

Go Ark; an ameliorative bio-product (*in vitro*) on Phenyl induced cytotoxicity

Research Article

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Abstract

Phenyl (PHY) is one of the chemicals which are used as a disinfectant in the world due to its toxic potential. Cleaning workers are directly exposed to it in institutes, hospitals and houses. Cow urine/Go Ark (GA) has been proved as a bioenhancer in many studies. The present study dealt with the *in vitro* analysis of PHY induced cytotoxicity (CT) on human peripheral blood lymphocytes and ameliorative potential of Distillate cow urine/Go Ark (DGA) and Fresh Go Ark (FGA) as GA is believed to be an elixir in Ayurved. MTT assay was used to study CT and Cell viability % on Human peripheral blood lymphocytes (HPBL) *in vitro*. CT of PHY was found to be higher than that of DGA and FGA treated groups. This showed that when PHY induced cells were treated with DGA and FGA, they showed increase in the cell viability %. It was also found that FGA had more potential for enhancing cell viability % of HPBL than that of DGA. We suggest that GA can be used as an ameliorative agent on PHY induced CT. It can be explored by *in vivo* experiments further for its detoxification properties. Now a day, PHY is used in combination with GA for cleaning purposes as "Gonyl", it may be safe for cleaning workers to use GA based disinfectants to diminish the CT induced due to PHY exposure at the time of cleaning.

Key Words: Ameliorative effect, Cytotoxicity, Go Ark, MTT assay, Phenyl.

Introduction

Cow urine

Cow which is also called as 'Gau Mata' is a mobile dispensary and wealth of medicines. The GA remedy can cure us from several incurable and curable diseases. The ancient Indian literature, like *Charak Samhita*, *Atharva Veda*, *Rajni Ghantu*, *Amritasagar*, *Vridhabhagabhata*, *Sushrut Samhita* and *Bhavprakash* describe about these things nicely. GA Treatment and Research Center have concluded that it can cure blood pressure, asthma, diabetes, eczema, psoriasis, heart attack, in arteries, blockage, fits, AIDS, piles, cancer, prostrate, migraine, thyroid, arthritis, ulcer, gynecological problems, abortion, constipation, acidity, nose and ear problems, and many other diseases on the basis of a lot of research already done in the previous few years. GA has shown that it contains sodium, manganese, nitrogen, sulphur, carbonic acid, iron, phosphate, silicon, chlorine, citric, tartaric, succinic acids, magnesium, maleic, and calcium salts, vitamin A, B, C, D, E, creatinine, hormones, minerals, enzymes, lactose and gold acids. When there is imbalance of the substances in the body, a person becomes diseased. The

GA contains the ingredients, which are available in the human body. Therefore, consumption of GA cures from incurable diseases and maintains the balance of these substances. (1)

GA abolishes the poisonous properties of residues and keeps body healthy. Electric currents (rays) retain body in fit status. These currents exist in our environment. These rays enter our body through copper in form of extremely small currents. Copper is found in cow urine. One of the qualities of copper is to attract these electric waves and we become healthy. (2)

Dhama K_{et al} (3) submitted in their research article that the recent medication has helped us to cure a number of ailments of mankind and creatures; but the existence of inveterate diseases like acquired immunodeficiency syndrome, diabetes, cancer, rheumatoid arthritis, increasing trends of antibiotics resistance, side effect of allopathy medicine and biopesticides have caused nutritional risk. This has made the situation more serious than ever before. Now, it has been the urge for scientist and researcher to find novel therapies. GA remedy has become cost effective which has the least adverse impact when compared with modern medicine.

Gulhane *et al* (4) found that GA has got distinct significance in Indian tradition. It is believed to have a pious cleansing effect also. *Panchagavya Chikitsa* (Cowpathy) is the treatment based on cow. The life style diseases like diabetes, AIDS, cancer, autoimmune diseases, etc. are increasing steadily in this existing era. Unreasoned use of antibiotics causes increase in antibiotic resistant infectious diseases. GA has been

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scientifically proven to have bacteriostatic action and it act as an immunomodulator.

