CAMPARATIVE STUDY OF THE PHYSICAL PARAMETERS IN DMBA INDUCED ORAL CARCINOMA RAT MODEL AND HERBAL EXTRACT FED ORAL CARCINOMA RAT MODEL.

Abstract: Cancer, a multifactorial disorder, arises due to defect in genetic makeup and exposure to unfavourable environmental conditions. Oral cancer, a life-threatening cancer worldwide, arises in the oral cavity due to ill-habits such as tobacco smoking and chewing (Capilla et al., 2007). Monitoring body weight is an essential aspect of any preclinical study, including DMBA-induced oral carcinoma rat models and models with potential therapeutic interventions like Zingiber and piper feeding. Changes in body weight, feeding, water intake can provide valuable insights into the general health and physiological effects of the experimental conditions.

Keywords: Body Weight, Squamous cell Carcinoma, Phytochemicals,*Zingiber Officinale, Piper nigrum*, DMBA.

INTRODUCTION: Oral cancer is the sixth most common cancer worldwide (Gil et al., 2008). Almost 90% of all oral cancers are categorized as squamous cell carcinoma (SCC) (Attar et al., 2012). In India, 20 per 100000 population are affected by oral cancer which accounts for about 30% of all types of cancer (Nair et al., 2005) Cancer, a multifactorial disorder, arises due to defect in genetic makeup and exposure to unfavourable environmental conditions. Phytochemicals have been fascinating scientists due to their property in altering cell cycle control and regulation, apoptosis, invasion, angiogenesis, interference with blood vascular system and metastasis. They have proved their efficacy in single treatment procedures or in connotation with other chemo preventive agents. Phytochemicals can be largely classified into vitamins, carotenoids and food polyphenols phenols like flavonoids, phytoalexins, sulphur surplus compounds and phenolic acid indoles. we extend these studies to rodent model primarily because of close genetic homology with humans (almost 68%) with addition of other relevant parameters. Accumulated indications pointed out histological, morphological, biochemical and molecular resemblances between DMBA induced oral tumors and human oral tumors (Tanaka, 2011)

AIM AND OBJECTIVE:

Monitoring the physical parameters in DMBA-induced oral carcinoma rat model and herbal extract fed oral carcinoma rat model serves several aims and objectives. These physical parameters play a crucial role in understanding the progression of oral cancer and evaluating the potential effects of herbal extracts on the disease. Below are the primary aims and objectives of monitoring these parameters:

1. **Tumor Development and Progression**: One of the main aims is to observe and document the development and progression of oral tumors in both models. Regular monitoring allows researchers to track the size, number, and location of tumors over time.

2. **Effectiveness of DMBA Induction**: In the DMBA-induced oral carcinoma rat model, monitoring physical parameters helps to confirm the successful induction of oral tumors with DMBA, ensuring that the model is consistent and reliable for the study of oral cancer.

3. **Herbal Extract Efficacy**: In the herbal extract fed oral carcinoma rat model, physical parameter monitoring aims to evaluate the effects of the herbal extract on oral cancer development and progression. The study focus on observation of several parameters including action of herbal treatment leads to tumor regression, inhibits tumor growth, or has any other beneficial effects.

4. **Body Weight and General Health**: Monitoring body weight and other general health parameters (e.g., activity, grooming) in both models helps assess the overall well-being of the rats. It ensures that the experimental procedures and treatments do not cause severe adverse effects or discomfort.

5. **Survival Rate**: We aim to track the survival rate of rats in both models the DMBA treated and DMBA along with supplementing with *Zingiber officinale*. This provides insights into the impact of oral carcinoma and the potential effects of herbal treatment on the overall survival of the animals.

6. **Safety and Toxicity Assessment**: Monitoring physical parameters also serves to assess the safety and potential toxicity of the herbal extracts. It is observed if the herbal treatment causes any adverse effects or damage to healthy tissues.

7. **Data Collection for Statistical Analysis**: By systematically monitoring physical parameters in a controlled manner,quantitative data for statistical analysis is gathered and further worked on.

