Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(5): 41-46

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Evaluation of Anticancer Potential of Caffeine

Patel Disha Rajesh Kumar*, Tirgar Pravin Rambhai, Jha Neha Ramanath

Rk University, School of Pharmacy, Rajkot-360020

ABSTRACT

Objective: *The present study evaluates anticancer potential of caffeine using various pharmacological models.*
Methods: *In case of In vitro hemolytic assay study the absorbance of the liberated hemoglobin and then checked % haemolysis using 450 nm λ_{max} in UV spectrophotometer. In Brine shrimp lethality assay checked % mortality as well as counting the viable naupliis at different time intervals. In case of Allium cepa (Onion) root model checked physical or macroscopical parameters like root number, root length. To check the significance of data, ANOVA, Tuckey's test, statistical tests were performed. $P<0.05$ and highly significant at $P<0.001$. Statistical analysis was performed using INSTAT statistical software.*
Results: *In vitro Hemolytic Assay shows concentration depended cytotoxic effect of Caffeine. When concentration of Caffeine increased % Hemolysis increased. In Brine shrimp lethality test shows concentration depended cytotoxic effect of Caffeine. Allium cepa (Onion) root model shows that caffeine reduce mitosis cell division process as it reduce number of root and number of length two different dose.*
Conclusion: *Caffeine shows potential cytotoxic activities hemolytic, brine shrimp and Alium cepa models and can be used as safer, cheaper as well as lower toxic herbal alternative phytoconstitue in management of incurable disease like Cancer.*

Keywords: Cytotoxic; Anticancer; Caffeine; Haemolytic study; *Allium cepa*

INTRODUCTION

Cancer is incurable group of disease which involving the abnormal growth of cell. It is undoubtedly a serious and the potentially life threatening condition [1]. Synthetic anticancer agent has a severity of the various side effects at the particular therapeutic dose [2]. High doses of radiation treatment are used to destroy the cancer cells inside the body. Various side effects occur because of radiation therapy can also damage the healthy cells and tissues near the treatment area of human body [3]. Using of isolated phytoconstituent rather than the combination of multi-constituent may increases the chance of accuracy of the treatment of cancer [4]. Caffeine is Purine alkaloids – Methyl xanthine category [5]. Naturally caffeine is found in the seeds, nuts, or leaves of a number of plants native to the South America and the East Asian country [6]. Hence, the present study is carryout to investigation of anticancer activity of caffeine. A lower concentration of caffeine can be induce gene p_{53}-depended apoptosis in case of the JB6 cells through the Bax and Caspase 3 pathways [7]. Although the well characterized as an inhibitor of the DNA damage-induced checkpoint reported to the delay G_1-S-phase of cell cycle [8] progression in the various pharmacological cell lines study. Here, we focused on the concentration depended cytotoxic effect of caffeine using pharmacological models for the preliminary evaluation of cytotoxicity used *in vitro* hemolytic assay. For evaluation of concentration depended anticancer activity of

caffeine we were used Brine shrimp lethality test and for determination of cellular mechanism of action we were used *Allium cepa* root (Onion root) model.

MATERIALS AND METHODS

Caffeine used as test sample. It was collected from LOBA CHEMIE PVT.LTD., Mumbai, INDIA. (Batch number G338807). All the chemicals as well as test sample reagents and organic solvents were used in this study were of analytical grade. Solubility of caffeine in water so it was dissolve in water [9]' for the authentication of sample we performed qualitative screening. We performed Melting point determination [10], TLC analysis [11] and chemical test for particular class of caffeine is Murexide test for Purine alkaloids – Methyl xanthine category of alkaloid [12,13].

***In vitro* Hemolytic Model**

In case of *In vitro* hemolytic assay fresh blood sample obtained from the animal with help of retro orbital method and it is (heparinized. Then diluted 1:1 with the sterile phosphate buffered saline solution (PBS pH 7.4). Red blood cells (RBCs) were separated with help of centrifugation (1000 g for 5 min) and resuspended in PBS pH 7.4. This procedure was repeated at three times. Make a final 2% RBCs suspension (v/v) was prepared by suspending RBCs in PBS (Positive control) 100 µl sterile Distilled water was used. Test drug as were serially diluted in the PBS. Test drug doses were 50, 250, 500, 750 µgm/ml. Standard drug dose were 20, 40 µgm/ml.2% RBC suspension were added to all wells of the plate. This procedure resulted in the eight dilutions of Test drug. The PBS solution and RBCs alone served as the 0% haemolysis for the negative control. Experiment was performed for three times. All plates were incubated for 3 hr at room temperature. Supernant were collected after centrifugation at 1000 RPM for 5 mins. The liberated hemoglobin was measured in the Double beam UV spectrophotometer in 450 nm λ_{max} [14]

$$\%\ Hemolytic\ activity = \frac{Abs\ sample\ -\ Abs\ negative\ control \times 100\%}{Abs\ positive\ control\ -\ Abs\ negative\ control}$$ [14]