Medicines have the potential to increase body immune system as per *Ayurveda Rasayana*. GA comprises of the similar *Rasayanatvatva* and it also acts as a bioenhancer agent. DGA has been found more effective bioenhancer than GA. (5)

Anti urolithiatic impact of Goo Ark

GA exhibited noteworthy effect against renal calculi as well as restoration of improper renal function. Probably this action of GA was found because it reduces excretion of calcium oxalate and inhibits crystallization. More research is requisite to know about its mechanism of action. (6)

Phenyl

PHY is a derived from phenol. It gives PHY group when we remove the hydroxyl group from the phenol molecule. Being an aromatic alcohol its chemical formula is C_6H_5OH . In its chemical structure a benzene ring and a hydroxyl group (-OH) is attached to it. It is a white crystalline solid in texture and volatile in nature. It is mildly acidic. It has the potential to give rise to chemical burns so it should be handled carefully. Coal tar was first used to extract Phenol. Today it is produced (about 7 billion kg/year) from petroleum extensively. (7)

These PHY compounds are derivatives of benzene (C_6H_6) in terms of their production. The PHY group is associated with a vinyl group in terms of its electronic properties. It is a strong deodorant and germ killer. It is used for disinfection of areas like hospitals, nursing homes, lavatory, toilets, cowsheds etc. It is broadly used for sanitation. Under the Drug Act it is notified as a "Drug" its production can be done necessarily with the prior permission and license from Drug Control Authorities. (8)

Structure of Phenyl

PHY groups possess six carbon atoms. These are attached together in a hexagonal planar ring out of which five are bonded to individual hydrogen atoms and the remaining carbon binds to substituent. In organic chemistry PHY groups are functional groups. The compound also has five hydrogen atoms due to the six-carbon ring conjugation. They don't have a three-dimensional structure. PHY groups are non-polar and extremely hydrophobic. They also do not undergo reduction or oxidation very easily. Due to the characteristics mentioned above, PHY groups are designated as highly stable substituents. They can be added easily to other compounds during chemical reactions. (8)

PHY is used by the females in houses for cleaning purpose. This PHY is directly exposed to them while cleaning the floors. Hence this investigation was done to analyze the cytotoxic of PHY (*in vitro*) on HPBL and the ameliorative effect of DGA on PHY induced CT using MTT assay.

Materials and methods

Lymphocytes isolation (whole blood)

It was done according to Gautam *et al* (9). Whole Blood (2.5 ml) from a male volunteer donor (with his consent) in a good health was collected. EDTA vacutainer was used for blood collection. This blood was mixed with PBS (1X).

In a centrifuge tube an aliquot of 2.5 milliliter of HiSep TM lymphocyte separation medium (LSM) 1077 (Hi media) was drawn and overlaid with diluted blood (7.5 ml). It was centrifuged (400 X g) for 30 minutes.

The supernatant was Discarded. The lymphocytes layer with half of HiSep layer was collected in a different sterile centrifuge tube carefully and washed with PBS (1X).

Haemocytometer based cell counting method was used. TC 199 medium (Hi media) supplemented with foetal bovine serum was used. The cells (3.03×10^5 per/ml of media) were used for the experiment.

MTT assay

The assay was accomplished as per Mosmann (10) with some alterations. Aliquots of ($180\mu\text{l}$) of suspension (3.03×10^5 cells per/ml) were added into a 96 well microtitre plate in 6 replicates of each sets *i.e.*, SET-1 to 4. SET -1 and 3 were performed to evaluate the CT of PHY at 2Hrs and 24 hours exposure respectively. SET 2 and SET 4 were performed to study the effect of DGA and FGA on PHY induced CT at 2 hours exposure respectively. One row (B) served as control (only medium and cells). PHY ($20\mu\text{l}$) was added to cell suspension in the concentrations of 100%, 70%, 40%, 10% and 1%. Each concentration of PHY was tested in six replicates for 2 hours exposure at 37°C with PHY at 5% CO_2 . After incubation, $20\mu\text{l}$ aliquots of MTT solution (5mg/ml PBS) were added to each well of SET-1. SET 3 was also given the same treatment as Set -1 except 24 Hours exposure. $20\mu\text{l}$ Distillate *Go Ark* was added in SET-2 on PHY induced cells for 2 Hours exposure and the plate was re-incubated for next 2 hours and for SET 2 for 24 hours. Dimethyl sulfoxide ($100\mu\text{l}$) was put in each well of SET-1. Added DMSO dissolves formazan crystals after overnight incubation.

