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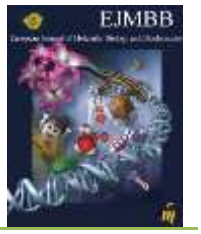

ANTICARCINOGENIC ACTIVITY OF PIPER NIGRUM EXTRACT AND ITS ACTIVE COMPONENT PIPERINE AGAINST 7, 12-DIMETHYLBENZ (A) ANTHRACENE INDUCED MOUSE SKIN CARCINOGENESIS

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ABSTRACT
Piper nigrum is famous as the spices king due to its pungent quality. Piper nigrum (*P. nigrum*) L. is a member of family Piperaceae. *P. nigrum* fruits are also used to produce white pepper and green pepper and are valued due the presence of piperine including its different isomers. Black pepper can be used for different purposes such as human dietaries, as medicine, as preservatives, as bio control agents, In the present investigation, the anticancer activity of *Piperine* and *P. nigrum* extract was evaluated using two stage in Swiss albino mice, induced by a single application of 7,12-dimethyadenz(a)anthrecene (104 µg/100 µl acetone) and one weeks later, promoted by repeated application of croton oil (1% in acetone/thrice a week) till the end of the experiment (16 weeks). The tumor incidence, tumor yield, tumor burdon and cumulative number of papillomas were found to be higher in the control (without *P. nigrum* treatment) as compared to experimental animal (*P. nigrum* treated). The difference in the values of the results of experimental group were statistically analyzed and found to be significant in comparison to the control group ($p<0.05$). In conclusion, the present study demonstrates the chemopreventive of *P. nigrum* on DMBA induced skin tumorigenesis in *Swiss albino* mice.

INTRODUCTION

Herbs are natural remedies for the disease with higher safety profile and efficacy. Country like India has got variety of climatic conditions and seasons favorable for growth of many species of plants. The family Piperaceae comprises 12 genera and about 1400 species mainly found in tropical region [1]. The genus Piper (L.) contains more than 700 species they grow in tropical and subtropical rain forest. Piper species grown in South India are economically important and among the important medicinal plants used in various systems of medicine.

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Several species of Piper are used in indigenous system of medicine in India [2]. Due to multidimensional effect on various systems of body, it has been described as antipyretic, diuretic, aphrodisiac, immune-stimulant, antioxidant, hepatoprotective, digestive, rubefacient, counter irritant, antiseptic, antispasmodic [3-7]. Besides it is also known to enhance the bioavailability of food and drugs [8]. The present study reveals relevant pharmacognostic, phytochemical, physicochemical, chromatographic and antimicrobial data of two piper species namely *Piper nigrum* and *Piper longum*. Black pepper consists of fully mature dried fruit of *Piper nigrum* Linn a climber, cultivated from Konkan southwards, especially in North Konkan, Kerala and also in Assam [9]. *P. nigrum* not only used in perfumery and food industry

but also very effective against fatal diseases caused by mutations [10] observed that when Drosophila melanogaster was exposed to mutation through promutagen-ethyl carbamate, in such induced situation the *P. nigrum* is effective to reduce mutational events. *P. nigrum* and its active derivatives especially peppercorn extract has been reported to inhibit tumours formation in experimental models [11]. Such reduced antitumor activity by the oral administration was also reported [12]. The alcoholic extract of peppercorn and piperine was effective in immunomodulatory, antitumor activity and Dalton's lymphoma [13]. Observed that piperine are involved in antimetastatic activity, in his experiment he documented that mice models when exposed to melanoma cells (B16F-10), the active agent piperine prevent and inhibit lung metastasis and finally concluded that piperine dramatically reduced tumour nodule formation. Moreover, piperine from *P. nigrum* reduced lung cancer by modulating lipid peroxidation and through the activation of antioxidative protection enzymes [14,15]. Therefore, we have plane to carry out anticancer activity of *Piper nigrum* extract using the two stage skin cancer model in Swiss albino mice.

MATERIALS AND METHODS

Animals

The study was conducted on random breed, 6-7 weeks old and 20 - 25 gm body weight bearing, male *Swiss albino* mice. Animals were maintained under controlled conditions of temperature and light (Light: dark 12:12 h.). They were provided standard mice feed and water ad libitum. The study protocol is approved by the Departmental Animal Ethical Committee (IAEC Ref. No. 01/IAEC/CCOST/2014).

