SUMMARY AND CONCLUSION
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Woodfordia fruticosa Kurz. (syn. Woodfordia floribunda Salisb.) belonging to the family Lythraceae, is a much branched beautiful shrub. It is the plant of tropical and subtropical regions with a long history of medicinal use. All parts of the plant possess valuable medicinal properties viz anti-inflammatory, anti-tumour, hepatoprotective and free radical scavenging activity but flowers are in maximum demand. The dried flowers are used in the treatment of a wide variety of disorders such as dysentery, sprue, rheumatism, hematuria, hemorrhoids, derangement of liver, as tonic in disorders of mucous membrane etc. in Indian traditional system of medicine. The flowers are used in the preparation of Ayurvedic fermented drugs called “Aristhas” and “Asavas”. The main objective of the study was to evaluate the antioxidant, anti-fibrotic and chemopreventive potential of Woodfordia fruticosa Kurz flowers in experimental models.

Preliminary studies were started with the extraction of dried flowers of Woodfordia fruticosa in petroleum ether (PEWF), chloroform (CEWF), acetone (AEWF), ethanol (EEWF) and methanol (MEWF) and the in vitro antioxidant activity of all these extracts were studied. Phytochemical evaluation of methanolic extract of W. fruticosa (MEWF) revealed the presence of phytochemical constituents such as alkaloids, flavonoids, phenols, tannins, glycosides, saponins, carbohydrates, proteins and amino acids. The results of in vitro antioxidant study reflects the activity of different extracts of Woodfordia fruticosa flower extracts in the following order MEWF > AEWF > EEWF > CEWF > PEWF. Here MEWF has higher antioxidant and free radical scavenging activity than the other extracts, which may be attributed to its higher phenolic and flavonoid contents. The antioxidant activity
of all the extracts were compared with that of standard compounds viz. ascorbic acid, quercetin etc. In addition, the LC-MS analysis for the phytochemical profiling of MEWF revealed the presence of major phytochemicals with proven antioxidant/cytotoxic/anticancer properties viz., octacosanol, malonic acid, oxaloacetic acid, octanoic acid, isocaryophyllene, confertin, Quercetin methyl ether, ellagic acid, ursolic acid, stigmasterol, hydroxy methyl flavan etc. Hence, further studies were conducted to establish the *in vivo* antioxidant, antifibrotic and anticancer efficacy of MEWF particularly its chemopreventive activity against liver cancer.

*In vivo* antioxidant and hepatoprotective potential of MEWF was evaluated in male Wistar rats intoxicated with thioacetamide (TAA) in both 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 MEWF (100 and 200 mg/kg; p.o.) for 9 days prior to TAA administration. In post-treatment groups, rats were administered with MEWF 2, 24 and 48 h after TAA intoxication. MEWF 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 MEWF in both the treatment regimens. Quantification of histopathological changes also supported the preventive and curative effects of the extract and the results were comparable with silymarin, the standard hepatoprotective drug. Since the MEWF showed antioxidant and hepatoprotective efficacy, a long term study was conducted to evaluate its antifibrotic efficacy.
Antifibrotic potential of MEWF was evaluated in carbon tetrachloride (CCl₄) induced hepatic fibrosis in male Wistar rats in preventive and curative models. Liver fibrosis was induced in male Wistar rats by exposure to carbon tetrachloride (150 µl/100 gm, p.o) for 10 weeks. CCl₄ was administered to the rats in all groups except normal and drug control. In pre-treatment groups, rats were treated with daily dose of MEWF (100 and 200mg/kg; p.o) for the entire period of 10 weeks. In post-treatment groups, rats were administered with daily dose of MEWF (100 and 200 mg/kg; p.o) for the next 2 weeks, after 10 weeks of CCl₄ intoxication. MEWF significantly ($p \leq 0.05$) prevented and reversed the elevation of serum AST, ALT, ALP, LDH, tissue malondialdehyde and hydroxyproline content in both the experimental models. Hepatic GSH, GST, GR, GPx and catalase levels were remarkably increased by the administration of MEWF in both the treatment groups. Quantification of histopathological changes and reduced level of expression of collagen III also supported the antifibrotic effect of MEWF in a dose dependent manner comparable with silymarin.

Many antioxidant substances have anticancer or anti-carcinogenic properties. Since MEWF exhibited potential antioxidant activity in both in vitro and in vivo experimental models and showed antifibrotic activity in chronic hepatic fibrosis model. Hence further studies were conducted to exploit its anticancer properties. The phytochemical constituents identified by the LC-MS analysis of MEWF such as confertin, quercetin methylether, ellagic acid etc are known for its antitumour properties. In view of this, future studies were conducted to evaluate the chemopreventive potential of MEWF against N - Nitrosodiethylamine (NDEA).
induced hepatocellular carcinoma in preventive and curative models was also evaluated.

