WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 4, 1286-1298. Research Article ISSN 2277– 7105

EVALUATION OF ANTIOXIDANT ACTIVITY, TOXICITY STUDY AND STANDARDIZATION OF TCCT TABLET

Vidyadhar Vaidya*

Founder of RV New Vision Healthcare Pvt. Ltd.

Article Received on
05 Feb. 2021,

Revised on 25 Feb. 2021,
Accepted on 17 March 2021

DOI: 10.20959/wjpr20214-20113

***Corresponding Author**
Vidhyadhar Vaidya
Founder of RV New Vision Healthcare Pvt. Ltd.

ABSTRACT

Background and objective: Oxidative stress is a common factor for various diseases like cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases. The incidence of such diseases is growing day by day around the globe and causes various complications. The disease not only affects the quality of life but also the mortality rate with these diseases are too high. Considering these factors, it is important to come up with a simple yet potential solution for reducing oxidative stress. Therefore, RV New Visions Health Care Pvt. Ltd. has developed Turmeric and Cinnamon chewable tablets (TCCT) as a potential treatment for reducing oxidative stress and fighting cancer. **Materials and Methods:** Antioxidant activity of Turmeric and Cinnamon chewable tablet was evaluated In-vitro by using DPPH scavenging assay, H2O2 radical scavenging assay, and reducing power assay. Further, toxicity testing in animal models and the standardization using the HPLC and HPTLC method was done. **Results:** The formulation Turmeric and Cinnamon chewable tablet showed significant anti-oxidant action when compared with standard ascorbic acid. The percentage scavenging activity at 50 - 500 ug/ml, 14.2 - 53.55 % by DPPH scavenging assay, 22.26 – 50.81 % by hydrogen peroxide scavenging assay. The reducing power was found to be increasing with increasing absorbance in the reducing power assay. **Conclusion:** The study thus proves, Turmeric and Cinnamon chewable tablet has significant antioxidant activity

KEYWORDS: Oxidative stress; Antioxidant; Cancer; Standardization; Herbal formulation; Toxicity testing.

INTRODUCTION

Oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products.[1]

Superoxide radicals (O2•), hydrogen peroxide (H2O2), hydroxyl radicals (•OH), and singlet oxygen (1O2) are commonly defined reactive oxygen species (ROS); they are generated as metabolic by-products by biological systems.[2,3] Processes, like protein phosphorylation, activation of several transcriptional factors, apoptosis, immunity, and differentiation, are all dependent on proper ROS production and present inside cells that need to be kept at a low level.[4] When ROS production increases, they start showing harmful effects on important cellular structures like proteins, lipids, and nucleic acids.[5] A large body of evidence shows that oxidative stress can be responsible, with different degrees of importance, in the onset and/or progression of several diseases (i.e., cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases).[6]

The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several examples have revealed the fact that plant which contains antioxidant potential demonstrate the beneficial effects in inflammatory diseases.[7]

Oxidative stress is a severe cause of various diseases, especially cancer, there is a great need to develop a potential solution to reduce oxidative stress.

Considering the benefits of herbal drugs in reducing oxidative stress, RV New Vision Pvt. Ltd. has come up with a formulation named Turmeric and Cinnamon chewable tablet as a potent anti-oxidant. The Turmeric and Cinnamon chewable tablets are composed of Ginger which possesses anticancer action, Dalchini is considered to be loaded with anti-oxidant activities, Nutmeg is believed to increase immunity and improve blood circulation, liqorice is an anti-viral and anti-HIV drug, as key ingredients.

The current study aims to evaluate of antioxidant activity of Turmeric and Cinnamon chewable tablets by in-vitro, in-vivo toxicity testing, and standardization by HPLC and HPTLC.

MATERIAL AND METHODS

Materials

Turmeric and Cinnamon chewable tablets were used for the analysis purpose aiming the evaluation of antioxidant activity, toxicity testing, and standardization of the same.

HPLC-grade solvents such as methanol were obtained from Merck Ltd. Bangalore India. Standard was purchased from Sigma Aldrich and Phyto concentrate India.