Chemicals

The chemicals, 7, 12-dimethylbenz (a)anthracine (DMBA) and croton oil were procured from Sigma Chemicals Co., St. Louis, USA. DMBA was dissolved at a concentration of 100 µg/100 µl in acetone. Croton oil was mixed in acetone to give a solution of 1% dilution.

Extract preparation

Fruits of *P. nigrum* were procured from local market of Raipur and authenticated at Central Laboratory Facility, Chhattisgarh Council of Science and Technology, Raipur, Chhattisgarh. The *P. nigrum* fruit washed thoroughly under running tap water then these fruit of *P. nigrum* were allowed to dry in shade after drying the dried pieces were then grounded to powder. Then the powdered of *P. nigrum* powder and solvent (Methanol) were taken in a soxhlet assembly. *P. nigrum* powder was taken in a soxhlet and 1:1 ratio solvent (Methanol: Water) were added. The extract was then kept in Oven at 50-60º C and the dried sample in form of fine crystals was then collected. On the day of the experimentation, the desired amount of powder was dissolved in double distilled water for the final administration.

Skin Carcinogenesis Bioassay

DMBA acts as an initiator and Croton oil is working as a promoter to induce skin papillomas and squamous cell carcinomas in skin carcinogenesis assay as described by Berenbrum *et. al.*, (1941 a, b) [16,17]. Male/Female inbred Swiss albino mice weighing 15-20 g will be procured from animal house of our research centre. They are taking synthetic pellet diet and water ad libitum and that will be used for the study. The animals are randomly divided in to 8 groups. Each group initially comprises of 10 animals. Their hair will be shaved in $2cm^2$ are with the help of hair removing cream in inter scapular region and after every 2 weeks hair will be removed with the help of scissors. The treatment will be provided topically on shaved area using the following protocol.

1. Group: (Untreated control)
No treatment was given and the animals will be kept under observations until the end of 16 weeks of experiment.

2. Group: (Vehicle control)
All animals in this group was subjected to topical applications of acetone, the vehicle (0.1 ml/mouse) on the shaved skin area twice week until the end of 16 weeks of experiment.

3. Group: DMBA alone
All animals in this group were subjected to a single topical application of DMBA (the initiator) at 104 µg on the shaved skin area. Ten days after the initiation, the treated skin will be applied topically with 0.1 ml of acetone twice a week until the end of experiment.

4. Group: Croton oil alone
All animals in this group was applied topically with 1% croton oil (v/v in acetone) the promoter twice a week throughout the experiment.

5. Group: Piperin alone
All animals in this group was received topical applications of PNE (95%Piperine) at 150 mg/kg bwt (the highest dose tested) twice a week throughout the experiment.

6. Group: DMBA + Croton Oil (Control Group)
Animals in this group were initiated with a single topical application of DMBA (104 µg /mouse). Ten days after the initiation, the shaved skin of each animal will be promoted with 0.1 ml of croton oil (0.5%, v/v in acetone) twice a week until the end of 16 weeks of experiment.

7. Group: DMBA + Piperine + Croton Oil (Positive Control)
All animals on this group were received topical applications of *P. nigrum* extract at 150 mg/kg bwt along with single application of DMBA followed by croton oil twice a week throughout the experiment.

8. Group: DMBA + *P. nigrum* extract + Croton Oil

All animals on this group were received topical applications of *P. nigrum* extract at 150 mg/kg bwt along with single application of DMBA followed by croton oil twice a week throughout the experiment.

RESULT

The body weight significantly decreased in *P. nigrum* extract treated animals as compared to control animals. The results of the present investigation have been summarized in Table 1 and 2. Single topical application of DMBA followed by croton oil, produced skin papillomas, which started appearing from the sixth week onward. The tumor incidence in the DMBA + croton oil treated mice (carcinogen control) reached 100% by the end of the experiment (16 weeks). The cumulative number of papillomas was recorded 19 and average number of papillomas per mouse (tumor yield) as well as the papillomas per papilloma-bearing mice (tumor burden) was found 3.16 in carcinogen control group.