8. **Clinico Pathological Studies**
   At the start of experimental work, rats were observed regularly for manifestation of clinical symptoms, Body weight changes and at the end of experiment organ weight of various organs under consideration of all the treated groups were compared with that of control group.
   Coat color changes and change in eye color was also noticed regularly throughout the experiments. Alteration in food consumption and water intake pattern along with quantitative data.

Overall, monitoring physical parameters in both the DMBA-induced oral carcinoma rat model and the herbal extract fed oral carcinoma rat model is essential for understanding the disease's biology, assessing treatment efficacy, and ensuring the ethical conduct of the research. The combined information from these parameters helps advance scientific knowledge in the field of oral cancer and may contribute to the development of new therapeutic approaches using herbal extracts or other interventions.

MATERIAL AND METHODOLOGY:

In research involving the DMBA (7,12-Dimethylbenz[a]anthracene) induced oral carcinoma rat model, the body weight parameter is an important factor to consider. The DMBA-induced oral carcinogenesis model is commonly used to study the development and progression of oral cancer in rats.

The body weight of the rats serves as an indicator of their overall health and well-being throughout the experiment. Monitoring the body weight of the animals is crucial to ensure that the treatment and exposure to DMBA are not causing severe adverse effects leading to significant weight loss or other health complications.

Male Wistar rats *Rattus Norvegicus* are used in this study. A total of 60 colony bred Swiss albino rats Rattus Norvegicus are used in this study. Rats were raised under laboratory conditions from an initial cohort of 20 rats via inbreeding for a period of 8 months. The entire experiment was scheduled in triplicates. Rats of 8-10 weeks of age, with average body weight of 150-180 gm, were obtained from Institute of Animal health and Veterinary and Biological Products, Rasalpura, Mhow, Madhya Pradesh. The animals were maintained at 22±3°C, with 50-70% relative humidity and 12:12 hrs of light and dark cycles and were kept in well-ventilated cages. The animals were fed with calculated amount of laboratory pellet diet procured from government agricultural college, Indore, India, and water *ad libitum*. Maintenance and cleaning were carried out twice a week and drinking water was replaced weekly or prior to that if required. The animals were observed daily to assess their general health. Animals were maintained as per the guidelines laid down by departmental ethical committee for handling and maintenance for experimental animals and the committee for the purpose of control and supervision on experiments in animals (CPCSEA NO-3565/IAEC/2014).

Rats were divided into four groups of five animals in each and were allowed free access to food and water for 20 days before the commencement of the experiment. As the drugs were given in pellet diet, so rats were closely observed and studied for a period of 20 days to evaluate the consumption of food. The entire experimentation was performed in triplicates.

Daily dose was calculated on the basis of the following equation:

DD = (SD X BW) / F1(Research diet)

DD= diet dose (mg cmpd/kg Diet), SD= single daily dose (mg cmpd /kg BW/day) BW= body weight (gm BW/animal), F1= daily food intake (gm diet/day). Four groups (5 animals per group) of Wistarrats were used for study. Rats were dorsally shaved with hair clipper. Group 1 rats were painted with DMSO alone. Group 2 rats were treated with DMBA alone (0.2%). Group 3 rats were orally fed with *Zingiber officinale*extract (500mg/kg BW) and were painted with DMBA also. Group 4 rats were orally fed with *P. nigrum* extract (100mg/kg BW) and also DMBA was painted in the oral cavity and the surrounding area with sable hair fine brush no 4.

After 16 days of DMBA application, rats were observed each week for incidence and size of skin tumors, the body weight and average latent period were recorded for 16 weeks with a frequency of measuring body weight once in 2 days. Food and water consumption were recorded on daily basis.

