International Journal of Aquatic Science
ISSN: 2008-8019
Vol 12, Issue 02, 2021

Indigenous Cow Ark With *Allium Sativum* -A Key To Therapeutic And An Effective Antioxidant To Hepatocellular Carcinoma

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ABSTRACT: Background : Cow distillate, popularly known as Cow ark, reportedly contains several therapeutic biomolecules and used as a remedy for diverse ailments including chronic and acute diseases. Hepatocellular carcinoma (HCC) is an aggressive disorder, causing significant cancer associated mortality worldwide.
Aim: In the present investigation, efficacy of cow ark on antioxidant ability and anticancer actions on HCC were determined and reported.
Methodology: Indigenous cow ark has been characterized and obtained aqueous fractions were validated using, series of assays. Investigation was performed on cow ark antioxidant ability by adopting DPPH radical scavenging and Superoxide measures, and results have shown to be remarkable effects, compared with prior reports. Findings favour the claim of Indian medicine health providers. Consequently, biochemical constituents of cow ark such as urea, phenol and amino acids were determined, and presented. Moreover, effects of cow ark on HCC was examined, using intra peritoneal administration of diethyl nitrosamine (DEN) to experimentally induce HCC (200 mg /kg b.w), with corn oil as vehicle in the wistar rats model. The hepatocytes were separated using collagenase perfusion measure and all tumor hepatocytes were segregated using flow cytometry. The effects of cow ark, as single, and enhancing plant extract (PE), and combined effects were analysed.
Results: Our study results exhibited that cow ark has the potential to accelerate Reactive Oxygen Species production ,and allow to increase membrane permeability (MP) and efficient discharge of Cytochrome c in HCC cancer cells while, no remarkable change was recorded in control hepatocytes. Conclusion: Our findings collectively endorsed that cow ark is a promising and novel therapeutic alternate and supported evident to the emerging cancer research in India.

Keywords: Antioxidant, Cow ark, hepatocellular carcinoma, Reactive Oxygen Species, Bos indicus

1. INTRODUCTION

Bos indicus is a domestic blessing animal and reported to be a sovereign remedy for several dreadful diseases to human health and is regarded as a " moving sanatorium"[1]. Cow urine has been living as an indispensable part of Indian heritages and rituals. Recent decades, cow urine distillates or cow ark (a distilled form of cow urine) attracted much attention of researchers and an increasing number of literature published on its therapeutic potentials and enhancing the ability of effectiveness to antimicrobial, antifungal and anti-cancer drugs [2]. Cow distillate confers strong effectiveness to the drugs as reported [3]. A recent study

deciphered that cow distillate exhibited its potential against Diabetes mellitus, Carcinoma diseases, Goiter, skin disease, and proved to be an antiseptic in Gynaecological disorder (Kishore et al,2015). Another group of study was reported that fresh Cow urine and its distillate contributed a remarkable anti-obesity activity against fat diet stimulated animal model (Sanjay Sharma et al 2017). Edwin et al (2008) have demonstrated that cow ark has been prescribed for anti ageing and it has been reportedly developed immunity and free radical reduction implicated in the aging phenomenon [4]. Moreover, cow ark was offered with US patents No.6896, 907 and 6,410 059) attributable for its therapeutic properties, specifically its novel effects on antibiotic, anti-fungal and anti-cancer drugs. It is noteworthy that cow ark elevated the effectiveness of paclitaxel, a widely used, clinical therapy against breast cancer cell line specifically, MCF-7, was reported [5].

Hepatocellular carcinoma (HCC) is a widely emerging threat and one of the aggressive diseases of liver cancer. Above 6 Lakh people were affected and lead to mortality every year. World Health Organisation alarmed to intensify the research both in medical and pharmaceutical disciplines. Mitochondria is a power bank of a cell and played a crucial role in calcium and energy metabolism [6]. Nevertheless, it has shown to be significant apoptotic machinery and harbor a place for reactive ROS production in tumour cells [7]. Moreover, a noticeable distinction between non cancer mitochondria and tumour was recently reported [8]. It is pointed out that dysfunction of mitochondria is frequently observed in cancer cells and this phenomenon is shown to be varied to the type of cancer cells and based on proliferation, differentiation and micro environment. However, there are several claims targeting the use of cow ark, essential and urgent need is antioxidant effects of cow ark and its effects on HCC studies remain still very limited. Hence, we formulated the main objectives of the investigation was to characterize indigenous cow ark for determining the efficacy of therapeutic properties, targeting its role on anti-hepatocellular carcinogenic bio enhancingbehaviour.

2. MATERIALS AND METHODS

2.1 Plant extract formulation

Leaves of the plants of *Allium sativum* at 1000gm were powdered using a grinder. Followed by the extraction of 250gm of *A.sativum* powder and added methanol 50% and aged for a night.Subsequently filtered extract was desiccated. 0.5g of dehydrated extract was watery in 2mL DMSO for mitochondrial intervention [9].