A significant reduction was observed in tumor incidence, tumor burden, tumor weight, tumor size, and cumulative number of papillomas in Piperine treated groups as well as *P. nigrum* extract treated group relative to the carcinogen treated control. No papilloma was observed in Untreated control, Vehicle control, DMBA alone, Croton oil alone and Piperine extract alone groups. The body weight of control and experimental animals in each group are shows as significant different. The body weight significantly decreased in DMBA treated animals as compared to control animals. The results are summarized in Table 1 and 2.

Table 1. Effect of *P. nigrum* extract on DMBA-induced papillomas in Swiss albino mice

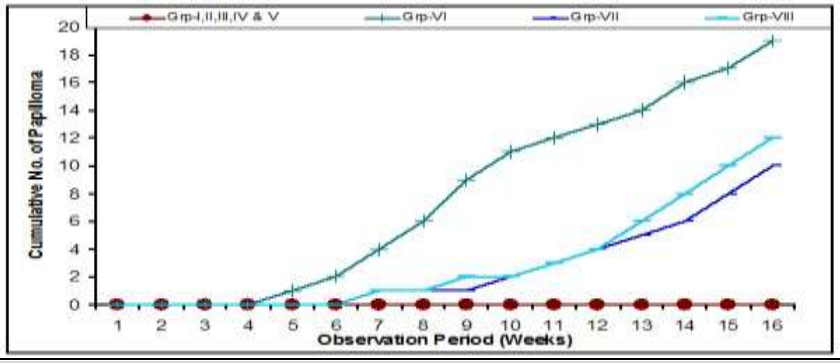
Sl	Groups	Body weight		Cumulative No. of Papillomas	% Tumour Incidence	Tumour yield	Tumour Burden
		Initial	Final				
1.	Untreated control	21.30±1.82	25.87±1.32	0	0	0	0
2.	Vehicle control	23.23±0.91	26.21±1.25	0	0	0	0
3.	DMBA alone (1 application)	22.15±2.06	25.60±0.98	0	0	0	0
4.	Croton oil alone	21.14±1.20	24.98±1.28	0	0	0	0
5.	Piperine alone	22.78±1.87	25.01±1.75	0	0	0	0
6.	DMBA+ Croton oil	23.12±0.87	21.54±0.69	19	6/6 (100%)	3.16	3.16
7.	DMBA + Piperine (150 mg/kg) + Croton oil	22.27±0.71	25.46±0.87	10*	3/6 (50%)*	1.66*	3.33*
8.	DMBA+ P. nigrum ext. (500 mg/kg) + Croton Oil	21.89±1.27	24.74±1.75	12*	4/6 (66%)	2.00*	3.00*

*****Denotes statistical significance in Student's `t' test ($p<0.05$) as compared to carcinogen control group.

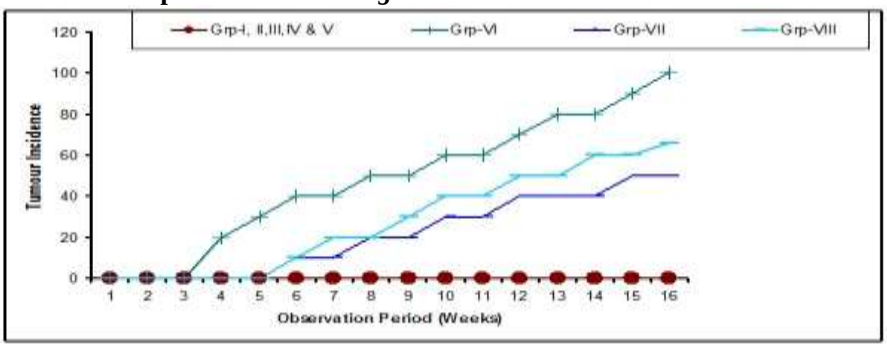
Table 2. Effect of *P. nigrum* extract on tumour incidence in weeks

