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Anticancer Capability of Go-Ark With *Plectranthus Amboinicus* Against Hepatocellular Carcinoma

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Abstract
Cancer is a life threatening disease and an emerging public health issue that affects humans worldwide. The main objective of the present investigation is to detect the effects of Cow ark, synergistic with ethanolic leaves extract of *Plectranthus amboinicus* on Diethylnitrosamine (DEN) and 2-Acetylaminofluorene (AAF), stimulated hepato carcinogenesis. Cow ark and 5% Ethanolic extract of *P. amboinicus* were systematically prepared. Identification of *in situ* DNA fragmentation was performed by TUNEL assay to explore apoptogenic characteristics of distillate with *P.amboinicus* leaves extract. Apoptotic study observed that a maximum number of apoptotic cells were detected in the liver cells belong to (AB) group, when compared with other tested groups. The Go-ark with *P.amboinicus* extract also showed chemo preventive activities by curing hepatocarcinogenesis activated by DEN/AAF carcinogens. This study proved to be a promising anti-cancer effect of distillate and *P. amboinicus* extract synergistically in increasing programmed cell deaths.

Key Words: *Plectranthus amboinicus*, apoptosis cells, hepatocarcinoma cells, *in vivo*

INTRODUCTION

Cancer is still a challenging disease and a major public health problem that affects individuals worldwide. Cancer stands as the second major cause of mortality in the United States. Recent data alarming that projected new cancer occurrences and mortality in the United States in 2022, offers a complete view of development of cancer. New cases are determined to increase by 70% in the forthcoming two decades. A statistical report reveals that in 2022, 609,360 people in the US are expected to die of cancer, accounting for 1700 deaths per days [1-4].

Hepatocellular carcinoma (HCC) is one of the most common malignancies reported in the world. Due to the rapid global pandemic of hepatitis B and C viral infections, the occurrence of HCC is known to be increasing in Asia and the majority of Western countries [5-8]. The treatment for advanced HCC is found to be very limited and novel approaches are essential [9-10].

Several investigations described that the traditional organic and animal origin products can be used for improved general health [11-12]. A study conducted by Cow Science Research Center reported that indigenous cow breed urine contains, therapeutic values in treating various kinds of HCC malignancy, Protective effects of Cow urine distillate such as, declining the enzymatic actions of SGOT, SGPT, ALP, GGT and total bilirubin with distillate tends to reduce the enhanced enzyme levels, indicating that it struck the action of free radical generations [13-17].

P. amboinicus is also a prolific plant used in Asia and African countries. This species was well recorded, for their use in curing, many ailments for human health A recent study elucidated that methanolic leaf extracts of *P. amboinicus* exerted a vital role in the management of oxidative stress, using their antioxidant mechanisms [17-18]. Another study in the existing line, revealed that hydro alcoholic extract *of P. amboinicus* was reportedly exhibited anti- inflammatory and anti-tumor activities [19]. Therefore, in the

present investigation was performed aiming at, to find the anti-cancer efficacy of Cow urine distillate, combined with *P. amboinicus* ethanolic extract in the hepatocytes of HCC cells *in vivo*.

MATERIALS AND METHODS

Collection of Cow urine

The fresh and very first flow of urine was collected in a container, a wide conical flask, from a healthy breed, Bos indicus, in the early morning. Collected urine was brought to the nearby laboratory, as early as, and filtered carefully, using Whatmann filter paper.

Preparation of Go-ark

As collected sample was subjected to the simple distillation set up for the urine distillation process. A wide mouth conical flask with broad rounded bottom, was placed on a heating mantle, and allow continuously, then it was linked with water cooled condenser, at one end, while, other side was connected with a beaker, which was incorporated into a large container for the distillate collection [19]. Clean filtrate distillate was received in a sterile container carefully, and it was kept as a stock solution. From this stock distillate, a varied concentration, such as 5% was formulated to detect anticancer activity potentials.

Collection of liver tissues

The rats were stimulated with carcinoma, by injecting 200 mg/kg-1 Diethyl Nitrosamine (DEN) dissolved in corn oil as vehicle, followed by a recovery of 15 days as food, blended with 2- acetylaminofluorene (0.02% AAF) as enhancer of hepatocarcinogenesis. At the end of the treatment tenure, the rats were weighed, and subsequently sacrificed by using ether anesthesia. Liver were detached as quick, and washed well in 1.15 concentrated potassium chloride solution suddenly. As processed liver samples were kept in care for Tunel assay and slices were viewed under confocal microscope for critical analysis.