The groups were as follows:

- C Control animals (DMSO painted)
- DMBA treatment(0.2%)
- T1,*Z.officinale* treatment(500mg/kg/BW).
- T2,*P. nigrum*treatment(100mg/kg BW)

Picture 1: Picture representing water intake, body weight and food intake changes during experimental duration

RESULT:

In all the experimental groups each rat was weighed accurately twice in the beginning of experimental schedule. It was made sure that all the experimental rats should fall in the range of 150-180 gm. The rats in group T1 and T2 which were painted with DMBA in the oral epithelium and orally administered with *Zingiber officinale* (500mg/kg B.W) and *Piper nigrum* (100mg/kg) showed no significant reduction in gain of body weight as compared to rats of control group. During the exposure period of DMBA to the animals the food consumption of DMBA treated group has been significantly reduced to 49.5±6.28 as compared with the control group 56.5±3.594. a drastic reduction in the water consumption in case of DMBA treated group has been found at 16 weeks where carcinoma has been well differentiated, but the water consumption pattern fluctuates in these 120 days period which is first marked by drastic increase in the water consumption initially at the 8th week reaches its peak point on 12th week but then there is abrupt continuous decline in the water consumption quantity with a value of 30.225 ±1.23 from initial value of 45.07 ±3.04. There is continuous reduction in the body weight of DMBA treated group during the entire treatment duration. In case of control group, the value is 147.50±9.32 while in case of DMBA administered group the value comes out to be 112.75±6.65. A gradual decrease in body weight was found in all rats of DMBA group during the last week of carcinogen application. DMBA treated animals weighted less than untreated animals by 5-7% probably due to metabolic stress caused by inflammation or irritation in the buccal epithelium.

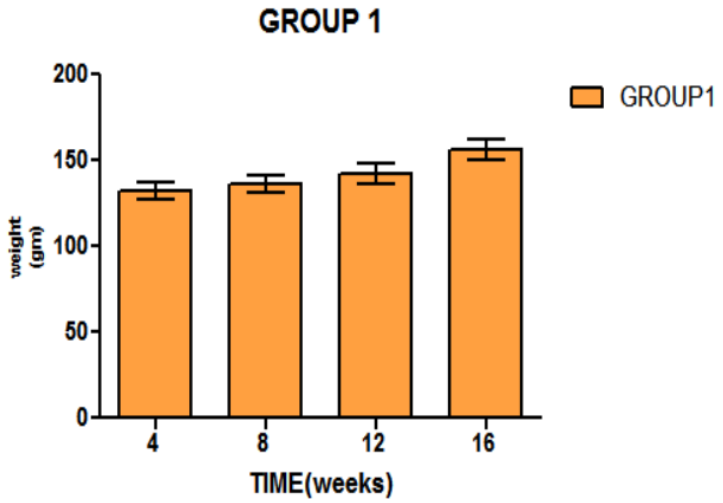

Fig1: Showing alteration in body weight of control group. Each vertical bar represents mean±S.E.M (n=5).

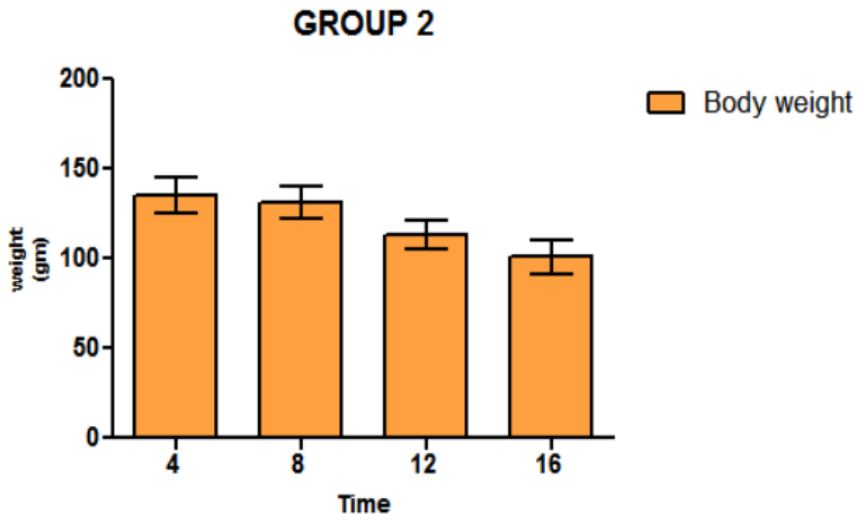

Fig:2 Changes in body weight of DMBA treated experimental animals in male rats. Each vertical bar represents mean±S.E.M (n=5), as compared to the control values.