Sl	Groups	Weeks															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1.	Untreated control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.	Vehicle control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.	DMBA alone (1 application)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.	Croton oil alone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.	Piperine alone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.	DMBA+ Croton oil	0	0	0	0	1	2	4	6	9	11	12	13	14	16	17	19
7.	DMBA + Piperine (150 mg/kg)+ Croton oil	0	0	0	0	0	0	1	1	1	2	3	4	5	6	8	10
8.	DMBA+ *P. nigrum* ext. (500 mg/kg) + Croton Oil	0	0	0	0	0	0	1	1	2	2	3	4	6	8	10	12

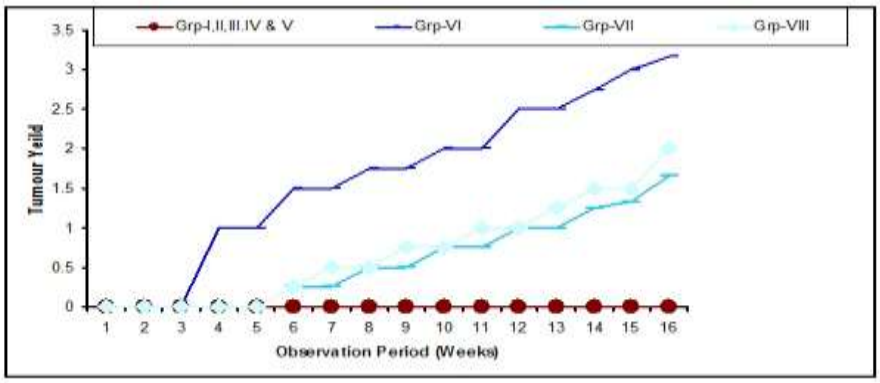
Graph 1. Effect of *P. nigrum* extract on Cumulative number of Tumour



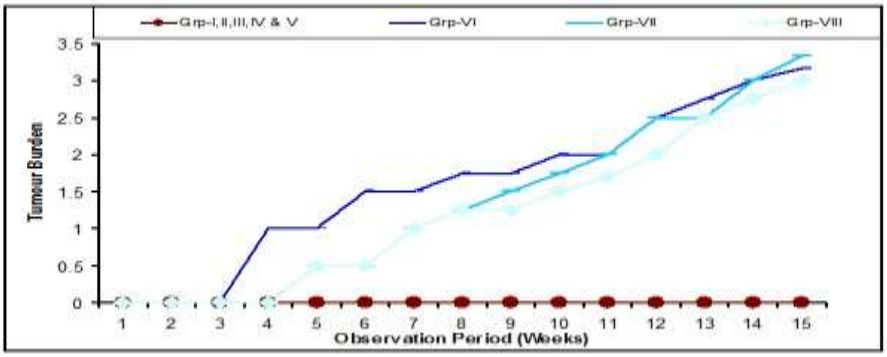
Graph 2. Effect of *P. nigrum* extract on Tumour incidence



Graph 3. Effect of *P. nigrum* extract on Tumour yield



Graph 4. Effect of *P. nigrum* extract on Tumour Burden



DISCUSSION AND CONCLUSION

Black pepper is important for its medicinal value. Medicinally black pepper can be used for digestive disorders like large intestine toxins, different gastric problems, diarrhea, and indigestion and also can be used against respiratory disorders including cold, fever and asthma. *Piper nigrum* L. is considered the king of spices throughout the world due to its pungent principle piperine. Peppercorn of *Piper nigrum* as a whole or its active components are used in most of the food items. Different parts of *Piper nigrum* including secondary metabolites are also used as drug, preservative, insecticidal and larvicidal control agents. Biologically *Piper nigrum* is very important specie. *Piper nigrum* is famous as the spices king due to its pungent quality [18]. Other related activities included Anti-inflammatory activity, thermogenic action, growth stimulatory activity, anti-thyroid activity and chemo preventive [19]. Secondary metabolites from *P. nigrum* play defensive role against infections by microbes, insects and animals [20, 21].

Piperamides extracted from *P. nigrum* had shown insecticidal activities [22, 23]. β-caryophyllene showed anesthetic activity [24]. Nerolidol is very famous secondary metabolite of *P. nigrum*, used to control mites. Another important component of pepper volatile oil is pipene, which is a famous odorants [25].

