oxidation by DDS-hydroxylamine.

The interference of PLV with DDS metabolism is clear with the observations that PLV prevented the rise in methaemoglobin levels initially but was not effective over the 12 weeks period. However, PLV reduced the deposition of hemosiderin in spleen, thus indicating that by regulating the DDS-hydroxylamine levels through GSH or through direct protective effects on RBC’s, PLV maintained of methemoglobinemia within the tolerance threshold. Further studies with higher doses of PLV may elucidate the mechanisms. Thus even in the presence of detectable MHb levels sederosis was
reduced. PLV induced increase in the spleen weight might reflect a reactive hyperplasia commonly seen in response to xenobiotics.

Rastogi, et al. (2001) have recently reported that PLV decreases the gamma glutamyl transpeptidase (GGT) levels in the liver and serum of aflatoxin B1 treated rats. However, these rats were not investigated for the presence of pre-neoplastic foci/nodules and therefore, whether the GGT was extrafocal (biliary) or focal is not clear. Nevertheless, a reduction in GGT was also observed in the livers of DEN-DDS rats, where GGT was detected histochemically and quantified using the Pitot’s image analysis program. This study to investigate the effect of PLV on hepatocarcinogenesis promotion by DDS using medium-term mechanistic models is the first of its kind.

5.2.2.2 Effect of PLV on DDS Promoted Hepatocarcinogenesis in Ito Model

The beneficial effects of PLV on liver focal volume and splenic hemosiderosis in DEN initiation and DDS promotion experiment in Ito model were largely identical to those found in Pitot model experiments. In addition PLV effectively prevented any rise in the methaemoglobin levels in the Ito model. The DDS promoted focal volume was reduced by 51% by PLV. This confirms the modulating effects of PLV on DDS metabolism and that the metabolites of DDS are responsible for the tumor promoting effects of DDS. PLV also reduced the spleen weight effectively. This observation is contradictory to that observed in the Pitot model, where a rise in the spleen weight was observed. This may be because the duration of exposure to the promoter (DDS) and the modulator (PLV) in the Ito’s model is six weeks, which is exactly half of that in the Pitot’s model, which is twelve weeks. Thus, the duration in case of the Ito’s model was short, and the drugs did not induce the reactive hyperplasia as in the Pitot’s model.
5.2.2.3 Effect of Betel Leaf (BTL) on DDS Promoted Hepatocarcinogenesis in Pitot Model

Betel leaf is known to contain a variety of compounds such as β-carotene, α-tocopherol and hydroxycavicol (Azuzine and Bhide, 1992). Some workers have studied the prevention potential of betel against carcinogenesis (Rao, et al, 1985) while others have studied betel for its cancer causing effects (Chen-Shen, et al. 1996). However, these investigators tested different types of extracts of betel leaf and none of them used the whole leaf powder that closely simulates the form in which, it is consumed by humans, especially in India. This is important because the extracts do not contain all the constituents of the whole leaf and consequently the proportion and quantity of the constituents in a given dose of whole leaf powder differ from that found in extracts. In the present study whole betel leaf powder (BTL) had no effect on the DDS promoted focal volume. In other words, BTL neither enhanced nor suppressed the DDS promotion effect. It also had no effect on the blood methaemoglobin levels during the 12 weeks treatment. However, BTL increased the spleen weight without enhancing hemosiderin deposition in spleen. Collectively these results indicate that BTL may not interfere at any level with the metabolism or tumor promotion potential of DDS.

5.2.2.4 Effect of BTL on DDS Promoted Hepatocarcinogenesis in Ito Model

As in the Pitot model, BTL, when administered for 6 weeks along with DDS, neither enhanced nor suppressed DDS promotion of DEN initiated foci in the Ito model. BTL however could restrict the rise in methaemoglobin levels initially though not in the later period but could not prevent the formation of hemosiderin in the spleen. Chronic administration of BTL along with DDS reduced the liver weight. In the Ito's model partial hepatectomy is performed at the third week after initiation with DEN and a week after the promotion begins. BTL may possess mild mito-inhibitory properties, which might be the cause for
mild retardation in the re-growth of liver after the partial hepatectomy conducted five weeks before the termination of experiments.