2.2 Characterisation of Cow Ark (Cow Urine Distillate)

A second flow of *Bos Taurus Indicus* (Red Shindhi) cow urine was poised in a metal container with contamination free, directly from *Bos indicus* in hygienic circumstances. Using a small distillation setup, 10L of cow urine was subjected to distillation, continuously with a temperature at 40-50° C to get 8-9 L of the cow ark in 17 hrs. Distillate (Cow Ark) was packed carefully in a glass container and sealed for further analysis. HPLC profile of cow ark was carried out using C-18 column with Agilent HPLC, water as a mobile phase. 80:20 acetonitrile was fixed and flow rate 1.0 ml / Min. Detection point was set at 230 nm Det.4 Ch J/230nm. To understand the therapeutic potential of cow ark, by performing HPLC, chemical characterization of cow ark was also identified following the protocol described.

200ml Cow ark was added with 100ml of Methanol and extracted with hexane. The hexane fraction was used for testing antioxidant activities. The aqueous fraction was extracted using ethyl acetate, and tested for the effect on hepatocellular carcinogenesis. The collected distillate urine samples were subjected to biochemical analysis Berthelot, end point assay was adopted for urea and uric acid estimation by Murray et al., method was used to find out the Phenol content [10].

2.3 Antioxidant assay by DPPH

The free radical scavenging ability (antioxidants) of distillates of indigeneous cow ark was investigated by adopting two methods. These include DPPH radical scavenging ability and superoxide scavenging activity [11]. Ascorbic acid was used as a control to compare the efficiency. In the methanolic solution of DPPH, 0.05 ml test compound dissolved in methanol was mixed with a different concentration (1-5 mg m/G'). Similarly Equal amount of ethanol was added to the control. Absorbance was noted at 517 nm with systematic intervals of 15 minutes for 30 minutes. The inhibition rate was computed as per the standard formula

$$\% \text{ reduction} = \frac{\text{Control} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

DPPH radical was measured using spectrophotometric instruments.

2.4 Superoxide Scavenging Assay

Alkaline DMSO method was adopted for finding superoxide free radical scavenging ability. In this method, solid potassium superoxide was kept with DMSO in dry condition for a period of 24 h. and subsequently it was filtered prior to performing further procedure. In filtrate solution, an amount of 200 mL was mixed with 2.8 ml of an aqueous solution possessing nitro blue tetrazolium (56 μmg) EDTA and potassium phosphate buffer was used with a quantity of (10mM). Indigenous cow ark was taken 1ml with different concentrations such as 1mg mlG' to 5mg mlG'. This sample was added carefully and absorbance ability was noted at 560 nm against control solution. Control represented a pure DMSO alternative to alkaline DM50. The inhibition percentage was computed using the following formula.

$$\% \text{ of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of examine solution}}{\text{Absorbance of control}} \times 100$$

2.5 Experimental design for Anti-Cancer activity

Selected male Wistar rats, weighing about(250-300 g) were hosted in a metallic cage with contamination free and were fed with standard commercial pellets and water lipidium were used according to the experimental protocols. Animals were purchased from the Animal Health and Veterinary Science department, Govt. King Institute, Guindy, Chennai, Tamil Nadu, India, and followed guidelines prescribed by the Animal Experimentation Committee of Alagappa University, Karaikudi, Tamilnadu, India. A constant temperature was maintained (20°– 25°) with normal relative humidity of 55% and exposed to 24h day light cycle.

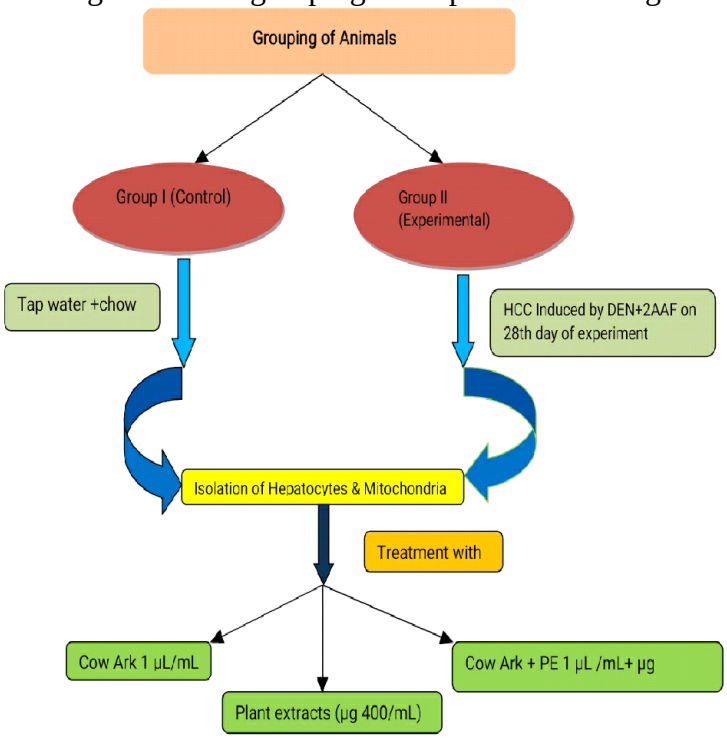
2.5.1 Grouping of Animals

Induction of Hepatic carcinogenesis was attempted by DEN used in corn oil with a limit of 200mg/kg body weight. This was administered intraperitoneally to the animals. After 15days of the treatment cancer cell growth was stimulated with 0.02% dietary 2-AAF habitually for 15 days. The animals bifurcated into 2 wide groups, consisting of 8 numbers in each.