Brine Shrimp Lethality Assay

The Brine Shrimp Lethality Assay is applied as an alternative bioassay method for evaluation of cytoxicity of plant constitute because Brine Shrimp having ability to rapid growing similar to cancer cell in human body [15]. It's a preliminary toxicity screen for further experiments on the mammalian animal models [16]. Brine shrimp ordered from Amazon. in as capsules. Which are filled with the thousands of dried cysts. Dried cysts were performed as the indicated above, and then incubated (1 g cyst per liter sea water solution) in a hatcher at 28-30°C with the appropriate aeration, under a continuous light regime. Approximately after 12 hr hatching the phototropic nauplii were collected with a pipette from the lighter side and concentration in a small vial. Ten brine shrimp were transfer using adequate pipette. Each test considered of exposing groups off 10 Artemia aged after 12 hr to different concentration and % of deaths was calculated. Larvae were considered dead if they did not exhibit any internal or external movement during the observation. The larvae should not receive food. To ensure that the mortality observed in the bioassay could be attributed to the bioactive compounds and not to starvation. We should compare the dead larvae in each treatment to the control. In case of hatched brine shrimp nauplii can survive for up to 48 hr without food they feed on their yolk-sac. Where control deaths were detected, calculated percentage of mortality [17].

% M=Percentage of survival in the control – Percentage of survival in the treatment [18].

***Allium cepa* (Onion) Root Model**

Caffeine is effective against cells that are proliferating and produce cytotoxic effect by damaging the DNA during the S-phase of cell cycle. Most of the plant derived anti-cancer drugs affect the microtubule dynamics of the cell and induce persistent modification of biological processes and signaling pathways that ultimately lead to apoptotic death (Mollinedo and Gajate, 2003) [19] So we were used *Allium cepa* (onion) root model to evaluate cytotoxic potential of caffeine. *Allium cepa* (onion) root model Onion bulbs (*Allium cepa* L) kept on different drug concentration (150, 250, 750, STD methotrexate 20 and 40 µgm/ml) containing flask. Then after 2, 4, 6 days interval measure physical parameters like root number, root length. Observed microscopical as well as macroscopical study of roots like, compared size of cell root cell with different concentration as well as standard drug with help of Phase contrast microscope [20].

Statistical Analysis

To check the significance of data, following statistical tests were performed:
ANOVA: To see the variability within all the groups.

Tuckey's test: For the same purpose mentioned in above test.
ANOVA, P-value, Degree of freedom, Standard deviation, etc.
Data were considered statistically significant at $p<0.05$ and highly significant at $p<0.001$. Statistical analysis was performed using INSTAT statistical software.

RESULTS AND DISCUSSION

As the absorbance of sample is increases more chances of haemolysis, which indicates the cytotoxic activity of different concentration of caffeine as well as standard drug. 500 µgm/ml concentration of Caffeine shows highest % Haemolysis. After analytical result we conclude that % haemolysis increased when concentration of drug increased that shows cytotoxic potential of caffeine (Table 1 and Figure 1).

Table 1: % Hemolysis

Drug concentration	Absorbance (λ_{max} 450 nm)	%Hemolysis
Water (+ve)	0.00	-
0%Phosphate buffer	0.508	-
Blood+PSS (-ve)	1.474	-
50 µg/ml	0.69	53.18%
250 µg/ml	0.36	74.21%
500 µg/ml	0.34	76.93%
750 µg/ml	0.38	78.8%
Std-1 (20 µg/ml) Methotrexate	0.48	99.52%
Std-2 (40 µg/ml) Methotrexate	0.79	99.91%