DDS has shown to reduce liver GSH levels in the previous studies also. The depletion may be related to the utilization of GSH in recycling the DDS-N hydroxylamine to DDS resulting in reduction in promotion pressure but this was not apparent. Aqueous and acetone extracts of betel leaf have shown to be non-mutagenic in the S. typhimurium strains (Nagabhushan, et al. 1987). Thus there appears to be no interaction between BTL and DDS.

Collectively, the results demonstrate that BTL has no modulating effect on DDS promoted hepatocarcinogenesis.

5.2.2.5 Effect of BTL on AAF Promoted Hepatocarcinogenesis in Ito Model

BTL failed to prevent the tumor promotion by AAF. The metabolites of AAF are mitoinhibitory and thus prevent normal hepatocytes from proliferating in response to partial hepatectomy. In contrast the initiated cells being incapable of producing mitoinhibitory metabolites proliferate in response to the growth stimulus and form foci and nodules. It appears that BTL does not interfere with the metabolism of AAF in normal hepatic parenchyma or with the proliferation of focal cells. However, the mechanism by which BTL reduces the liver GSH levels remains to be investigated.

It is concluded that BTL has no modulating effect on AAF promoted hepatocarcinogenesis.

5.2.2.6 Effect of BTL on PB Promoted Hepatocarcinogenesis in Ito Model:

BTL did not prevent the tumor promotion by PB. BTL alone also showed no promoting effect; thus it appears that betel leaf is neither a promoter nor does it have any chemopreventive or modulating effect on PB promoted hepatocarcinogenesis. Similar lack of tumor promoting or promotion-suppression effect was reported by Rao, et al. (1985) in 7,12-
dimethylbenz(a)anthracene induced mammary tumors in rat using methanolic extract of betel leaf. In the present study administration of BTL reduced the liver weight. The reason for this is not readily apparent but it is possible that BTL is mildly mito-inhibitory to normal hepatocytes but not initiated hepatocytes which could have affected the liver regeneration in response to partial hepatectomy performed 5 weeks before termination of the experiment. In the Ito's model partial hepatectomy is performed at the third week after initiation with DEN and a week after the promotion begins. Metabolites of AAF are mito-inhibitory, since in the initiation-selection protocol, AAF is administered, which is metabolized to its N-hydroxy metabolites by the normal liver parenchyma, thus exerting its effects only in the normal cells. Since the initiated cells, posses lower metabolizing abilities due to reduced CYP_{450} levels, (Gravella, et al. 1975; Okita, et al. 1976) they are protected from the toxic effects of AAF metabolites and continue to proliferate. Till date there are no reports on the promotion or promotion modulating potential of BTL in liver carcinogenesis. In the present study, BTL has reduced the foci volume; thus BTL does not promote DEN-initiated hepatocarcinogenesis.

5.2.2.7 Effect of BTL on the Initiation Stage of DDS Promoted Hepatocarcinogenesis in Ito Model

Administration of BTL for two consecutive days before and after DEN administration neither increased nor decreased the number of foci generated by DEN. Changes in the foci volume were not considered relevant in this study because only the number of foci reflects the initiation potential of an agent. As explained before, foci are clonal in origin wherein a single genetically altered (initiated) cell proliferates to give rise to a focus. Since BTL did not induce genetic damage in cells, there was no addition to the clonally derived number of foci over those already produced by DEN. For several reasons, the available evidence on the carcinogenic and anti-carcinogenic potential of betel leaf is inconclusive. In limited studies on humans, betel quid (consisting of betel inflorescence, areca nut etc) use has been implicated in the development of
hepatocellular carcinoma (Chung-Ji, et al. 2000). However it should be pointed out here that the betel quid in the above study did not contain betel leaf, but only betel inflorescence, which is rich in the liver carcinogen, safrole. Moreover, areca nut, another constituent of the betel quid, has also been shown to be carcinogenic (Wang and Peng, 1996). Recently, Murakami, et al (1998) reported that methanolic extract of betel leaf inhibited the tumor promotion in 12-O-hexadecanoylphorbol-13-acetate-induced Epstein-Barr virus activation assay in vitro, which is used as an indicator of tumor promotion. In animal studies, Azuine, et al. (1992) reported that daily dietary (or in drinking water) administration of betel leaf (aqueous/ethanolic/methanolic) extract for 13 months inhibited the incidence of methyl (acetoxyethyl) nitrosamine induced buccal pouch mucosal cancer in hamsters. However none of the above investigators used whole betel leaf as such and at the average quantities consumed by human beings and as employed in our present investigation. This is important because, firstly the extracts do not contain all the constituents of the whole leaf and consequently the proportion and quantity of the constituents in a given dose of whole leaf powder and extracts are not identical. Secondly, the effect of betel leaf extract has been found to be apparently site-specific in reducing the incidence of oral mucosal tumors. And finally, in vitro screening systems such as those used by Murakami, et al. (1998) are indirect indicators of promotion in which, neither pre-neoplastic or neoplastic lesions are used as end points. They also fail to simulate the in vivo situation with respect to metabolic activation of the test agent. Nevertheless, it appears that the absence of an initiating effect or initiation-suppression effect of whole betel leaf powder in the present study is probably specific to rat liver and/or that the duration of exposure is short and/or that the whole leaf contains a mixture of carcinogenic and anti-carcinogenic compounds which, depending upon the proportion present, may initiate or suppress carcinogenicity. The carcinogenic and anti-carcinogenic constituents and properties of plants have been reviewed by Ames, (1983).
5.2.2.8 Effect of BTL on the Initiation Stage of AAF Promoted Hepatocarcinogenesis in Ito Model