Group-I (Vehicle) This group was treated as control, and were given water as vehicle.

Group-II (Treatment) On the 28th day of the experiment, animals were sacrificed, as soon as, liver hepatocytes as well mitochondria were segregated from the Control group and Investigational group respectively. Subsequently, both groups were treated with Cow Ark, a medicinal plant extract of*Allium sativum* and combined extract of Plant extract + Cow Ark 1μL / ml concentration (Fig-1).

Fig-1. Animal grouping for experimental design



2.6 Determination of membrane potential of mitochondria (MPM)

To assume MPM, we used cationic fluorescent dye, viz., Rhodamine 123, for predicting the membrane potential of mitochondria. Fractions of isolated Mitochondria (0.5mg Protein/ML) were subjected to incubation at 87° C after treating the cow ark and plant extract independently and cow ark + plant extract synergistically. Rhodamine 123 was added (10 Um) to the tested solution with MPM assay buffer (220mM sucrose, 10mM KCL, KH2PO4 68mM D-Mannitol,2UM Rotenone). Fluorescence was assessed using fluorescence spectrometers at a wavelength of 490 nm and 535 nm respectively [12].

2.7 Isolation of Hepatocytes

Hepatocytes of the experimental animals were acquired, using, a method of collagenase perfusion of the liver and viability were determined by PL membrane disruption that was assessed by trypan blue exclusion test. Cells were deferred with 10^6 cells mL^{-1} in a bottle, revolving in a water bath at 37° C in a buffer solution,accompanied with HEPES(12.5mM)and exposed to an atmosphere of 10% Oxygen, 85% Nitrogen and 5% carbon dioxide. Each bottle possesses, 10ml of hepatocytes deferment. Among them, Hepatocellular Carcinogenic Hepatocytes(HCC) and non Hepatocellular carcinogenic (NHCC) were carefully recognized and out-of-the-way.

2.8 Hepatocytes isolation

A standard method was applied in the preparation of secluded hepatocytes is commonly performed in two ways by collagenase liver perfusion measure [13]. Mitochondria were removed from hepatocytes (30 x 10^6 cells/mL) by diluting and resuspended in Krebs medium, complemented with 5mM glucose within the created, comfortable an atmosphere of 95%% Oxygen and 5% carbon dioxide in a trembling bath at 37° C aged for 2 h [14]. Cells were crushed and resuspended in 10mL of solution A (combination of 0.25M sucrose, 0.01M Tricine, 1mM EDTA, 2mM mgcl2, 10nM NaH2PO4) add-on 0.4% chilled BSA at – 80° C

allowed for 10 min. to interruption the PL membrane, followed by centrifugation at 760g for the period of 5 minutes. The gainedclear supernatant was left for some time ,where the pellet is homogenized using homogenizer for 10 minutes, subsequent centrifugation of 760 g. for 5 Min. The supernatants established from earlier two steps were mixed and centrifuged for 20 min. at 8000g. Finally, mitochondrial pellets were again suspended in Tris buffer at 4° C. To measure RO Species invention, obtained were suspended in respiration buffer. The segregation of mitochondria was ascertaine by measuring mitochondrial complex II (Succinate dehydrogenase) effectiveness [15].
Examined mitochondria were found fresh in each experiment and used within an hour of incubation limit. A series of steps was involvedand conducted in ice to deliver high quality mitochondria. The concentration of selected plant and cow ark with a concentration such as 200, 400 and 800 µg/mL were determined on dosage regime (Data not shown) Mitochondriawere reared in Tris buffer in varied concentrations for 1 hour.

2.9 MTT assayonComplex II activity

The ability of anti-cancer of mitochondria complex-II was confirmed by measuring the decline of MTT. In concise, a quantity of 100 µL of Mitochondrial deferment (0.5mg protein/mL) was gestated with various concentrations of plant extract with cow ark such as, 200, 400 and 800 µg/mL) at 37° C aged for 20 minutes. A quantity of 0.4% of MTT was mixed further to the medium followed by incubation for 30 min.. The production of Formazan Crystals was softened in 100µL DM80 and absorbance was noted at 570 nm, using ELISA reader [16].

2.10 Determination of level of ROS in inaccessible mitochondria

Level of ROS in mitochondria was performed by using fluorescent, DCFH-DA. A detailed methodology was discussed in the ref. [15].

