SUMMARY AND CONCLUSION
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*Amorphophallus campanulatus* (Roxb.) Blume belonging to the family of Araceae is a perennial herb commonly known as elephant foot yam. It is basically a tuber crop of south East Asian origin and is largely cultivated throughout the plains of India for using its corm (bulb) as food. This plant is also valuable as medicine especially the corm has been used traditionally for the treatment of liver diseases, abdominal pain, abdominal tumors, piles, enlargement of spleen, asthma and rheumatism. The main objective of the study was to evaluate the antioxidant and chemopreventive potential of *A. campanulatus* tuber in experimental models since there was no scientific data available regarding its medicinal use to verify traditional claims.

Preliminary studies were started with the extraction of the tubers of *A. campanulatus* with n-hexane and methanol. Phytochemical evaluation of n-hexane extract showed the presence of steroids. But the screening of methanolic extract revealed the presence of alkaloids, tannins, glycosides, phenols, flavonoids, saponins and carbohydrates. *In vitro* studies revealed that *A. campanulatus* tuber methanolic extract (ACME) has higher antioxidant and free radical scavenging activity than *A. campanulatus* tuber n-hexane extract (ACHE), which may be attributed to its higher phenolic and flavonoid contents. The antioxidant activity of ACHE and ACME were compared with that of standard compounds viz. ascorbic acid, quercetin etc. In addition, the LC-MS analysis for the phytochemical profiling of ACME revealed the presence of eight major phytochemicals with proven antioxidant/cytotoxic/anticancer properties viz., Cinnamaldehyde, Ferulic acid, Retinol, Quercetin, Quercetagetin, 1-Caffeoyl-β-D-glucose, Triacontanol and Asiatic acid. Hence,
further studies were conducted to establish the *in vivo* antioxidant potential and anticancer efficacy of ACME particularly its chemopreventive activity against colon and liver cancer.

*In vivo* antioxidant and hepatoprotective potential of *A. campanulatus* tuber was evaluated in male Wistar rats against Thioacetamide (TAA) induced liver damage in preventive and curative models. Single dose of TAA (100 mg/kg; s.c.) was administered to the rats in all groups except the normal control. In pre-treatment groups, rats were treated with daily dose of ACME (125 and 250 mg/kg; p.o.) for 9 days prior to TAA administration. In post-treatment groups, rats were administered with ACME 2, 24 and 48 h after TAA intoxication. ACME significantly (*p* ≤ 0.05) prevented and reversed the elevation of serum AST, ALT, ALP, LDH, and tissue malondialdehyde levels in both the experimental models. Hepatic and renal GSH, GST, GR, GPx, and catalase levels were remarkably increased by the administration of ACME in both the treatment regimens. Quantification of histopathological changes also supported the preventive and curative effects of extract and the results were comparable with silymarin, the standard hepatoprotective drug. Further, in both the experimental model, the extract exhibited its antioxidant potential in a dose dependent manner.

Many antioxidant substances have anticancer or anti-carcinogenic properties. Since ACME exhibited potential antioxidant activity in both *in vitro* and *in vivo* experimental models, further studies were conducted to exploit its anticancer properties. The phytochemical constituents identified by the LC-MS analysis of ACME such as Ferulic acid, Retinol, Quercetin and Asiatric acid are known for its anti-colon cancer properties, whereas Cinnamaldehyde is reported to induce
apoptosis in human hepatoma cells. In view of this, we have evaluated the chemopreventive potential of ACME against 1, 2-dimethylhydrazine (DMH) induced colon carcinogenesis and N-Nitrosodiethylamine (NDEA) induced hepatocellular carcinoma in rats.

Colon cancer is a major cause of morbidity and mortality in developed and developing countries. In a dose response study, the chemopreventive potential of ACME was evaluated on 1, 2-dimethylhydrazine (DMH) induced colon carcinogenesis in male Wistar rats. Rats were assorted into five groups, viz., group I served as control and group II animals received ACME alone (250 mg/kg body weight p.o.) daily for 15 weeks, group III rats received DMH (20 mg/kg body weight) subcutaneously once a week for the first four weeks, groups IV and V rats received DMH along with ACME (125 and 250 mg/kg body weight p.o./day) for the entire period of 15 weeks. ACME at a dose of 250 mg/kg was able to exert a more pronounced effect, as shown histologically by a significant reduction in the extent and severity of lesions in colon tissue, abridged expression of proliferating cell nuclear antigen (PCNA) and also biochemically by the modulation of hepatic, intestinal and colonic antioxidant status and lipid peroxidation. These results indicate that ACME can exert a dose dependent preventive effect against DMH-induced colon carcinogenesis.