BTL showed no modulating effect in Ito’s model of hepatocarcinogenesis in which the standard selection pressure inducer AAF was used. The mechanism of action of the tumor promotion by DDS and AAF are different. At low doses the metabolites of AAF are mitoinhibitory and thus prevent normal hepatocytes from proliferating in response to partial hepatectomy. In contrast the initiated cells being incapable of producing mitoinhibitory metabolites proliferate in response to the growth stimulus and form foci and nodules. The lack of changes in organ weight and liver GSH content in BTL exposed rats further indicate the absence of a modulatory effect for BTL. The inability of BTL to either increase or decrease the number of foci/liver in this model of hepatocarcinogenesis suggests that it is not a genotoxin and has no effect on DEN initiation process. As explained earlier, the number of foci/nodules per liver is an index of initiation whereas the growth of foci measured, as focal/nodular volume is a function of promotion.

5.2.2.9 Effect of Bengal Gram on DDS Promoted Hepatocarcinogenesis in Pitot Model

Withdrawal of Bengal gram, from the diet beginning at least a week before DEN initiation till the end of the observation period did not affect the focal volume but caused an increase in the number of foci/liver suggesting that Bengal gram interferes with DEN initiation. Apart from the known nutritive value, Bengal gram is also known to contain lysine, tryptophan, aromatic and sulfur containing amino acids and B-vitamins (el- Adawy, 2002) and isoflavanoids (Simmonds and Stevenson, 2001). Some of the isoflavanoids present in Bengal gram are 5-O-Methylgenistein, Biochanin A, Biochanin A 7-O-glucoside, Daidzein and Formononetin (Norman, et al. 1975). There are no reports on the significance of Bengal gram in the process of carcinogenesis, although there are reports on flavanoids offering protection in skin tumor promotion in mice (Wei, et al. 1990).
Quercitin, kaempferol and luteolin have shown to inhibit various TPA induced phenomena such as increased Ornithine decarboxylase (Kato, et al. 1983) and protein kinase C (Levy, et al. 1984) which are believed to represent nonspecific markers of tumor promotion. Withdrawal of Bengal gram caused a increase in the number of foci/liver, thus the reduced number of foci seen in the DEN-DDS group supplemented with Bengal gram may be attributed to the presence of above mentioned isoflavonoids in Bengal gram, which are known to suppress breast cancer by their antiestrogenic properties. Thus, the present study, for the first time reports the protective effect Bengal gram has on DEN-initiated DDS promoted hepatocarcinogenesis. Withdrawal of Bengal gram caused an increase in the liver and spleen weights and a rise in the liver GSH levels, the significance of which in the reduction of foci number is not known at present.
Cancer today is the leading killer disease. Environment (which includes chemicals), diet and life-style habits are the predominant contributors to the etiology of cancer. Drugs and pharmaceuticals, as with other chemicals, have caused cancers in humans. But, unlike other environmental chemicals, exposure of humans to pharmaceuticals is intentional for improving human health, and therefore can be easily controlled. This is achieved by extensive evaluation of the compounds for their safety, including the assurance that their long-term use will not cause cancer. Drug regulatory authorities also ensure that those drugs with carcinogenic potential are not released into market and withdraw the existing ones, if post marketing surveillance or new evidences indicate their carcinogenic potential. However, keeping in view the enormous expenses involved in discovering a new drug (one out of several thousands synthesized in about 25 years becomes a drug), alternative strategies are currently being developed to reduce or eliminate the toxicity and carcinogenic potential of useful drugs. These include development and use of newer carcinogenicity test methods and use of substances of synthetic or plant origin to reduce the toxic or carcinogenic effects of drugs.