TUNEL Assay (Apoptotic observations)

TUNEL is a dynamic staining method for detecting dead cells. It has been demonstrated as a powerful tool for determining manifestations of cell death in biological systems, in particular, while used as part of a panel of complementary biomarkers. Terminal deoxynucleotidyl transferase – aided dUTP nick end labeling the free S -hydroxy terminal. Tissues isolated from the rats of each group were cut into pieces from the largest lobe and were well fixed in 4% paraformaldehyde, developed in paraffin blocks, subsequently, sections were done at dead end. A TUNEL fluorometric system was performed, based on the manufacturer instructions which was displayed. Such stained slides with TUNNELS were brought under fluorescent microscope. Prepared slides were tested and a Scoring measurement was also carried out, at x200 magnifications and chosen 5 location spots, randomly [20]. In each spot, Green coloured cells were enumerated as TUNEL positive apoptotic cells of examined tissues. Fluorescence images obtained, using fluorescence imaging systems.

Statistical application

The values expressed in the graph as mean + or -SD. By applying SPSS software package, ANOVA test was performed to know if the statistically significant difference between varied groups at $p<0.05$ were determined.

RESULTS

Detection of Apoptosis cells, using TUNEL system

The tested and stained liver tissues of DEN/AAF stimulated rats, exhibited that a small number of fluorescent Green stained cells were detected, whereas, Red and Orange colored stained cells were

declared as viable cells, still present time. Our TUNEL staining results showed the presence of apoptotic cells with abundant green fluorescence of liver slices, which were received from DEN/AAF activated rats, treated with 5% Cow urine distillate (Fig-1-3). The present observation is obviously evident that many prominent intensified signals of DNA breaks were clearly appeared, while, distillate along with extracts of *P. amboinicus*, treated liver cells, in specific, HepG2 cells, when compared to vehicle control group (C) rat liver cells.

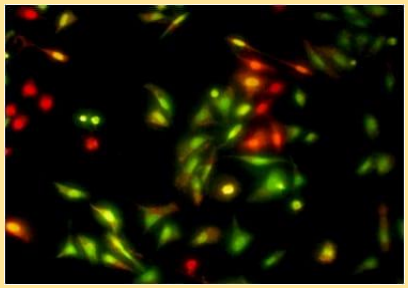

Fig 1 **control**

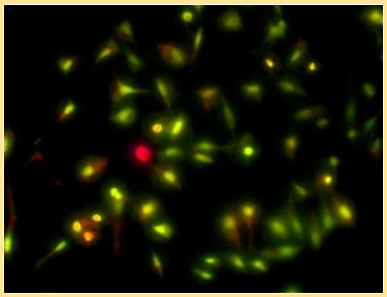

Fig 2 **Go-ark treated on liver tissues**

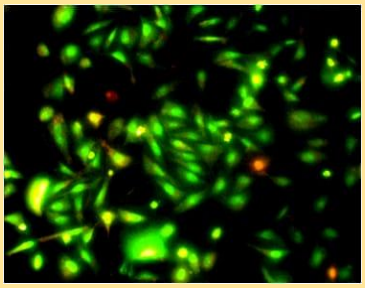

Fig 3 **Go-ark *with P. amboinicus treated* on liver tissues**

MEASUREMENT OF TUNEL POSITIVE CELL SCORING

Counting of dead cells (apoptotic cells) was also performed by using TUNEL positive cells score. Results obtained from TUNEL assay, subsequently, positive cells scoring were determined. The findings were depicted in the graphical representation as in Fig 4 which reveals that there was a significant difference between Distillate with *P. amboinicus* extract treated group with other designed rat groups at $p<0.05\%$ in apoptotic cells detected.