2.11 Determination of Cytochrome c discharge

Cytochrome c concentration was measured using Mouse cytochrome immune assay kit, procured from Apex Biotech Pvt. Ltd. Chennai, India. In concise, monoclonal antibody related cytochrome c was mildlycovered on the microplate. 75 µL of conjugates (Comprising monoclonal antibody exact for Cyto-c conjugated to horseradish peroxidase). 50 µL of control and positive control were added to the each well. 1 micrograms of protein from each supernatant fraction was poured into the sample wells. Entire standard, control, experimental samples were added in two wells of microplate.
After 2 h. of nurture, substrate solution (100µL) was added to each well and incubation at 30 min. After 100µL of the stop solution was extra to each well to read optical density of each well of the microplate, using spectrophotometer to 450nm [14].

3.RESULTS

3.1 Characterization of cow ark by HPLC Profile

The observed chromatogram of indegenous cow arks exhibited the following HPLC profile, which included three imposible peaks. The peaks eluted with a Retention Time (RT) of 1.557,1.634,and 2.113nm. RT is an indicator and key for identifying peak profile. The changes in the RT is directly associated with the inverse square of the temperature. In our investigation, elution of biomolecules of indigenous cow ark, is known to be therapeutic molecules, injected as a mobile phase in a Chromatography detector. The detection was performed at 230nm, using Det.4 ch1/230nm. Results reported that presence of Hexanes that eluted at 2.113nm, and other peaks and respective Retention Times were tabulated in Table -

1. Chromatogram was illustrated in Fig-2. A standard solution was also maintained (ethyl acetate) and eluted at 1.317 with an area of 8203464, in the similar condition of tested cow ark. It is noteworthy that height of the peak stipulates, irrespective of the concentration of unknown compounds. It is evident that Presence of abundant hexanes (polysaccharides) has favoured and markedly contributed therapeutic properties of cow ark.

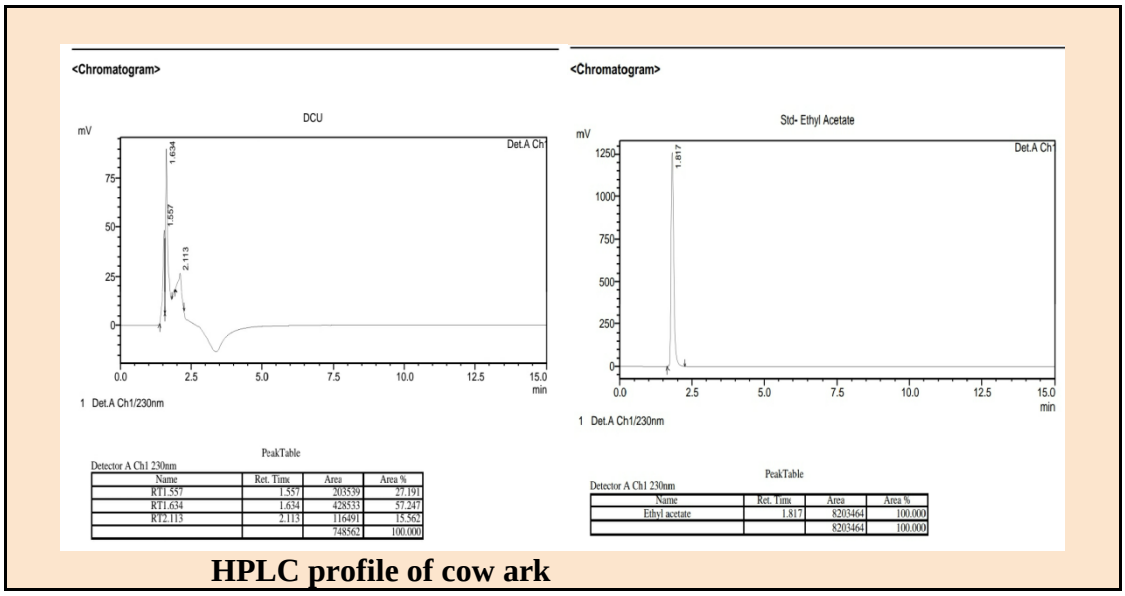


Fig-2.Characterization of Cow ark, using HPLC technique

Table-1showing biochemical constituents of Cow ark

Results	Estimated quantity
Urea	2.101 g %
Uric Acid	28.05 mg %
Phenol	43.62 mg %
Amino Acid	154.21 mg %

3.2 Antioxidant determination

A General observance on intake of food and other behaviour.

During the total study period, intake food and water by the animal andbehaviours of both groups were monitored. It was noticed that a decline was recorded in the treated group, when compared to control animals. The body weight of treated animal group was found to be decreased at different stages of the experiment. The results of antioxidant ability of cow ark was also tabulated in Table-2.