In our two stage skin carcinogenesis study the *Piper nigrum* extract show the most potential against carcinogens. The body weight significantly decreased in the carcinogen group. Single topical application of DMBA followed by croton oil, produced skin papillomas, which started appearing from the sixth week onward. The tumor incidence in the DMBA + croton oil treated mice (carcinogen control) reached 100% by the end of the experiment (16 weeks). The cumulative number of

papillomas were reduced as compared to carcinogen control group and average number of papillomas per mouse (tumor yield) as well as the papillomas per papilloma bearing mice (tumor burden) was also found decrees as compared to carcinogen control group. A significant reduction was observed in tumor incidence, tumor burden, tumor weight, tumor size, and cumulative number of papillomas in Piperine treated groups as well as *P. nigrum* extract treated group relative to the carcinogen treated control. Many herbal products have been reported to antitumour promoting activity using two stage skin cancer models. These include *Solanum lycopersicum* [26], *Lawsonia inermis* [27], *Bahunia variegate* [28], *Aloe vera* [29] etc. Therefore, we have planned to make to screen the anticancer activity of piperine as well as *P. nigrum* extract. The out comes of the present study is very significant.

Our results suggest that anticancer activity of *P. nigrum* in skin carcinogenesis tumour model. This work explores the anticancer activity of *P. nigrum* extract in reducing the number of tumours in test model. The underlying molecular mechanisms now require attention. These results are important because this plant is a worldwide known is traditional medicinal property. These finding indicate that *P. nigrum* extract can be used as a supplementary agent for cancer treatment. The work conduct so far reveals that tradition wisdom of Ayurvedic can be of immense utility in enhancing the bioavailability of allopathic action but their therapeutic utility is marred on account of poor bio-availabity hopefully the tradition wisdom coupled with modern technology would open new vistas in human health care.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

REFERENCES

1. Barroso GM. (1978). Sistematica de angiospermas do Brazil. *Epu Usp. Sao Paulo*, (1), 45.
2. Parthasarathy U, Saji KV, Jayarajan K, Parthasarathy VA. (2006). Biodiversity of Piper in South India - application of GIS and cluster analysis. Current Science. 91(5), 652-658.
3. Atal CK, Dhar KL, Singh J. (1975). The chemistry of Indian Piper species. *Lloydia*, 38(3), 256-264.
4. Addae-Mensah. I, Torto FG, Dimonyeka CI, Baxter I, Sanders JKM. (1977). Novel amide alkaloids from the roots of Piper guineense. Phytochemistry, 16, 757-759.
5. Sunila ES, Kuttan G. (2004). Immunomodulatory and Antitumor activity of Piper longum Linn. And Piperine. *J. Ethnopharmacol*, 90, 339-346.
6. Stohr J R, Xiaso P G, Bauer R. (2001). Constituents of Chinese piper species and their inhibitory activity on prostaglandin and leukotriene biosynthesis in vitro. *Journal of Ethanopharmacology*, 75, 133–139.
7. Nongyao S, Kitja S, Pathana P. (2004). Effect of Piper longum fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall on caecal amoebiasis in mice. *Journal of Ethno pharmacology*, 91, 357–360.
8. Westhuizen JHV, Ferreira D, Roux DG. (1980). Phytochemical deoxygenation of an ketol, the dihydroflavonol- flavanone conversion. *Journal of Chemical Society Perkin Transactions*, I4, 1003–1006.
9. Indian herbal pharmacopoeia. (2002). Revised New edition. Indian Drug Manufacturer's Association Mumbai. 317-320.
10. El-Hamss R, Idaomar M, Alonso-Moraga A, Munoz-Serra A. (2003). Antimutagenic properties of bell and black peppers. *Food Chem Toxicol*, 41, 41-47.
11. Lin Z, Liao Y, Venkatasamy R, Hider RC, Soumyanath A. (2007). Amides from *Piper nigrum* L. with dissimilar effects on melanocyte proliferation in-vitro. *J. Pharma. Pharmaco*, 59, 529-536.
12. Ahmad N, Fazal H, Abbasi BH, Rashid M, Mahmood T, Fatima N. (2010). Efficient regeneration and antioxidant potential in regenerated-tissues of *Piper nigrum* L. *Plant Cell, Tissue and Organ Culture*, 102, 129-134.
13. Appiah I, Milovanovic S, Radojicic A, Nikolic-KokicA, Orescanin-Dusic Z, Slavic M, Trbojevic S, Skrbic R, Spasic MB, Blagojevic D. (2009). Hydrogen peroxide affects contractile activity and anti-oxidant enzymes in rat uterus. *Br. J. Pharmacol*, 158, 1932-1941.
14. Selvendiran K, Sakthisekaran D. (2004). Chemopreventive effect of piperine on modulating lipid peroxidation and membrane bound enzymes in benzo (α) pyrene induced lung carcinogenesis, *Biomed. Pharmacotherapy*, 58, 264-267.
15. Mona AM, Abo-Zeid, Ayman A, Farghaly. (2009). The Anti-mutagenic Activity of Piperine against Mitomycine C induced Sister Chromatid Exchanges and Chromosomal Aberrations in Mice. *Nature and Science*, 7, 72-78.
16. Berenblum I (1941a). The mechanism of co-carcinogenesis. A study of significance of cocarcinogenic action and related phenomena. *Cancer Res*, 1, 807-814.
17. Berenblum I (1941b). The co-carcinogenic action of croton resin. *Cancer Res*, 1, 44-51.
18. Srinivasan K. (2007). Black pepper and its pungent principle-piperine. A review of diverse physiological effects. *Crit. Rev. Food Sci. Nutr*, 47, 735-748.
19. Panda S, Kar A. (2003). Piperine lowers the serum concentration of thyroid hormones, glucose and hepatic 5D activity in adult male mice. *Horm. Metab. Res*, 35, 523.