Methods

1. Evaluation of antioxidant activity

a) DPPH radical scavenging activity

The ability of the formulations to scavenge DPPH radicals was determined by using the following method. 50 µl aliquot of each formulation, in 50 mm Tris-HCl buffer (pH 7.4), was mixed with 450 µl of Tris–HCl buffer and 1.0ml of 0.1mM DPPH in methanol. After 30 min incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517 nm. The percentage inhibition was calculated.

b) Hydroxyl radical scavenging activity

The degradation of Deoxyribose generated by the Fenton reaction was measured spectrophotometrically in the presence and absence of the test compound. The final reaction mixture in each test tube consisted of 0.3 ml each of Deoxyribose (30 mM), ferric chloride (1mM), EDTA (1 mM), H2O2 (20mM), in the phosphate buffer having pH 7.4 and 0.3 ml of test compound at different concentration. the test tubes were incubated for 30 min at 37oC after incubation, trichloroacetic acid (0.5 ml, 5%) and the thiobarbituric acid (0.5 ml, 1%) were added and the reaction mixture was kept in a boiling water bath at 30 min. it was then cooled and the absorbance was measured at 532 nm. The result was expressed as a % of scavenging of hydroxyl radical.

c) Reducing power activity

The reducing power of the formulations was determined. Extracts at different concentrations in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml potassium ferricyanide [K3Fe (CN) 6] (1%), and then the mixture was incubated at 500C for 30 min. Afterward, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml FeCl3 (0.1%), and the absorbance was measured

at 700 nm. Increased absorbance of the reaction mixture indicated an increase in reducing power.

2. Toxicity testing

Groups of five male and five female mice were administered Turmeric and Cinnamon chewable tablets by oral gavage daily at the dose of 1000 mg/kg bodyweight for 28 days and were sacrificed on day 28 to evaluate its toxicity. The concurrent control group receiving the vehicle was also maintained. Additionally, satellite groups of five mice per sex receiving test articles at 1000 mg/kg level were further observed for 14 days for assessment of reversibility, persistence, or delayed occurrence of toxicity.

3. Standardization by HPLC method

Preparation of Standard curcumin

5 mg of standard curcumin was accurately weighed and transferred into a 20 mL volumetric flask. 15 mL of methanol was added and then sonicated in an ultrasonic water bath for 30 minutes. The solution was cooled and volume was made up to the mark with methanol. Then filtered through a 0.45 µ syringe filter. The resulting solution was used as a standard solution.

Preparation of the test solution

300 mg of tablet powder was accurately weighed into a 100 mL volumetric flask. 70 mL of diluent was added and sonicated in an ultrasonic water bath for 30 minutes. The resulting solution was cooled and volume was made up to the mark with methanol. The content of the volumetric flask was filtered through Whatman filter paper No. 41 and then 0.45 µ syringe filter. The resulting solution was used as a test solution.

Chromatographic conditions

HPLC was performed using a Shimadzu LC20AD system with a 2996 photodiode array detector (PDA). The standard compound was resolved on a reverse-phase 250 × 4.6 mm, 5-µm, Phenomenex C18 column 5µ (4.6 X 250 mm). The mobile phase was prepared from 0.1% ortho-phosphoric acid in the water of pH 2.5 (solvent-A) and Acetonitrile (100 v/v) (Solvent-B). The mobile phase was degassed and filtered through a 0.45-µm filter before use. The gradient program used is given in Table 1.

Table 1: The gradient program used for HPLC.

S. no.	Time	Flow	%A	%B
1	0	1.00	58.0	42.0
2	25.00	1.00	48.0	52.0
3	26.00	1.00	10.0	90.0
4	30.00	1.00	10.0	90.0
5	31.00	1.00	52.0	48.0
6	35.00	1.00	42.0	58.0

The mobile phase flow rate was kept at 1 ml/min. Before the first injection, the column was saturated for 30 min with the initial mobile phase. The column temperature was maintained at 25°C. Injection volume was decided to maintain at 10 µl. The PDA was set by optimizing the wavelength at 425 nm to acquire the chromatogram. The standard compound was identified by comparing the retention time and spectra obtained from the sample and standard solutions. The present work was performed in an air-conditioned room maintained at 25°C.

Preparation of calibration graph

Seven different concentrations were prepared by diluting the standard stock solution. The calibration graph of each standard was constructed by plotting concentrations against peak area for the respective standards.

4. Standardization by HPTLC method

a) Preparation of mobile phase

9 mL n-hexane, 3 mL Ethyl acetate, and 0.3mL Glacial acetic acid was measured individually using a graduated measuring cylinder and transferred into a 50 mL stoppered conical flask. The mobile phase was properly mixed, sonicated, and transferred into a 10x10 cm TLC development chamber and allow equilibrating for 15 minutes.

b) Preparation of test solution

About 200 mg tablet powder was weighed and transferred into a 20 mL volumetric flask; about 15 mL of methanol was added. It was sonicated for 15 minutes in cold water. It was diluted up to the mark with diluent. The solution was filtered through Whatman filter paper no. 41. The resulting solution was used as a test solution**.**