Table -2 showing Free radical scavenging ability of cow ark using DPPH and NBT measures

Tested sample	DPPH	NBT assay
Cow Ark	5.0	5.1
plant extract	3.5	3.8

Cow Ark+PE	5.9	6.0
Ascorbic acid	3.2	3.0
IC 50 m/g/µg/g		

3.3 Effects of Cow Ark on MPM in Group II mitochondria

MPM is a unique determinant for the mitochondrial permeability. Results showed that MPM of treated group-II significantly declined the MPM ability based on the duration P>0.05) in mitochondria isolated from diethylnitrosamine/2 Acetylaminofluorene induced rats. (Fig-3& 4) Meanwhile MPM was found to be decreased and statistically significant in both groups (Tab-3&4).

Table-3 Effects of Cow Ark on MPM in Group II mitochondria

Group	30min	45min	60min
Control	2683 ± 17	3124 ±120	4021 ±19
Plant extract (µg 400/mL)	4827 ±91	5732 ±29	5960 ±103
Cow Ark 1 µL/mL	5231 ±107	5724 ±81	6235 ±47
Cow Ark + PE 1 µL /mL+ µg 400/mL	5913 ±112	6315 ±74	6973 ±35

Fig -3. Effects of cow ark on MPM of Group II mitochondria

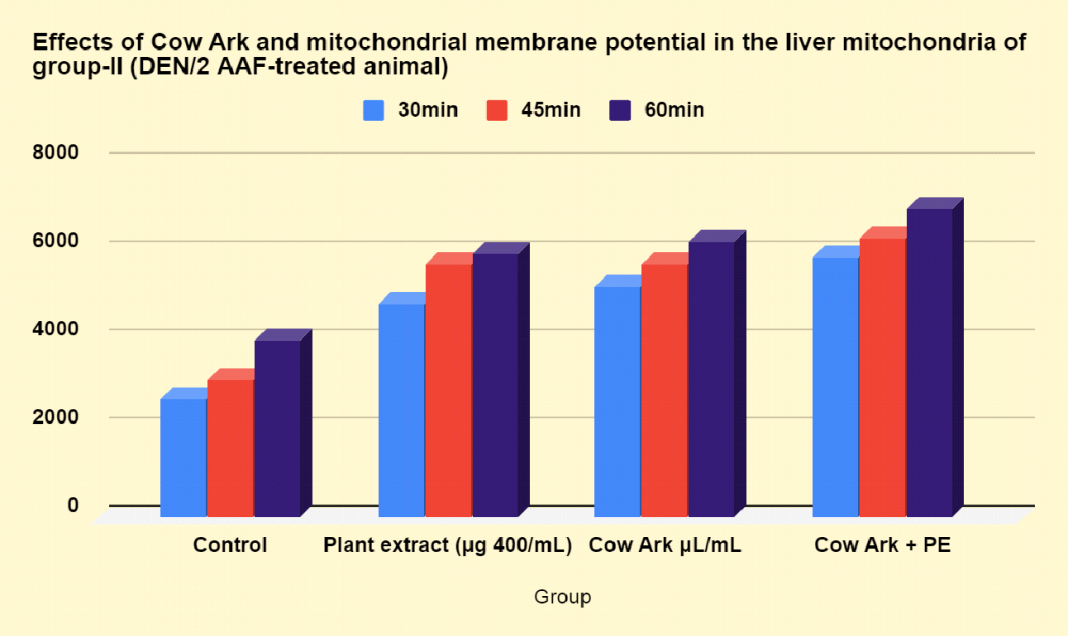


Table-4 Effects of cow ark on the MPM in the liver of group I animals

Group	30min	45min	60min
Control	2425 ± 34	2934 ±73	2015 ±19
Plant extract (µg 400/mL)	2842 ±17	2931 ±112	3063 ±84

Cow Ark 1 µL/mL	3736 ±72	3863 ±18	4100 ±60
Cow Ark+PE 1µL/mL+ µg 400/mL	4023 ±102	4842 ±112	5422 ± 05