On the basis of our current understanding of the carcinogenic process, carcinogens are broadly differentiated into initiators or genotoxic/mutagenic agents whose effects are irreversible and promoters or non-genotoxic agents, that stimulate the growth of initiated cells and whose effects are reversible and dose dependent. Rodent models capable of differentially identifying initiating and promoting carcinogens are now available and are currently recommended by drug regulatory authorities like Food and Drug Administration (FDA) for a rationalized testing of pharmaceuticals. Carcinogenicity testing using such mechanistic models is especially relevant for those drugs intended for long-term use. The two established and most widely used rodent hepatocarcinogenicity models for evaluating not only carcinogenic potential
but also modulation potential of agents are those developed by Pitot et al. and Ito et al. In both the assays appearance of preneoplastic liver foci/nodules expressing specific markers such as glutathione S-transferase-placental form (GST-P) and gamma glutamyl transpeptidase (GGT) are taken as endpoint.

Dapsone (DDS) is the most widely used drug for the long-term treatment of leprosy and dermatitis herpetiformis. Currently it is also being used for AIDS-related Pneumocystis carinii pneumonia and toxoplasmosis. Available data indicate that DDS therapy is associated with certain cancers in human patients and conventional 2-year bioassays showed that it induces tumors in rodents. However, DDS is not mutagenic, and does not induce sister chromatid exchanges or chromosomal aberrations. Whether the ability of DDS to induce tumors is due to its non-genotoxic promoting effect is not known. This elucidation of mechanism is important, since this stage is regarded as a reversible stage and, thus, there are sound reasons for treating such agents in an entirely distinct manner as regards health risk analysis.

DDS exerts several adverse hematological effects such as hemolytic anemia, methemoglobinemia, agranulocytosis, aplastic anemia and other blood dyscracias. Metabolites of dapsone such as DDS-N-hydroxylamine and N-acetyl-DDS have been shown to be responsible for methemoglobinemia. Some well known drugs such as the H₂-receptor antagonist cimetidine (CMT) and the hypoglycemic sulphonylurea tolbutamide (TLB) have been shown to reduce the extent of DDS metabolism by inhibiting the generation of DDS-N-hydroxylamine, through inhibition of specific CYP₄₅₀ isoforms that are responsible for DDS metabolism; CMT inhibits CYP3A4, and TLB is known to inhibit CYP2C9. Whether these agents also have the ability to suppress tumor promotion is not yet known.

Several naturally occurring substances are known to exert protective effects against cancer and aging. These agents are mostly antioxidants that scavenge free radicals known to damage cells. Picroliv (PLV) is a hepatoprotective drug developed by CDRI and consists of purified glycosides
of *Pierorhiza kuroa*. It has also been shown to possess antioxidant property. However, its ability to suppress tumor promotion remains to be investigated.

Another natural product, the leaf of *Piper betel* (BTL) is consumed with areca nut and lime in south Asian countries and the preparation is known as betel quid. There are increasing reports of oral cancer in people consuming the quid, though isolated reports indicate that extracts of the leaf alone have anti-tumor effects. However, no information is available on its effects on tumor promotion.

The present study was therefore aimed at:

- understanding the tumor promotion properties of DDS,
- the relation between DDS metabolism and its tumor promotion potential using CMT and TLB as probes/modulators and
- investigating the modulating effect of natural products, PLV and BTL on the promotion potential of DDS.

For this purpose, two well-established rodent models; the 8 week model of Ito and the 14 week model of Pitot, were used in the present study.