20. Umit A, Kadir I, Akgun KO. (2008). Antifungal activity of aqueous extracts of spices against bean rust (Uromyces appendiculatus). *Allelopathy Journal*, 24, 0973-5046.
21. Ahmad N, Fazal H, Abbasi BH. (2011). In vitro Larvicidal potential and Antioxidative enzymes activities in Ginkgo biloba, Stevia rebaudiana and Partheniumhysterophorous. *Asian Pacific Journal of Tropical Medicine*, 169-175.
22. Scott IM, Gagnon N, Lesage L, Philogene BJ, Arnason JT. (2005). Efficacy of botanical insecticides from Piper species (Piperaceae) extracts for control of European chafer (Coleoptera, Scarabaeidae). *J. Econ. Entomol*, 98, 845-855.
23. Naseem MT, Khan RR. (2011). Comparison of repellency of essential oils against red flour beetle *Tribolium castaneum* Herbst (Coleoptera, Tenebrionidae). *J. Stored Prod Postharvest Res*, 2, 131-134.
24. Santra M, Santra DK, Rao VS, Taware SP, Tamhankar SA. (2005). Inheritance of β-carotene concentration in durum wheat (*Triticum turgidum* L.ssp.durum). *Eucalypta*, 144, 215-221.
25. Jayalekshmy A, Menon AN, Padmakumari KP. (2003). Essential oil composition of four major cultivars of black pepper (Piper nigrum L.). *J. Essential Oil Res*, 15, 155-157.
26. Agrawal, RC, Wasim Raja, Rachna Jain, Ovais M. (2009). Anticarcinogenic Effect of *Solanum lycopersicum* fruit extract on swiss albino and C57BL mice. *Asian Pacific Journal of Cancer Prevention*, 10, 379-381.
27. Wasim Raja, Agrawal RC and Ovais M (2009). Chemopreventive action of *Lawsonia inermis* Leaf Extract on DMBA-induced skin papilloma and B16F10 Melanoma Tumour. *Pharmacology online*, 1043-1249.
28. Sonam Pandey and RC. Agrawal. (2009). Effect of Bauhinia Variegate Bark Extract on DMBA-Induced Mouse Skin Carcinogenesis, a Preliminary Study. *Global Journal of Pharmacology*, 3(3), 158-162.
29. Agrawal, RC and Sonam Pandey. (2013). Evaluation of Anticarcinogenic Activity of herbal Medicinal Plant *Aloe vera* in Swiss albino mice. *International Journal of Scientific and Research Publications*, 3(2), 1-6.