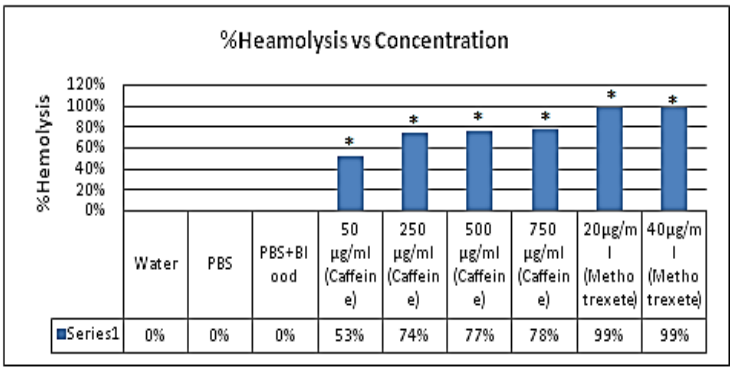

Figure 1: Graph of % Hemolysis vs. Concentration

After the time intervals the viable naupliis were counted. The numbers of survivors were counted and % death was calculated. Larvae were considered dead if they did not having any internal or external movement during several seconds of observation. With help of this method we compared cytotoxic activity of caffeine. After 12 hr 750 µgm/ml shows 100% Mortality of Brine shrimp (Figure 2). So, we conclude that the brine shrimp lethality assay is an excellent predictive tool for the evalution of cytotoxic potential of drug in humans and the caffeine was evaluated as cytotoxic agent (Table 2).

Table 2: % Mortality of Brine Shrimp

Time (hr)	Control	150 µgm/ml (Caffeine)	250 µgm/ml (Caffeine)	750 µgm/ml (Caffeine)	STD-20 µgm/ml (Methotrexate)	STD-40 µgm/ml (Methotrexate)
0	0%	0%	0%	0%	0%	0%
2	0%	20%	20%	30%	50%	70%
4	10%	20%	40%	50%	50%	80%

6	20%	40%	40%	60%	70%	90%
8	40%	50%	60%	80%	80%	100%
10	40%	60%	60%	80%	90%	100%
12	40%	70%	80%	100%	100%	100%
14	70%	90%	100%	100%	100%	100%

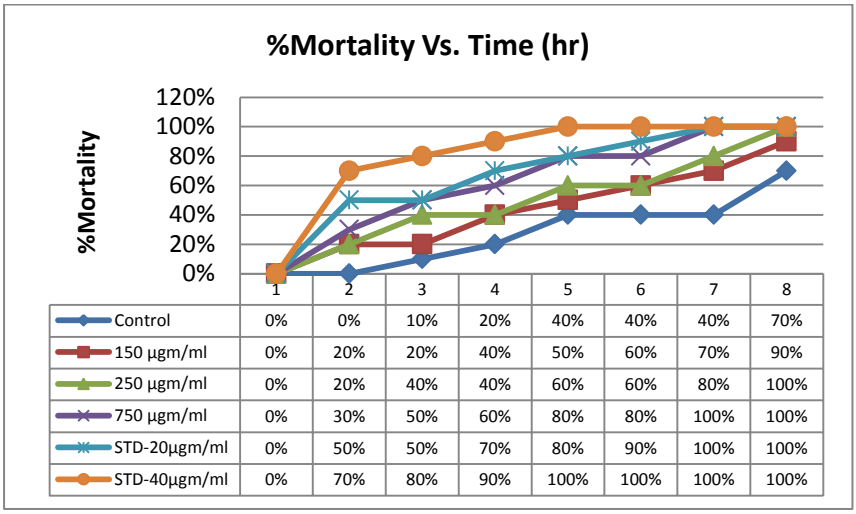

Figure 2: Graph of % Mortality Vs Time (hr)

For determination of mechanism of drug we used *Allium cepa (Onion*) root model as preliminary model (Figure 3). In this model we were find out cellular mechanism of caffeine. In *Allium cepa (Onion*) root model when concentration of caffeine increased, decreased number as well as length and number of Onion roots. 750 µgm/ml concentration of Caffeine shows highest cytotoxic effect of Caffeine. So we conclude that caffeine act on rapid growing cells (Figures 4 and 5). Root tip of *Allium cepa* having rapid growing cell, our drug inhibit growth of root so we conclude that caffeine having cytotoxic potential (Tables 3 and 4).