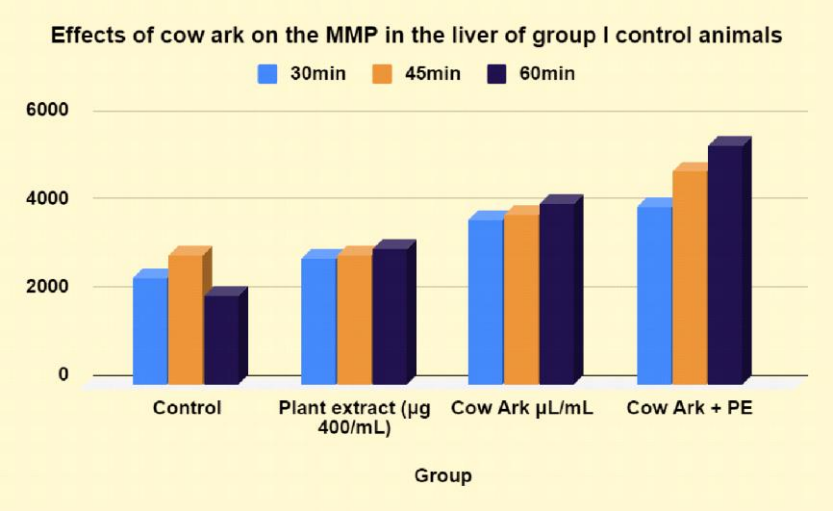


Fig- 4 Effects of cow ark on the MMP in the liver of Group I (control) animals

The effects of Cow Ark and with Plant extract exhibited that, When, plant extract MP was insignificant at (p>0.05%) in three indicated time periods. Extinguishing Rhodamine is directly proportional to the efficiency. Therefore, MMP reported in our present study was opposite symmetrical.

3.4 Effects of Cow Ark treatment on ROS production

As shown in Table (5&6) effects of Cow Ark, Cow Ark+ PE and plant extract were examined and tabulated. Plant extract alone stimulated and accelerated significant hydrogen peroxide formation (P>0.05) in mitochondria isolated from DEN+2AAF induced rats (Group-II) Consequently, cow ark and combined plant extract treated showed a significant hydrogen peroxide formation, whereas PE alone reflects insignificant variation in the mitochondria isolated from control group-1 animals were reported.

Table:5 Effects of cow ark in ROS production in the Liver of DEN/2 AAF induced rats (Groups II)

Group	**30min**	**45min**	**60min**
Control	1529 ± 69	1699±15	2005 ±10
Plant extract (µg 400/mL)	1917 ±107	2621 ±118	2807 ±115
Cow Ark 1 µL/mL	2246 ±76	2769 ±67	3349 ±116
Cow Ark + PE	2677 ±15	2996 ±183	3635 ±162
Value denoted as Averagewith Standard Deviation(p<0.05)			

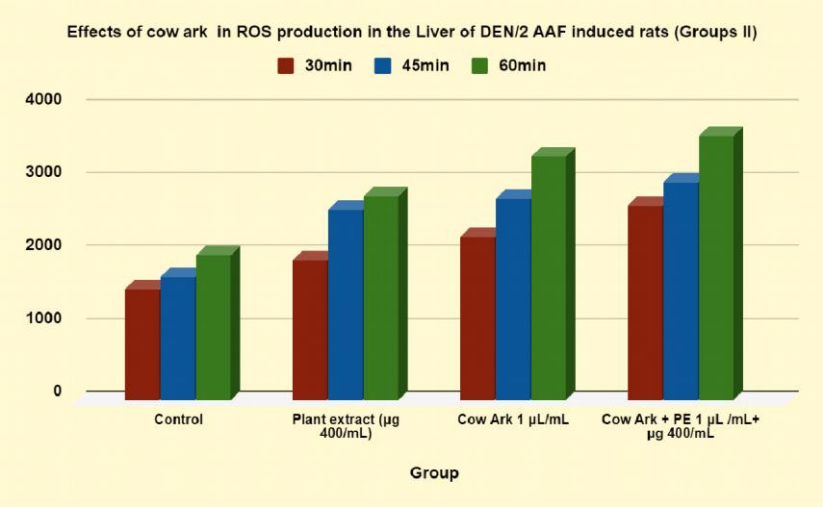


Fig-5Effects of cow ark in ROS production in the Group II Liver of DEN/2 AAF induced rats

Table:6 Effects of cow ark in ROS production in the Group I Liver of control

Group	**30min**	**45min**	**60min**
Control	192 ± 5	207±5	237 ±7
Plant extract (µg 400/mL)	201 ±2	235±7	245 ±12
Cow Ark 1 µL/mL	276 ±5	289 ±12	296 ±3
Cow Ark + PE	291 ±7	301±7	343 ±13
Values denoted as Average with Standard Deviation (p<0.05)			