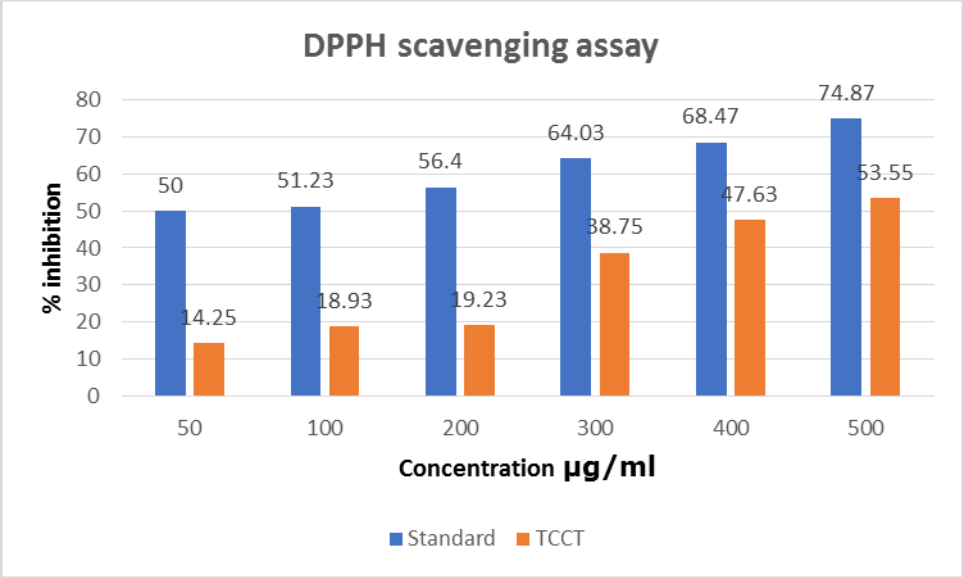
RESULTS

1) Evaluation of Anti-oxidant activity

a) DPPH radical scavenging activity

All the concentrations were capable of scavenging DPPH radicals at pH 7.4 in a

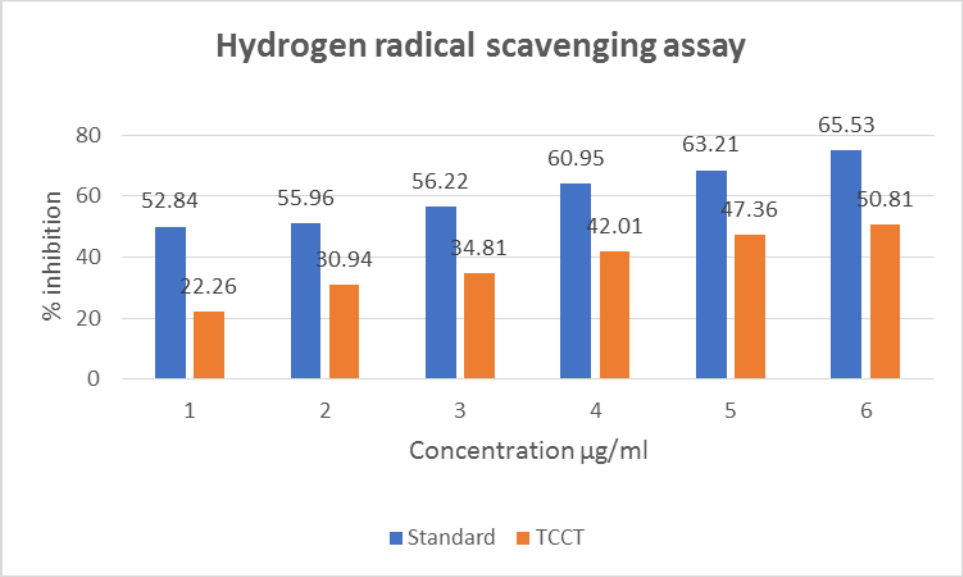
dose-dependent fashion. All the concentrations of the test compound were capable of scavenging the free radicals and had shown anti-oxidant activity. Graph 1 depicts the percentage inhibition of Turmeric and Cinnamon chewable tablet at different concentrations of test compounds and also the comparison with different concentrations of Standard i.e., ascorbic acid. The % inhibition of test formulation at concentrations of 50 ug/ml, 100 ug/ml, 200 ug/ml, 300 ug/ml, 400 ug/ml, 500 ug/ml were found to be 14.2%, 18.93 %, 19.23 %, 38.75 %, 47.63 %, 53.55 % respectively. When compared statistically between the groups, the P-value was found to be 0.0010 and the test results were found to be significant.