In the Ito model, groups of male rats received a single intraperitoneal administration of the initiator N-nitrosodietilamine (DEN; 200mg/kg) and after a 2-week rest period, received daily oral administration for 6 weeks of the test substance or one of the two standard promoters: 2-acetylamidofluorene (AAF, 20mg/kg/day) and phenobarbitone (PB, 90mg/kg/day) or vehicle only, with or without concurrent administration (1hr. before promoter) of test modulator; CMT (50mg/kg/day), TLB (50mg/kg/day), PLV (10mg/kg/day) or BTL (200mg/kg/day). In one experiment BTL (200mg/kg) was administered for 5 consecutive days around the DEN dosing (2 days before and after DEN and on the day of DEN dosing) for assessing the initiation-modulating potential of the agent. One week after starting the promoter or test substance (with or without modulator) the rats were subjected to 2/3 partial hepatectomy.

In the Pitot model, groups of female rats received a single oral administration of DEN (10mg/kg) 24 hours after 2/3 partial hepatectomy and
after a 2-week rest period, received daily oral administration for 12 weeks of the test substance. Methemoglobin was monitored fortnightly.

At the end of week 8 post-DEN in the Ito model and week 14 post-DEN in Pitot model, the rats were killed and liver glutathione content was estimated. Liver sections were stained for GGT (cryosections) and immunostained for GST-P (paraffin sections) for the identification of foci/nodules expressing these markers. Liver sections were also stained for general histopathology and with periodic acid-Schiff for glycogen. Spleen sections were stained with Perl's iron method for hemosiderin deposits. The number of foci/liver and the volume of foci/nodules are estimated using a dedicated computer image analysis program developed by Pitot et al. for stereological quantitation of foci/nodules.

The major findings in the present study are:

1. DDS, at a dose of 50mg/kg, is a promoter of hepatocarcinogenesis as shown by both the short-term Ito model and the longer term Pitot model. DDS increased the focal volume by 103% in the Ito model and 158% in the Pitot model. The promoting potential of DDS was less than that shown by standard promoters, PB and AAF, and could be graded as DDS<PB<AAF. The dose of DDS used in the present study is less than that used by earlier workers (~57mg/kg/day x ~2years) in life-term bioassay of the drug.

2. Dose response studies in the Pitot model revealed that the promoting effect of DDS is dose related from 0 to 25mg/kg dose levels and there was no further increase in the effect at 50mg/kg. At the latter dose there was a small decrease instead. This finding clearly demonstrates that the promotion effect of DDS can be detected even at very small doses and in a short period in the current initiation-promotion model compared to that used by earlier workers in the 2-year life-term bioassays.

3. DDS did not induce any changes in the numerical density of the foci. This finding clearly indicated that DDS is not an initiator of
carcinogenesis and confirmed the non-mutagenic/non-genotoxic nature of using a definitive cancer-related end point.

4. DDS also induced a dose dependent increase in methemoglobin formation and hemosiderosis in the spleen.

5. DDS at 50mg/kg reduced the liver glutathione content in the Pitot model, which was not observed in the Ito model. Methemoglobinemia was also lower in these rats compared to that in Ito model rats. This observation in Pitot model is consistent with the known role of glutathione in the recycling of DDS metabolites back to DDS. The absence of such effect in Ito model suggests that it may be sex-related, since male rats were used in Ito model and female rats in Pitot model.

6. The CYP3A4 inhibitor CMT at 50mg/kg-dose level inhibited the promoting effect of DDS in both the Ito and Pitot's models; the volume of foci/nodules was reduced to an extent of 78% and 72%, respectively. That is, the inhibition was seen both in the male and female rats after 6 weeks and 12 weeks' exposure duration, respectively. CMT significantly reduced the methemoglobin levels increased by DDS in Ito model but not in the Pitot model. This finding suggests a sex related difference in the ability of CMT to suppress MHB as the Pitot model uses female rats and the Ito model employs male rats. However, CMT reduced the severity of hemosiderosis in the spleen in both the models indicating the effective suppression of DDS metabolism by CMT, with respect to the metabolites that are responsible for hemosiderosis. CMT treatment also reduced the glutathione in the livers, in the Ito model.

7. The CYP2C9 inhibitor TLB at 50mg/kg-dose level inhibited the promoting effect of DDS in the Ito’s and Pitot’s models; the volume
of foci/nodules was reduced to an extent of 79% and 68% respectively. That is, the inhibition was seen both in the male and female rats after 6 weeks and 12 weeks' exposure duration, respectively. TLB reduced DDS induced methemoglobinemia in lto model but not in the Pitot model and a reduction in the severity of hemosiderosis in the spleen was observed in only the Pitot model but not the lto model. This may again be related to the sex of the rats used. The degree of suppression was however not as efficient as CMT. This difference may also be attributed to the difference in CYP450 in the two sexes, since the degree of methemoglobinemia too was different in both the sexes. Males had higher levels where as females had lower levels. TLB was able to suppress the hemosiderosis only in the female rats, but was unable to reduce it in the male rats. The study thus indicates a difference in the metabolic pattern in between the two sexes, which may be related to the differences in the CYP450 in the two sexes.