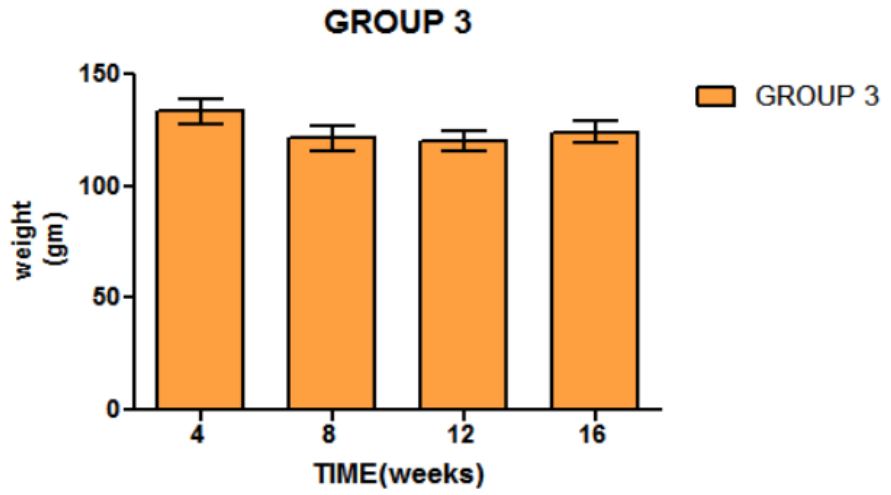

Fig.3: Changes in body weight of DMBA treated experimental animals along with *Z. officinale* extract administration in male rats. Each vertical bar represents mean ±S.E.M(n=5), as compared to the control values.

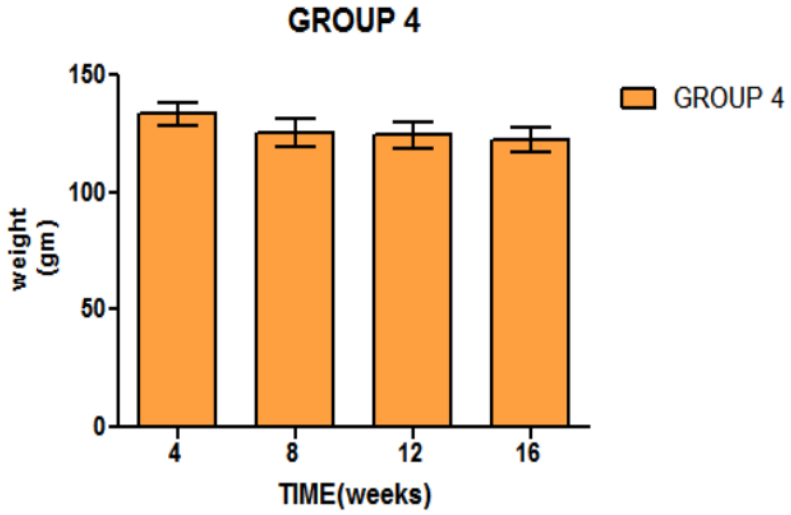

Fig.4: Changes in body weight of DMBA treated experimental animals along with P. nigrum extracts in male rats. Each vertical bar represents mean ± S.E.M(n=5), as compared to the control value.