Hepatocellular carcinoma (HCC) is one of the world’s deadliest cancers, ranking third among all cancer related mortalities. The potential chemopreventive activity of MEWF was evaluated against N-nitrosodiethylamine (NDEA) induced hepatocarcinogenesis in male Wistar rats in preventive and curative models. 0.02% NDEA (2ml, 5days/week) was administered to the rats in all groups except normal and drug control. In pre-treatment groups, rats were treated with daily doses of MEWF (100 and 200mg/kg; p.o) for the entire period of 20 weeks. In post-treatment groups, rats were administered with daily dose of MEWF (100 and 200 mg/kg; p.o) for the next 28 days, after 20 weeks of NDEA intoxication. After the experimental period the biochemical indices of serum, the changes in morphology, histology, antioxidant status, expression of cancer markers like PCNA, VEGF and Cyclin D1 in the liver were examined to assess the preventive and curative effect of the extract. MEWF administration significantly prevented and reversed the increase of the hepatic nodule incidence and nodule multiplicity induced by NDEA, improved hepatocellular architecture and significantly inhibited the elevation of AST, ALT, ALP, LDH, GGT, AFP and total bilirubin in a dose dependent manner. The biochemical analysis of hepatic tissue also demonstrated that MEWF counteract NDEA induced oxidative stress in rats exemplified by the prevention and restoration of the level of GSH, activity of GST, GR, GPx and CAT in preventive and curative models. Immunohistochemical analysis of liver tissue showed the localization of overexpressed vascular endothelial growth factor, proliferating cell nuclear antigen and cyclin D1 around the periportal area in N-nitrosodiethylamine treated rats. The
over expression of all these cancer markers were inhibited by the treatment with MEWF at a dose of 200mg/kg, b.w in both treatment groups indicating its inhibitory role of the extract on neo-vasculature formation in rat liver. Notably 200 mg/kg MEWF supplementation showed better results than the treatment with 100 mg/kg MEWF and silymarin (100 mg/kg), a known tumour suppressive agent against HCC in both the experimental groups. This significant defense exhibited by MEWF 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 *W. fruticosa* flower extract is a strong chemopreventive agent against liver cancer.

The dose-dependent (50 and 100 µg/mL) cytotoxic and apoptotic activity of MEWF and the sub fractions of MEWF viz. petroleum ether fraction (PEF), chloroform fraction (CHF), ethyl acetate fraction (EAF) and methanolic fraction (MEF) were studied in human hepatoma PLC/PRF/5 cells. Antiproliferative effects of the extracts were studied by MTT assay, apoptotic activity was assessed by DAPI and JC-1 staining. The chemotherapeutic drug, 5-fluorouracil (5-FU) was used as positive control. Cytotoxicity study by MTT assay and apoptotic study by DAPI and JC 1 staining indicated that the number of apoptotic cells were higher in drug treated cells than untreated and DMSO controls. In DAPI staining, PLC/PRF/5 cells treated with the drugs showed marked nuclear fragmentation and chromatin condensation which are clear indications of apoptosis. In JC-1 staining, the loss of mitochondrial membrane potential ($\Delta \Psi_m$) is indicated by the decrease of red fluorescence and the increase of green fluorescence. 18 h treatment of PLC/PRF/5 cells with 100 µg/ml of CHF followed by the JC-1 staining resulted in green fluorescence in majority of
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The results demonstrated that chloroform fraction (CHF) is the most effective one. LC-MS analysis of most promising CHF of MEWF revealed the presence of confertin, quercetin methyl ether, ellagic acid and stigmasterol. Here confertin, quercetin methylether and ellagic acid are compounds reported to possess antitumour activity. So the components in single or in combination with other components present in the CHF might be responsible for the cytotoxic and apoptotic activity against human hepatoma PLC/PRF/5 cells.

Thus the present studies on *Woodfordia fruticosa* conclude that the flower possess excellent antioxidant, hepatoprotective, antifibrotic and anticancer properties. These properties of the extract are mediated through antioxidant and/or free radical scavenging activities. The medicinal properties attributed to this flower may be due to the single or combined activity of the identified phytochemicals such as confertin, quercetin methylether, ellagic acid etc. The isolation and purification of the active phytochemical constituents from *Woodfordia fruticosa* flower and the determination of their individual antioxidant, antifibrotic and anticancer properties will be further performed.