The effect of ACME on lipid peroxidative damage, antioxidant status and aberrant crypt foci formation was also investigated in a long term preclinical model of DMH induced colon carcinogenesis in rats. The male Wistar rats were divided into six groups, viz., group 1 control rats received modified pellet diet; group 2 rats received ACME (250 mg/kg body weight) orally along with modified pellet diet;
group 3 rats received DMH (20 mg/kg body weight) subcutaneously once a week for the first 15 weeks; groups 4, 5 and 6 rats received ACME along with DMH during the initiation, post-initiation stages and the entire period of study respectively. All the rats were sacrificed at the end of 30 weeks and the liver, intestine and colon tissues from different groups were subjected to biochemical and histopathological studies. The results showed decreased levels of liver enzymic and non-enzymic antioxidants and increased levels of lipid peroxidation in DMH treated rats, which were significantly (P<0.05) reversed on ACME supplementation. Moreover, the intestinal and colonic lipid peroxidation (MDA) and also the antioxidants such as catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR) and reduced glutathione (GSH) were significantly diminished in DMH treated rats, which were significantly (P<0.05) elevated on ACME supplementation. ACME administration also significantly suppressed the formation and multiplicity of aberrant crypt foci (ACF) and lowered the levels of serum marker enzymes viz. AST, ALT, ALP and LDH. These results indicate that ACME could exert a significant chemopreventive effect on colon carcinogenesis induced by DMH.

The potential chemopreventive activity of ACME was also evaluated in Hepatocellular carcinoma (HCC). HCC is one of the world’s deadliest cancers, ranking third among all cancer-related mortalities. In this investigation the efficacy of ACME on N-nitrosodiethylamine (NDEA) induced hepatocarcinogenesis in Wistar albino male rats were assessed. The animals were divided into five groups. Group I rats served as normal control in the experiment. All the rats except group I were administered with 0.02% NDEA (2ml, 5 days/week) for the first 20 weeks of
the experiment to induce HCC. After 20 weeks of NDEA intoxication, group III rats received silymarin at a dose of 100 mg/kg for the last 28 days. Whereas group IV and group V animals were supplemented with 125 mg/kg and 250 mg/kg of ACME respectively, for the last 28 days, after 20 weeks of NDEA challenge. After the experimental period, the biochemical indices of serum, the changes of morphology, histology, antioxidant status and the expression of PCNA in the liver were examined to assess the curative effect of the extract. ACME administration significantly inhibited the increase of the hepatic nodule incidence and nodule multiplicity induced by NDEA, improved hepatocellular architecture and significantly inhibited the NDEA induced elevation of serum biochemical indices (AST, ALT, ALP, LDH gamma-glutamyl transferase (GGT), alpha fetoprotein (AFP) and total bilirubin) in a dose dependent manner. The Biochemical analysis of hepatic tissue also demonstrated that ACME counteract NDEA induced oxidative stress in rats exemplified by the restoration of catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activity, and the level of reduced glutathione (GSH) in liver. Notably, 250 mg/kg ACME supplementation showed better results than the treatment with 125 mg/kg ACME and silymarin (100 mg/kg), a known tumor suppressive agent against HCC. The significant defense exhibited by ACME against NDEA induced hepatocarcinogenesis might be related with the enhancement of the antioxidant activity and the inhibition of cell proliferation. From this, we can hypothesize that A. campanulatus tuber extract is a strong candidate as chemopreventive agent for liver cancer.
The dose-dependent (50 and 100 µg/mL) cytotoxic and apoptotic activities of the sub fractions of ACME viz. petroleum ether fraction (PEF), chloroform fraction (CHF), ethyl acetate fraction (EAF) and methanolic fraction (MEF) were also studied in human hepatoma, PLC/PRF/5 cells and human colon carcinoma cell line HCT-15. Antiproliferative effects of the extracts were studied by MTT assay. Apoptotic activity was assessed by DAPI, annexin V-FITC and JC-1 staining. The chemotherapeutic drug, 5-flurouracil (5-FU) was used as positive control.

A pronounced result of chemopreventive activity were observed in the cells treated with 5 - FU and CHF, whereas, EAF and MEF treated cells exhibited a moderate result and the least effect were observed in PEF treated cells. LC-MS analysis of the most promising chloroform fraction of ACME revealed the presence of ferulic acid, a phenolic compound reported to possess antiproliferative activity against colon cancer. These findings indicate that among the sub fractions of ACME, CHF possess significant dose-dependent antiproliferative and apoptotic activity against PLC/PRF/5 cells and HCT-15 cells.

Thus, the present study on *A. campanulatus* conclude that the tuber possess excellent antioxidant and anticancer properties. These medicinal properties attributed to this tuber may be due to the combined activity of the identified phytochemicals such as Cinnamaldehyde, Ferulic acid, Retinol, Quercetin, Quercetagetin, 1-Caffeoyl-β-D-glucose, Triacontanol and Asiatic acid in the methanolic extract. It is possible that the anticancer effect of the extract is mediated through antioxidant and/or free radical scavenging activities. This investigation further demonstrates that *A. campanulatus* tuber extract is a promising
chemopreventive agent for colon and liver cancer and might be useful clinically after further molecular chemopreventive studies.

The isolation of the active phytochemical constituent from *A. campanulatus* tuber and the determination of their individual antioxidant and anticancer activity will be further performed along with the pharmacological studies at molecular level to establish the mechanism of the action of the drug against colon cancer and hepatocellular carcinoma.