Graph 1: The graphs show % inhibition of Turmeric and Cinnamon chewable tablet as compared to standard at different concentrations of test compound and standard.

b) Hydrogen radical scavenging activity

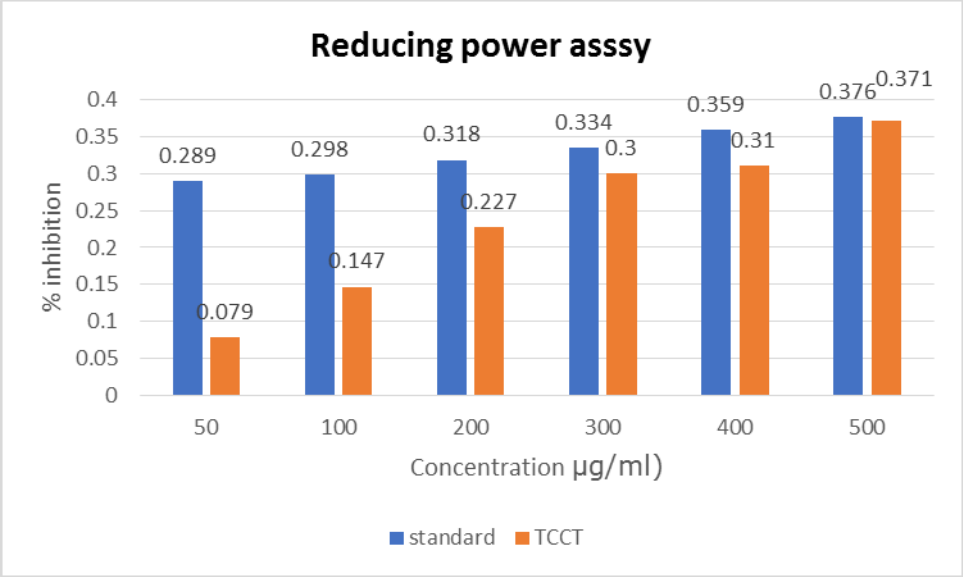
Hydroxyl radicals are very reactive, can be generated in biological cells to the Fenton reaction. The test compounds exhibited concentration dependent scavenging activity against hydroxyl radical generated in the Fenton reaction system. Turmeric and Cinnamon chewable tablets demonstrated scavenging of hydroxyl radicals ranging from 22.26 % to 50.81 %. The P-value was calculated and the results were found to be significant. The results are depicted in Graph 2.



Graph 2: It indicates the % inhibition of Turmeric and Cinnamon chewable tablet in comparison with Standard.

c) Reducing power assay

For the measurements of the reductive ability of test compounds, the Fe^{3+} – Fe^{2+} transformation was investigated in the presence of test compounds. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of each antioxidant sample. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. The reducing power of test compounds is enhanced by increasing the concentration of samples. The absorbance of the samples increased together with the reducing power. The reducing power of test compounds was found to be concentration-dependent. The results are depicted in Graph 3.



Graph 3: The above graph depicts the absorbance of Turmeric and Cinnamon chewable tablets in comparison with the standard.

2) Toxicity testing

The results for toxicity testing were found to be prominently significant. At the dose of 1000mg/kg, there were no toxicity signs for Turmeric and Cinnamon chewable tablets.

a) Bodyweight

During the study period of 28 days, the changes in body weight were observed and were wound to be with a weight gain of 14.37 and 12.92% in females and males respectively.

b) Clinical Signs and Mortality

Clinical signs and mortality were assessed for 28 days and no abnormal clinical signs and mortality were observed in both male and female rats.

c) Individual animal organ weights

After 28days the animals were sacrificed and organs were dissected and weighed. The individual animal organs' weight was noted and is depicted in table no 2.

Table no. 2: Individual animal organ weights (absolute values) (g).

Animal ID	Adrenals	Ovary	Brain	Kidney	Liver	Heart	Spleen	Epididymis	Thymus	Uterus
AVG Wt.	0.004	0.073	0.493	0.379	1.434	0.209	0.088	NA	0.073	0.149
SD	0.000	0.015	0.049	0.043	0.064	0.032	0.014	NA	0.014	0.029
Animal ID	Adrenals	Testes	Brain	Kidney	Liver	Heart	Spleen	Epididymis	Thymus	Uterus
AVG Wt.	0.004	0.018	0.509	0.417	1.396	0.229	0.103	0.059	0.059	NA
SD	0.001	0.002	0.056	0.060	0.184	0.048	0.017	0.019	0.019	NA

d) Average hematological parameters:

The hematological parameters like total leukocyte count, RBC count, Hemoglobin, Hematocrit, MCV, MCH, MCHC, platelet count, etc. were done for all the groups were checked and results are depicted in table no 3.