- The culture plate was then placed in and Absorbance of SET-1 was taken (600nm) after 22 hours with the help of Microplate reader (Alere).
- After completion of 24 hours of exposure of DGA in SET-2 at 37°C , each well was added with $20\mu\text{l}$ aliquots of MTT solution and re-incubated for two hours at 37°C .
- Then $100\mu\text{l}$ of DMSO was added to each well of Set-2 to dissolve formazan crystals followed overnight incubation at 37°C for 22 hours.
- Absorbance of SET-02 was also read after 22 hours of incubation in DMSO. The readings were analyzed after making due adjustment with these data. The effect of DGA on PHY toxicity was calculated.
- SET-04 was tested to analyze the effect of FGA. Hence, the treatment was similar to SET-02 except FGA in place of DGA.

Results

Results indicated that the drop in cell viability % was directly proportional to the exposure of time of PHY. When cells were treated 100%, 70%, 40%, 10% and 1 % PHY for 2 Hrs exposure, the viability % was found to be 79.58 and 96.32 on 70% and 1% PHY exposure (Table 1). The cell viability was inversely proportional to the concentration of PHY. The decline was found to be significant at $p < 0.05$. (Figure 2, Table 2).

When the cell viability % was compared at 2 and 24 hours exposure of PHY, it was found to be 88.07 and 70.65 respectively which showed that when the exposure time is increased, the CT also increased proportionally (figure 1). When the cells were treated with DGA and FGA, the cell viability % was higher than that of 24 hours PHY exposure group (Table 3, 4). FGA and DGA treated group showed higher cell viability % that might be due to ameliorative effect of GA.

We found that GA was found to show ameliorative effect against the CT produced by PHY on HPBL (figure 3). The highest cell viability was found in FGA treated SET.

Discussion

In vitro studies on HPBL can provide us a way regarding the toxic effect of PHY on HPBL and the potential of DGA as a Drug in controlling the toxicological effect of PHY. Recently there are limited research reports that provide the influence of PHY and fresh *Go Ark* on lymphocyte cultures of human.

Randhawa Gurpreet K (11) found that GA has an effective antimicrobial potential. Most of the studies quoted are *in vitro* studies. GA can be used as a natural and newer drug in modern therapy.

PHY toxicity has not been studied using MTT assay. However, its toxic potential has been observed in many researches. It is used as a cleaner due to its toxic potential. Hence, we have studied its toxicity on HPBL. Other workers have worked on toxicity of some pesticides using MTT assay.

Gautam *et al* (12) have concluded in their study the proliferative potential of GA on HPBL. The potentiality of FGA was observed higher than that of DGA with reference to enhancement of cell viability%. We also found the same when tested GA on PHY induced CT. Patel *et al* (13) exposed Chinese hamster ovary (CHO) cells to cypermethrin, pendimethalin and dichlorvos (1 μM , 10 μM , 100 μM , 1000 μM and 10,000 μM cypermethrin, pendimethalin and dichlorvos) for 3 h and assessed CT with MTT assay. They found dichlorvos and pendimethalin exhibited higher extent of CT as compared to cypermethrin. Cypermethrin caused a significant ($p < 0.05$) DNA damage only when exposed to higher concentrations (1000 and 5000 μM). Suman *et al* (14) also has measured mitochondrial metabolism in cell culture providing information of percentage of cell survival by MTT assay.

Randhawa GK (15) explained GA distillate as a 'bioenhancer'/'biopotentiator' that boost the bio-efficacy and bioavailability of active substance. 'Yogvahi' principle in *Ayurved* revealed the bioenhancing assets of medicines. It increases the oral bioavailability which results in lowering their dose and side effects. Go ark has also been granted US Patents (No. 6,896,907 and 6,410,059) for its medicinal properties, particularly as an antibiotic, bioenhancer, antifungal and anticancer agent. It also showed rise in the potentiality of "Taxol" (paclitaxel) against MCF-7 which is a human breast cancer cell line, while performing *in vitro* experiment (US Patent No. 6,410,059)

Gautam *et al* (16) studied cypermethrin CT on HPBL using MTT assay. The cells were exposed with cypermethrin (1 $\mu\text{g/ml}$, 2.5 $\mu\text{g/ml}$, 5 $\mu\text{g/ml}$, 7.5 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$). It was found that the cell viability % on 2hrs exposure group at 1 $\mu\text{g/ml}$ was 67.09 \pm 0.07%. It was found to be 87.10 \pm 0.25 % on 18 hrs exposure group at the same concentration. The dose related decline in the cell viability was observed in the direct proportion to concentrations of Cypermethrin. They found that the decrease in cell viability% at less exposure time was high. This might be found owing to self-recovery potential of HPBL. We also found that the concentration of PHY was directly proportional to the CT *i.e.*, a dose related decline in the survival of HPBL. GA can be good ameliorative bioproduct for minimizing the toxic effect of PHY when exposed at the time of cleaning our houses.