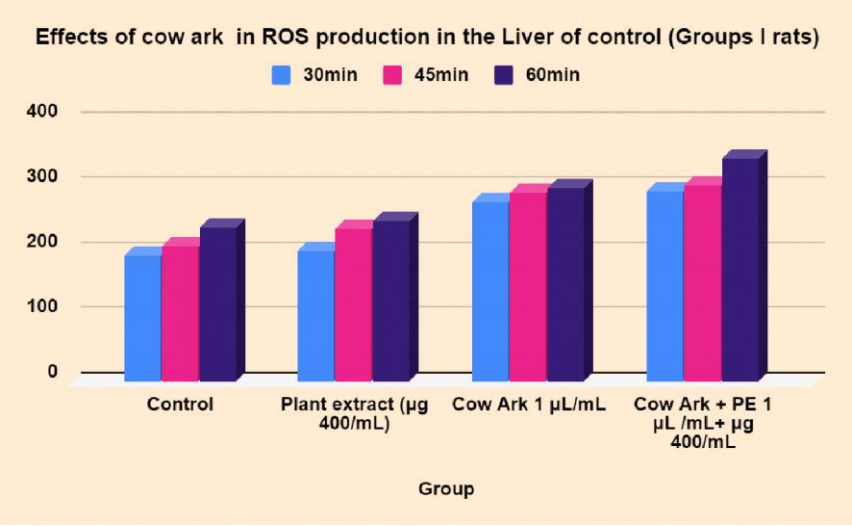


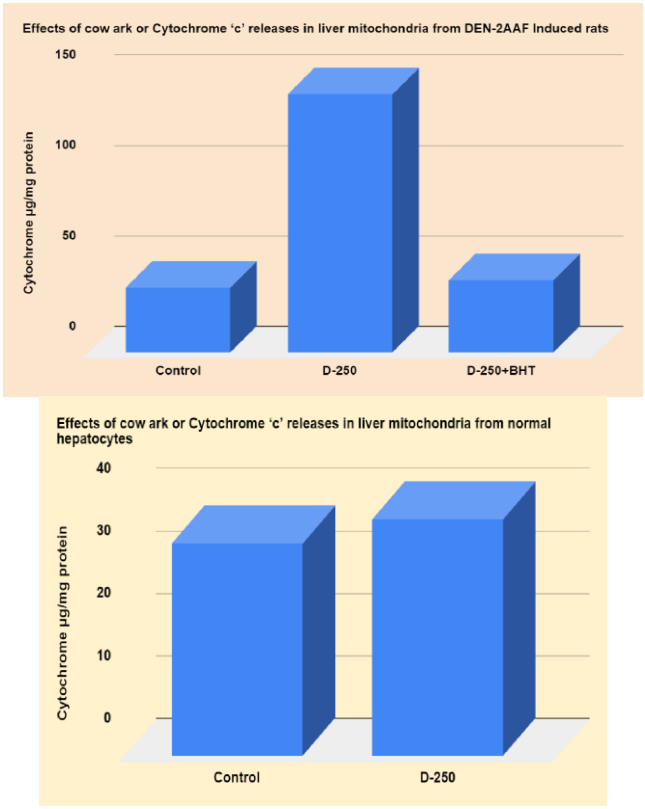
Fig-6 Effects of cow ark in ROS production in the Groups I rats

3.5 Effects of cow ark and cytochrome C discharge

The present investigation clearly reveals that cow ark combined with plant extract significantly distracts the mitochondrial membrane potential, thus it causes a discharge of cytochrome –C from mitochondria. Our study results reported that as indicated in Fig-7&8

cow ark with plant extract (250µg/ml) has stimulated a significant discharge of mitochondria in DEN/2/AAF induced experimental group. Whereas no such changes observed in control group 1 rats. Pre-treatment of cow ark treatment withwith MPT inhibitor CsA and antioxidant such as, BHT, inhibited cytochrome C discharge, as compared to the treated group. Results indicating the crucial role of reducing and compensating the oxidative stress and MPT pores opening in cytochrome C proclamation, were due to the effect of cow ark.

Fig- 7&8 - Showing effects of cow ark on Cytochrome 'c' releases in DEN-2AAF Induced rats



4.DISCUSSIONS

In Indian medicine, cow urine has been reported to be a potential compound for improving general human health. Previous study reported that the estrus urine of Yorkshire pigs having major sources of pheromones [17] and also the gene mutation can be increased the binding strength to Pheromone compound[18]. In the previous study stated that manifold biomarkers in Bos Indicus (Kangayam cows) estrous urine and particular volatile compounds are detected [19]. Earlier study reports strongly demonstrated that cow urine distillate (Cow Ark) exerted diverse actions such as antioxidant, immunomodulator and served as a better enhancer for drug effectiveness. The present intervention was to explore and validate the effectiveness of active fraction of cow ark on the ability of antioxidant and therapeutic properties against Hepatocellular carcinogenesis in induced rat models. Cow urine reportedly contains several chemical properties, constituents that have the potential to target illness, imbalance in the body [20- 21]. The characterization of cow ark contributed a therapeutic composition of dried active fractions that could be very active in expressing anti-oxidant and anti-cancer effects. Similar observations were demonstrated in earlier study reports and were in line of agreement with our study results. HPLC chromatogram reported the vibration of

hexane with a retention time of 2.113 in mobile phase of indigenous cow ark, implicating its therapeutic potency.

The molecules that expressed any kind of actions either in suppressing or accelerating a biological activity is termed as bio molecules. In the present investigation, active fraction comprising bioactive molecules have directly implicated in inhibiting HCC development and antioxidant actions. Sumen Preet et al (2002) have reported that active biomolecules fractions of cow ark could be used in formulating additives for several diseases including anti cancer and drug resistance bacteria and virus [22].An important study was described the therapeutic properties of cow distillate with an emphasis on its bio chemical compositions [23]. In the present investigation we here report, the biochemical constituents such as urea, uric acid, phenol and free amino acids content were high when compared to the previously reported [24]. This may be attributed to the nature of indigenous traits collection time, gestation, and indicative of renal function [25] Amino Acid content was recorded as 154.21 mg % and it was slightly increased from previously reported [26].