Figure 3: Result of *Allium cepa* model at Day 6

Table 3: Number of roots

Day	**Number of roots**					
	Control (Water)	**150 µgm/ml (Caffeine)**	**200 µgm/ml (Caffeine)**	**500 µgm/ml (Caffeine)**	**Std 1 (Methotrexate) (20 µg/ml)**	**Std 2 (Methotrexate) (40 µg/ml)**
0	0	0	0	0	0	0
2	40	20	16	13	7	1
4	54	22	18	16	13	10
6	56	34	20	16	19	17

Table 4: Length of root

	Length of Roots (cm)					
Day	**Control (Water)**	**150 µgm/ml (Caffeine)**	**200 µgm/ml (Caffeine)**	**500 µgm/ml (Caffeine)**	**Std 1 (Methotrexate) (20 µg/ml)**	**Std 2 (Methotrexate) (40 µg/ml)**
0	0	0	0	0	0	0
2	3	2.5	1.7	1.7	1	0.5
4	6.5	5.2	4.5	4.3	4	4
6	7.5	5.5	5	4.6	4.1	4

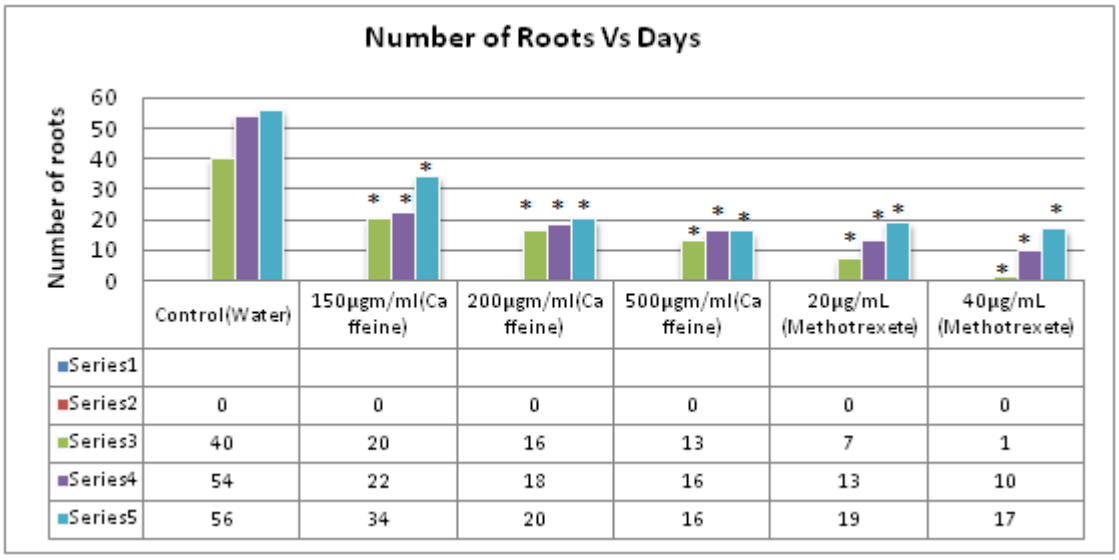

Figure 4: Number of roots vs. Day

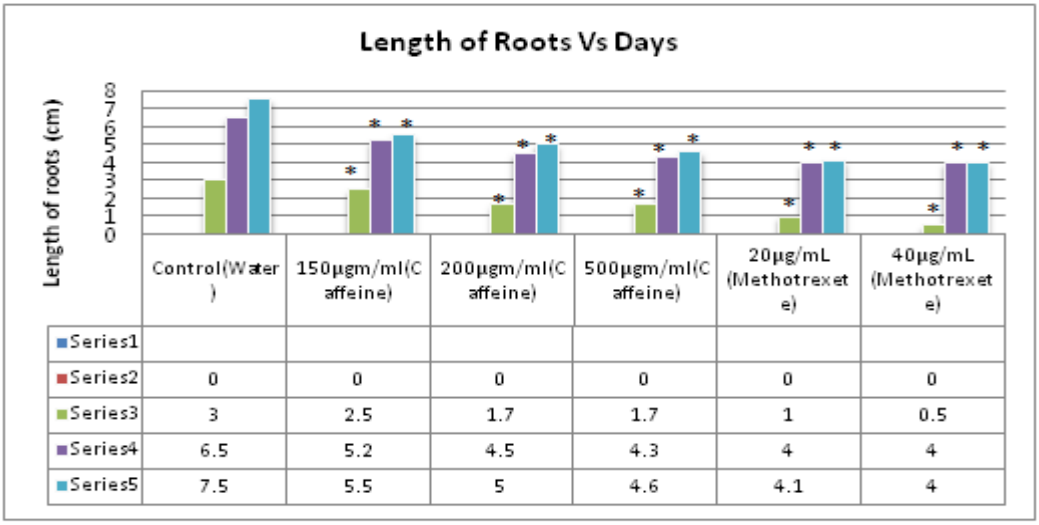

Figure 5: Length of Roots vs. Day

CONCLUSION

In nutshell of above discussion we conclude that caffeine shows significance cytotoxic potential at 500, 750 µgm/ml concentration in hemolytic, brine shrimp and *Aliium cepa* pharmacological model. Probalbe mechanism of this action might be inhibiting mitosis process in cell division. So caffeine could be the next better, safer and cheaper herbal alternative in management of chronic disease like cancer.

