SUMMARY

In vitro study on production of cell biomass and secondary metabolites of Gymnema sylvestre R.Br. of pharmaceutical importance was investigated. Different explants such as node, juvenile leaf, shoot tip and internode were selected. More than a few findings are elaborately discussed on the effect of plant growth regulators on tissue response.

Maximum primary callus initiation was achieved with nodal explant on MS basal medium supplemented with 2,4-D (4.52 μM) and BA (2.22 μM). It was found that each growth regulator had its own distinct effect on callus induction and synthesis of active compounds. Auxins 2,4-D and NAA had double the effect on fraction-IV yield compared to leaf.

Remarkable callus biomass to the tune of 370 g/l (FW) and 23.12 g/l (DW) was obtained on medium fortified with 2,4-D (2.26 μM) + NAA (13.43 μM) + BA (2.22 μM). Sucrose concentration also found to exert its effect on increasing biomass yield. But the maximum total active priciple fraction was obtained in sucrose at 3.5 %. Interestingly an increased sucrose concentration (4%) showed profound effect on the yield of Fraction IV, which was 200 %.

Age of culture showed significant effect on biomass yield and different active principles fractions in a standardised medium. Fraction-I was noteworthy on the 12th and 15th day old cultures and Fraction – II was noteworthy on the 24 day, Fraction-III was profoundly high (157%) on the 30th day and Fraction-IV was enormously high (251%) on the 15th day.
Effect of different light did not show any significant effect on biomass yield whereas in dark resulted a comparable biomass yield to that of control was seen. But different light played more significant role on compound synthesis. Exposure to fluorescent light for 8 h showed amazing effect on Fraction-IV (315%) yield and higher total fraction. Red light played more prominent role on Fraction-II and IV yield. Even though culture grown in dark showed increased biomass, the yield of fractions was drastically reduced. Similarly continuous fluorescent light exposure also yielded poorly. But 24 h exposure to red light showed significant yield of total fractions. This clearly indicates that the compound synthesis is light dependent.

Elicitors showed a remarkable enhancement in compound yield from cell biomass and its culture filtrate.

The electrophoresis study implies that there are more polypeptide compounds in callus than that of in vivo leaf.

**CONCLUSION**

There is no known report of production of active principles of *G. sylvestre* by callus. This is the first successful attempt of production of secondary metabolites where, production levels can be manipulated with appropriate PGRs. Further studies will be directed towards large scale production, testing the efficacy of secondary metabolites through animal cell lines and exploring market potential.
FUTURE PLAN

The demand in Pharmaceutical industries for plant based raw materials is ever increasing. The present study is a stepping stone for in vitro production of required active principles of Gymnema sylvestre R.Br.

The Gymnemic acid and gurmarin are derivatives of G. sylvestre having more medicinal importance. The presence of these active principles in the leaf is controlled by various environmental factors. Thus the present in vitro study clearly demonstrates that the amount of secondary metabolites present in the callus is higher than the cultivated leaf and has scope for further improvements. The study has also shown the presence of additional compounds that may be beneficial or harmful. Pharmaceutical activity of the callus fractions for the treatment of diabetes and other related treatments have to be standardised.

Laboratory scale study shows that G. sylvestre tissue culturing can be scaled up for commercial ventures. The use of biotechnological tools like immobilisation, elicitors, metabolic engineering and bioreactors for continuous production can further be fine tuned for mass production.

The production of active principles of secondary metabolites from G. sylvestre through callus by tissue culture technique opens a new market potential for naturaceuticals. This can ensure the standard quality, continued availability. This technique also produces pure active principles in short time and the required active principles can be obtained by manipulation of media. This also does not require vast area of land maintenance etc., as opposed to the whole plant.