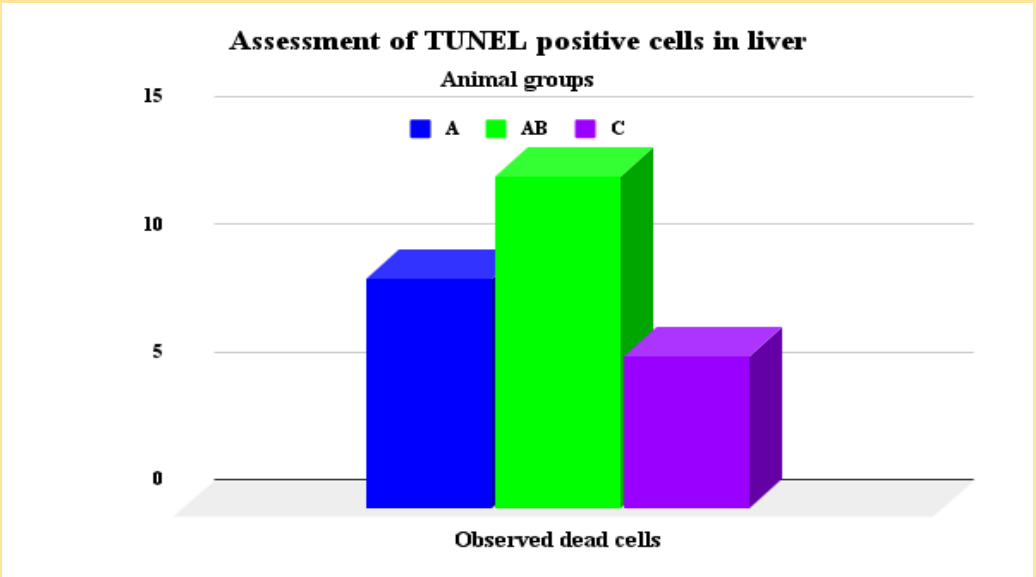

Fig 4: values expressed as mean with Standard deviation. A significant apoptosis detected in cancerous tissues treated with 5% concentrated Go-ark with *P.amboinicus,* when compared to other groups.

DISCUSSION

The rational formulation of therapeutic entities from traditional medicine to treat dreadful diseases and pathogenic infections since Rig Veda and it was compared as nectar [21-23]. Cow urine acts as a 'bio

enhancer' to the specific antibiotics previously reported. The different fractions of cow urine distillate have been reported to be antimicrobial, anticancer and larvicidal properties. Existing lines of studies shown that cow urine distillate is an immune enhancer [24- 31]. Moreover, cow urine possess ,antioxidant characteristics and helps to lymphocytes to survive by suppressing, their suicidal cells and repairing the injured DNA. Hence, cow urine distillate used as effective anti cancer therapy [32-36]. Cow urine was observed in a study, as a chemopreventive ability and resulted, the incidence of tumor was statistically less was recorded [37-41]. However, cow urine distillate affects the other disorders or other cancer related have never been highlighted in earlier investigations. Therefore current study conducted, as a complementary investigation, for its anti cancer effects of cow urine distillate along with ethanol extract of *P. amboinicus in vivo*, in using hepatocarcinogenesis models. This would offer effective and authentic information to Cancer research.

Apoptosis is associated either with external stimulus or stress inducing agent of pathological consequences. In chemotherapeutic treatments, it was reported to induce apoptotic mechanisms of tumor cell death, and it could be a positive means for present cancer treatment [42-43].) Besides, apoptosis is an innovative target for removing cells which are transformed into advanced stages, whereas other practiced mechanisms exhibited were unsuccessful to block the upstream cancer development [44-45], The therapeutic activation of *P. amboinicus* has been analyzed by many research groups and reported to have strong cytotoxic and anti tumor potentials [46].

In several investigations, identification of chemo preventive stimulated a programmed cell death in HCC in animal models were demonstrated, including Humans [47-49]. Analyzing DNA fragments *in situ* , using TUNEL assay is a popular technique to detect apoptosis and its visualizations, labeling of DNA fragments, due to apoptosis [50]. In earlier stages of liver carcinoma, apoptosis, extremely compensate, cell proliferation [51].

Quantitative assessment of apoptosis used to reveal the tendency to enhance in the conversion of normal rat apoptosis to malignant cells. In our investigation variation in difference in TUNEL apoptotic positive cells were found to be markedly different ($p < 0.05$) between control and cancer stimulated rat groups.

Moreover, a good agreement with previous studies, such as Hae-Jeung et al, [52] described that supplementation of *P. amboinicus* with distillate have shown to be a significant anti cancer effects against DEN/AAF stimulated HCC, were ideal by TUNEL positive cells count in the examined liver slices. Our findings showed that an abundant number of death cells were observed in the treatment of go-ark with ethanol extract of *P. amboinicus* than other treatment schedules. This is apparent evidence that pre-neoplastic cells, activated by DEN/AFF are more vulnerable to the apoptotic cells, when compared to normal cells. This might be attributed to the synergistic effects of cow urine distillate and *P. amboinicus* ethanol extract, in killing cancer cells in the treatment. Interestingly, normal cells were not affected by the distillate and extract treatment. These findings proved that distillate and *P. amboinicus* extract altogether, showed notable hepatoprotective effects in the liver cells [53]..

CONCLUSION

Above comprehensive study affirmed that Go-ark extract of *P. amboinicus* played a significant role in increasing apoptosis of cancer cells in stimulated liver carcinogenesis.

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