Conclusion

We found that the exposure time and the concentration of PHY was directly proportional to the CT. CT of PHY was found to be more than that of GA treated groups which showed *in vitro* ameliorative effect of GA. It was also found that FGA had greater ameliorative potential than that of DGA.

We suggest that CT induced by PHY can be treated using GA at some extent as our *in vitro* study revealed. Further its detoxification properties can be explored by *in vivo* experiments. Nowadays, PHY is used in combination with GA for cleaning purposes as "Gonyl", so it may be safe for cleaning workers (usually females in houses) to use GA based disinfectants instead of PHY only to reduce the harmful effects of PHY exposure. We suggest that the cleaning workers to apply safety measures like wearing gloves, using masks during work to reduce the cutaneous exposure of toxicant.

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Conflict of Interest – None

Abbreviations: - PHY – Phenyl, GA - Go Ark, FGA - Fresh Go Ark, DGA - Distillate Go Ark, HPBL - Human Peripheral Blood lymphocytes, CT - Cytotoxicity

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Table 1-Effect of PHY on cell viability % on HPBL

Concentration of PHY	Cell viability%	
	2 hours Exposure group	24 hours Exposure group
Control	100	100
100%	80.15	69.80
70%	79.58	59.61
40%	85.79	59.45
10%	86.60	62.26
1%	96.32	72.77

Table -2 Two-way ANOVA for analysis of CT of PHY at 2 and 24 hours

ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Rows	1274.279	5	254.8558	4.856582	0.053881	5.050329
Columns	910.6117	1	910.6117	17.3528	0.008776	6.607891
Error	262.3818	5	52.47636			
Total	2447.272	11				

Table 3: Effect of PHY and treatment groups (DGA & FGA) on HPBL

Cell Viability %			
	24Hrs Exp of PHY	DGA treated Group	FGA treated Group
Ctrl	100	100.00	100.00
100%	69.80	35.12	61.71
70%	59.61	63.07	62.57
40%	59.45	71.55	66.99
10%	62.26	76.33	71.55
1%	72.77	89.75	79.10

Table-4: The effect of DGA & FGA on PHY induced CT (Two way ANOVA)

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	3816.254	5	763.2507	7.936629	0.002943	3.325835
Columns	28.03421	2	14.01711	0.145756	0.866172	4.102821
Error	961.6812	10	96.16812			
Total	4805.969	17				

Figure 1. Phenyl toxicity on human PBL

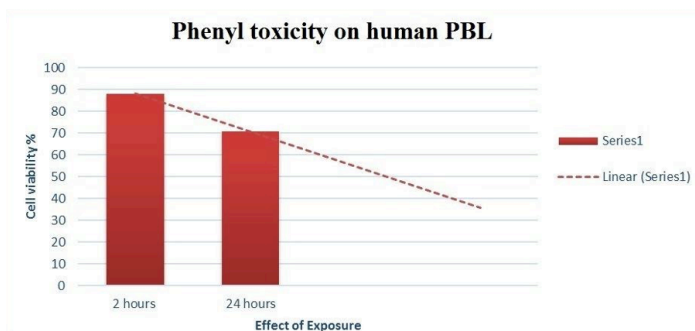


Figure 2. Effect of phenyl using ANOVA (Trend line)

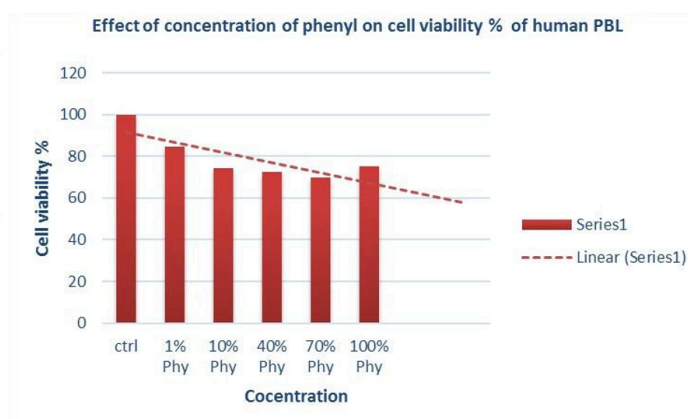


Figure 3. Effect of Go Ark on PBL using ANOVA (Trend line)