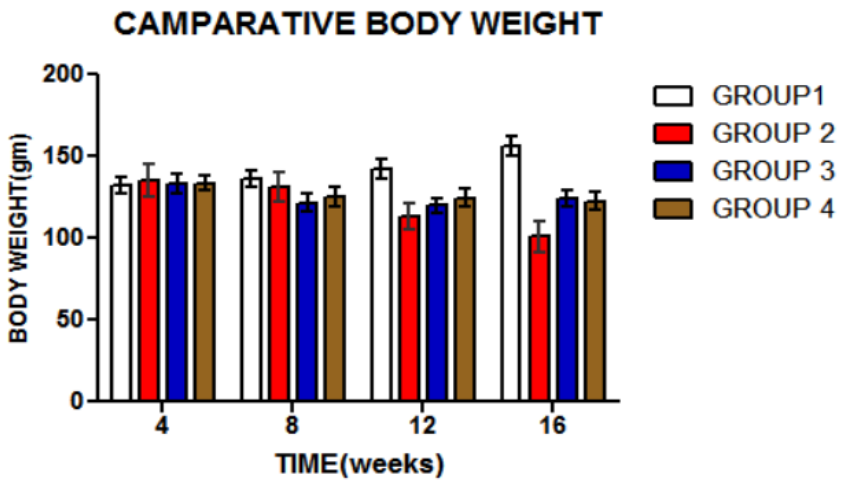

Fig.5: Comparative analysis of body weight alteration in all experimental groups. Each vertical bar represents mean ± S.E.M(n=5),as compared to the control values.

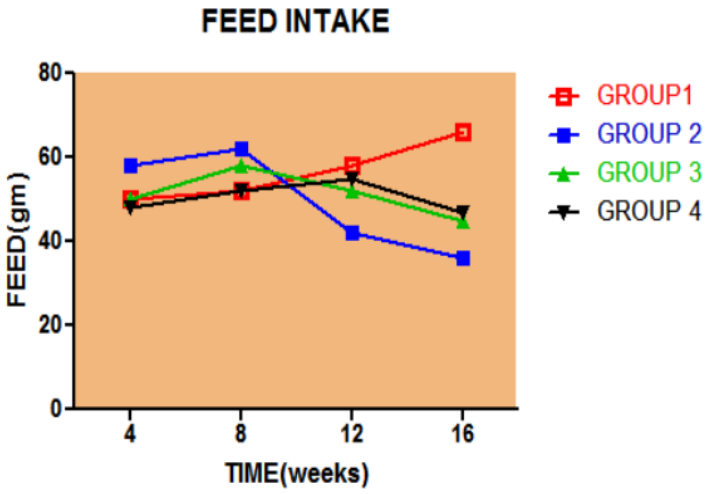

Fig 6: Comparison of food intake in experimental animals (n=5) following carcinogen and herbal extract administration in male rats

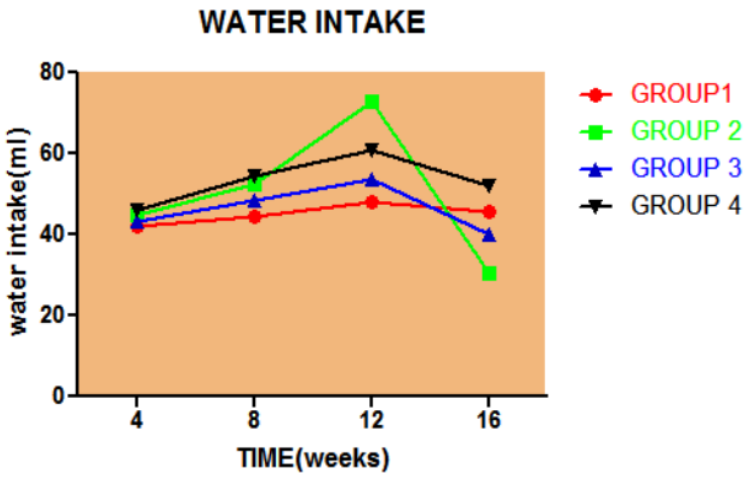

Fig.7: Comparison of water intake in experimental animals(n=5) following carcinogen and herbal extract administration in male rats.