8. PLV at 10mg/kg inhibited tumor promotion induced by DDS in both the lto and Pitot's models. PLV inhibited the promoting effect of DDS in the two models by 51% and 67%, respectively. That is, the inhibition was seen both in the male and female rats after 6 weeks and 12 weeks' exposure duration, respectively. PLV significantly reduced the methemoglobin levels increased by DDS in lto model but the reduction was observed only initially in the Pitot model. This finding suggests a sex related difference in the ability of PLV to suppress MHB as the Pitot model uses female rats and the lto model employs male rats. However, PLV reduced the severity of hemosiderosis in the spleen in both the models indicating the effective suppression of DDS metabolism by PLV, with respect to the metabolites that are responsible for hemosiderosis. PLV reduced the glutathione content in the livers in the Pitot model and also the degree of hemosiderosis in spleen. This, as mentioned above,
indicates that glutathione was utilized in the conversion of the toxic DDS metabolites back to DDS and demonstrated better utilization of glutathione in the female rats.

9. BTL at 200mg/kg dose had no effect on the tumor promotion by DDS, PB or AAF in both the Ito and Pitot models. BTL also did not effect the initiating ability of DEN in the Ito model since it did not effect the number of foci induced by DEN. In the Ito model BTL was effective in reducing the DDS-induced methemoglobin and hemosiderosis only in the Ito model but not in the Pitot model. However, BTL reduced the liver GSH content in the Ito model only and not in the Pitot model. This again shows utilization of GSH for the recycling of toxic DDS metabolites to DDS. When tested for its promoting effects, BTL did not exhibit any tumor promoting effects.

Thus it can be concluded from the present study that DDS is a potent promoter of hepatocarcinogenesis and the metabolites responsible for the hematological side effects may not be entirely involved in for the tumor promotion potential of DDS. The H₂ antagonist cimetidine, the hypoglycemic tolbutamide and the hepatoprotective natural product picroliv potentially inhibit DDS induced tumor promotion. While the betel leaf has neither beneficial nor tumor promoting effects in rat.
BIBLIOGRAPHY
BIBLIOGRAPHY


Buchara, M., and Fuchs, R. P. P. 1985. DNA binding and mutation spectra of the carcinogen 
N-2-amino-fluorene in Escherichia coli: A correlation between the conformation of the pre-


Vol. 9, No. 11, 2nd edition, Ed. G. M. Cooper. Jones and Bartlett Publishers, Boston. 1309-
1315.

- 7.


4, 207-250.

Rev. Toxicol. 2, 419-443.

24, 267-303.


induced focal and nodular lesions in the livers of newborn mice. Toxicol. Pathol. 13, 3-9.

Chander, R., Diwedi, Y., Rastogi, R., Sharma, S. K., Garg, N. K., Kapoor, N. K. and Dhawan,
B. N. 1990. Evaluation of hepatoprotective activity of picroliv (from Picrorhiza kuruza) in

Picrorhiza kuruza are scavengers of superoxide anions. Biochem. Pharmacol. 44, 180-183.


Lee-Chen, S. F., Chen, C. L., Ho, L. Y., Hsu, P. C., Chang, J. T., Sun, C. M., Chi, C. W. and Lin, T. Y. 1996. Role of oxidative DNA damage in hydroxychavicol-induced genotoxicity. Mutagenesis. 11, 519-23


initiation, promotion, and progression in one model of hepatocarcinogenesis in the rat. *Toxicol. Pathol.* 17, 594-612.


initiation, promotion, and progression in one model of hepatocarcinogenesis in the rat.  


Vage, C. and Svensson, C. K. 1994. Evidence that the biotransformation of dapsone and monoacetyl dapsone to their respective hydroxylamine metabolites in rat liver microsomes is mediated by cytochrome P<sub>450</sub> 2C6/2C11 and 3A1. *Drug Metab. Dispos.* 224, 572-7