ACKNOWLEDGEMENT

I acknowledgement is to my Grandparents and my lovely parents whose blessings are always with me for doing something new in my life. And special thanks to my dear brother Mr. Patel Parth. I can't forget to appreciate the efforts of my dearest person Mr. Maniar Parth for supporting me with positivity in every step of my life.

I also express my sincere thanks my co guide Mr. Ganatra Tejash as well as thanks to Shamjibhai Harjibhai Talaviya Cheritable trust and Management of RK University, School of pharmacy for providing me all the necessary facilities for caring out my work.

REFERENCES

[1] Overview: Introduction to Cancer '' Jessica Evert, Md, **2010**.
[2] "41 Percent Of Americans Will Get Cancer." Upi.Com. http://Www.Upi.Com/Health_News/2010/05/06/41-Percent-Of-Americans-Will-Get,Cancer/Upi-75711273192042c. **2013**.
[3] National Cancer Institute: Radiation Therapy Side Effects Www.Cancer.Net/Navigating-Cancer-Care/How-Cancer-Treated/Radiation-Therapy/Side-Effects-Radiation-Therapy.
[4] Picheswararao Polu; Udupa Nayanabhirama; Saleemulla Khan. *Herbal Medicinal Plants as Anticancer Agents.* **2015**.
[5] AB Nehlig A; Daval Jl; Debry G. *Brain Research Reviews.* **2001**, 17 (2), 139-170.
[6] Mitchell Diane C; Knight Carol A; Hockenberry Jon; Teplansky Robyn; Hartman Terryl J. *Food and Chemical Toxicology.* 63: 136-142.
[7] Zhiwei He; Wei-Ya Ma; Takashi Hashimoto; Ann M Bode; Chung S Yang; Zigangdong. Induction of Apoptosis by Caffeine is Mediated by the P53, Bax, And Caspase 3 Pathways. **2003**, 63(15).
[8] Caffeine May Be Anti-Aging and Anti-Cancer Agent. Caffeine-May-Anti-Aging-Anti-Cancer-Agent.Html, Caffeine-Health/278, **2004**.
[9] Shalmashi A; Golmohammad F. Solubility of Caffeine in Water, Ethyl Acetate, Ethanol, Carbon Tetrachloride, Methanol, Chloroform, Dichloromethane, and Acetone between 298 and 323 K. **2010**, 40, 283-285
[10] Chem.Wisc.Edu/Courses/342/Fall2004/Melting_Point. Doi **2004.**
[11] Guidote AD; Del Rosario AA; Abuzo A. Experiencing Organic Chemistry. Quezon City: Office of Research and Pulication, Ateneo De Manila University. **2004**, 158.
[12] Agarwal OP. Advances Practical Oraganic Chemistry. 26th edn. Meerut, India: Goel Publishing House. 53.
[13] Nayeem AA, Khatun A, Rahman MS, Rahman M. *Journal of Pharmacognosy and Phytotherapy.* **2004**, 3(8): 118-123.
[14] Daniel B Alencar; Arthur A. Melo1; Giselle C Silva; Rebeca L Lima; Kelma MS; Pires-Cavalcante. *Anacad Bras Cienc*. **2015**; 87(2).
[15] Mentor R Hamidi; Blagica Jovanova; Tatjana Kadifkova Panovska. Toxicological Evaluation of the Plant Products Using Brine Shrimp. **2014**.
[16] Harwig J; Scott PM. *Appl Microbio.* **1971**, 21: 1011-1016.
[17] José Luis Carballo; Zaira L Hernández-Inda; Pilar Pérez; María D García-Grávalos. *Bmc Biotechnology*. **2002**, 2.
[18] Sreeshma LS; Bindu R Nair. *Int J Pharm Pharm Sci.* **2014**, 6(4), 582-586.
[19] R Sehgal; S Roy; VL Kumar. Evaluation of Cytotoxic Potential of Latex of Calotropis Procera and Podophyllotoxin in *Allum Cepa* Root Model. **2006**, 30(1), 9-13.
[20] Elisamgela D; Michel L; Leolberto S; Daiana L; Veronica C. *Allium Cepa* L As A Bioindicator to Measure Cytotoxicity of Surface Water of the Quatoze River Located In Francisco Beltrao, Parana, Brazil. **2013**.