DISCUSSION:

Change in Body Weight, food and water consumption during Experiment. The hallmarks of stress response always include decreased body weight or body weight gain, difference in food consumption pattern, reduced or enhanced activity and altered organ weights of selected organs or any other kind of alteration (Parno, 2000).The dose of *Z. officinale* and *P. nigrum* did not alter the final body weight and the weights were comparable to that of control. Although percentage differences in mean terminal body weights between the dietary control group and the group supplemented with DMBA were different. A significant protection against oral carcinogenesisis conferred by Z. *officinale* and *P. nigrum*. At study termination, oral cancer incidence in rats receiving chronic dietary exposure to *Z. officinale* and *P. nigrum* were essentially identical as compared to dietary control group. Both the extracts protected agents against tumor associated body weights loss during last week of the study. Although cancer incidence, cancer invasiveness and survival are common endpoints for efficacy evaluations of chemo preventive agents in animal model, the relationship between chemo preventive efficacy, mortality and body weight loss in DMBA rat and cancer model appears to be highly appropriate and specific model. In several studies conducted in our laboratory using this model, we have found that virtually all mortality occurring during or after the completion of carcinogen exposure is tumor related. In essentially all cases, mortality is associated with the development of one or more large exophytic neoplasms at the base of the tongue. As a result of their strategic locations, these lesions may interfere with normal feeding pattern, resulting in reduced food consumption, body weight loss and subsequent moribund kills or incurrent deaths. In the present study dietary administration of both the drugs *Z. officinale* and *P. nigrum* decreased mortality in carcinogen treated rats and prevented the body weight loss that was seen in dietary controls beginning at approximately study week 12. As such reductions in size of strategically located oral cancers may underlie the improvements in survival and body weight that was seen in groups fed with *Z. officinale* and *P. nigrum* extracts. In our study the weight of all animals in each group was kept almost same that was in range of 150-180 gm, as shown in results. The purpose of keeping the same weight was that, all the proposed parameters should not be altered on the basis of age or

weight of Wistar rats. Physiological development of rat did not influence the haematological, biochemical or molecular parameters. Rather an alteration in these indices was purely restricted to *Z. officinale* and *P. nigrum* fed to Wistar rats and not due to age or weight of wistar rats. Weight is one the most crucial clinical parameter to study any kind of effect on body. (Larson, 1962; Ambrose et al., 1972). Gingerols, Shogaolsin *Z. officinale* and piperidine in *P. nigrum* reduced the genotoxicity of DMBA overall altering all the biological endpoints. These compounds may exercise their protective effects through their anti-inflammatory, immune-modulating activity. Our findings were very well in consistency with Mans et al., 2018. These findings clearly suggest the high specificity, improved bioavailability, slow release, and low systemic toxicity of our herbal extracts. Finally, clinical studies (in-vivo) examining both the safety and efficacy of herbal extracts and their derived phytochemicals has been investigated and there is marked change in most of the clinical parameters (no reduction in weight) in treatment group. Oral administration of *Z. officinale* and *P. nigrum* (500 mg/kg body weight and 100mg/kg body weight) to DMBA-treated rats for 16 weeks inhibited the tumour incidence not in an absolute but qualified manner and restored the physical parameters near to normal levels. These findings were in orderliness with Rajendran et al., (2019). DMBA induced rats showed a significant reduction in body weight due to their cancer cachexia which is directly correlates to cancer progression in experimental subject perhaps this may be due to the damage of skeletal muscle and adipose tissue.*Z. officinale* and *P. nigrum* treatment opposes the loss of body weight by its counteractive and antioxidants property which is evident from the gross monitoring of weight from time to time during the entire schedule of 120 days. Besides, the constant intestinal absorption of *Z. officinale* and *P. nigrum* facilitates increased bioavailability, body weight gain and radical scavenging effects this could be positively coincided with Mani et al., 2018. Thus, it clearly suggested that decreased tumour multiplicity, tumour incidence, and cancer neoplasm could be attributed in the setback of cancer initiation.Besides, the constant intestinal absorption of *Z. officinale* and *P. nigrum*facilitates increased bioavailability, body weight gain and radical scavenging effects which is found to be positively coinciding with the findings of Maniet al., 2018.

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