4.1 Antioxidant

Considering in view, unmatched number of benefits and therapeutic potentials of the Cow Ark, a scientific validation was attempted.In the present investigation we tried to explore the antioxidant activities of the cow ark. It exerted more effectiveness against free radicals biomolecules, that prevents the oxidation of other chemical constituencies and to form free radicals. Cow ark might have protected important cell organelles by offering neutralizing effects of free radicals and act as neutralizing agents and commonly they are by products of cell metabolism [27]. A large number of studies were reported that free radicals implicates and inter linked with enormous number of disorders covering diabetes cancer and ageing [28]. The earlier studies reported that cow ark with *Acalypa indica*, *Tamarindus indica*, *Gymnema sylvestre and Murraya koienigii* have highly potential of antioxidant and antimicrobial against pathogenic bacteria[29].

Present study reporting antioxidant property of cow ark by exerting the potential therapeutic value against oxidative stress. Taking everything into account, obtained results strongly endorsed that antioxidant ability was due to the free radical scavenging action of cow ark constituent and this would play in controlling the ageing process and participated in the anti cancer activities.

4.2 Anticancer

Despite cow urine offering several success stories in the treatment of cancer, drug resistance capacity of cancer cells is challenging in the present scenario. Cow Ark curtailed apoptosis in lymphocytes process, and allowed them to withstand and survive. DNA repairing damaged cells was also reported. However, treating cancer cells is a serious and complex process in medical care. The isomers of conjugated Linoleic acid (CLA) from Indigenous cow milk have high significant potential against Hep-G2 and MCF-7 cells [30,31]

Advancement in the biomedical field, novel medicines, developed to inhibit specific cellular proteins and targeting signalling. HCC is a dreadful disease and a major cause of hepatic cirrhosis [32]. A remarkable number of anti tumour agents, recently used in the clinical practices are of natural origin. Several drugs, Internationally approved anticancer drugs are represented from natural origin and their derived products [33,34]. HCC is a chronic infected, with hepatitis B virus alcoholism and obesity reportedly, found to be the other factors [35]. Natural produced derived compounds are rich resources of novel therapeutic agents [36]. Cow ark is reportedly a relevant alternate source and can be used for the

treatment of various cancers in Ayurvedic medicine. Alteration or modification in sub cellular mitochondria is directly associated with cancer [37].

In our study, special emphasis was given to ascertain the ability of cow ark in regulating and controlling the cancer activity in HCC, /DEN/2AAF induced in Wister rats animal model. In this investigation, a decreased body weight indicated the HCC was induced by DEN+2 AAF in the experimental group. Cow ark has significantly decreased the MMP in mitochondria of HCC hepatocytes in a time phased manner. But, Plant Extract of *A.sativum* (PE), independently showed insignificant effect on MMP of liver mitochondria, isolated from the control group. Similarly, Hydrogen peroxide production in the liver mitochondria of HCC hepatocytes was found to be increased in DEN/2 AAF induced rats (Fig) whereas the observed results in H2O2 activity in PE has no considerable effects in control group rats treated with PE alone. A significant increased activity was observed in H2O2 production and the synergistic effect of Cow Ark with PE was found to be effective was noticed. This has confirmed the enhancing ability of Cow ark with PE in scavenging radicals to the antioxidant ability. Moreover, cow ark synergistic with PE treated mitochondria showed an increase trend of cytochrome c release by cleaving mitochondrial membrane integrity in liver mitochondria from DEN + 2AAF treated, whereas no such effects was noticed in the control group. ($P< 0.05$).

Several therapeutic approaches have been exposed, based on cytotoxic molecules that are involved in induction of mitochondria. These effects depend on the mitochondrial permeability transition pores (MPT). In few occasions permeability may increase by elevating ROS generation leads to release of cytochrome c and other apoptogenic proteins from mitochondria of cancer cells were described [38].

5.CONCLUSION

Finally, it was presumed that cow ark contains natural bioactive molecules and exerts its effects on mitochondriaby inducing, ROS production, in turn opening the MPT pores followed by the destruction of inner membrane potential. When discharge of cytochrome c , subsequently apoptosis signalling has been activated often. From the above observations, Cow ark with plant extract remarkably activated H_2O_2 production, MM potential, cytochrome c release was strongly endorsed its bio enhancing ability. We suggested cow ark may be considered to be a candidates anticancer agent and used as alternative targeting HCC cell mitochondria.

AKNOWLEDGEMENT

I, extend my great gratitude to the Authorities of the Alagappa University, Karaikudi, Tamil Nadu, India, for given me, Financial assistance pertaining to execute the research work, widein MHRD-RUSA 2.0-F.24/51/2014-U, Policy (TNMulti-Gen), Dept. Of Edn.Govt.of. India.

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