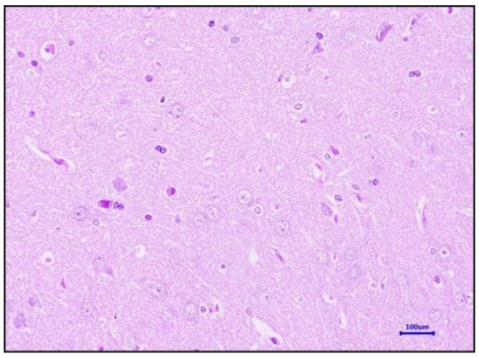
Table no. 3: Average hematological parameters.

Test	Total Leukocyte Count (X 10^3/µL)	RBC Count (X 10^6/µL)	Hemoglobin (Hb) (g/dL)	Hematocrit (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Platelet Count (X 10^3/µL)
AVG	1.42	6.09	11.34	33.44	55.80	18.88	34.92	465.30
SD	0.43	0.65	0.66	4.53	11.42	2.78	7.79	178.91

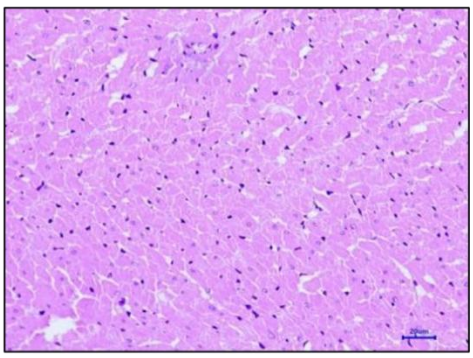
e) Histopathology

Histopathology of one animal in each group was performed at the end of the studies and the results were found as depicted in the following figures.

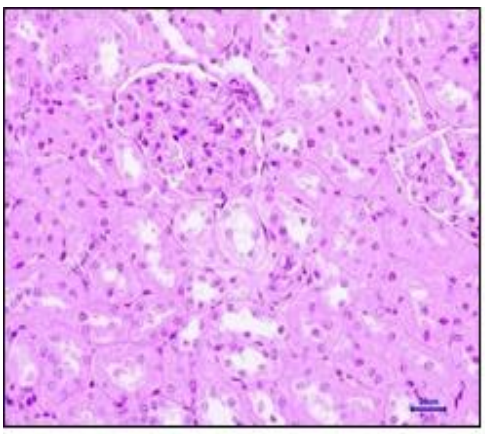
All the figure depicts the histopathology of specific organs in males and females.

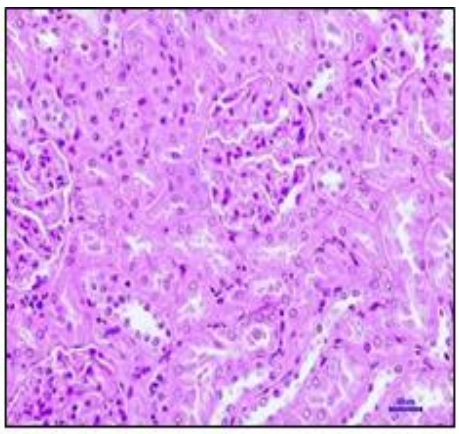


Brain in Male

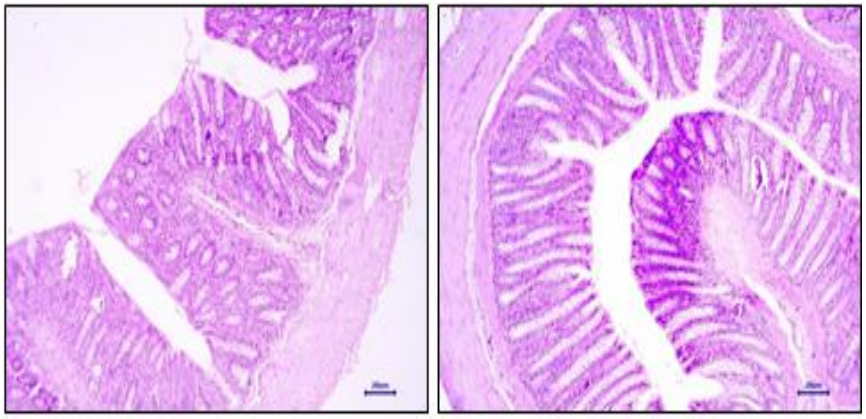


Heart in Male





Kidney in Male and Female

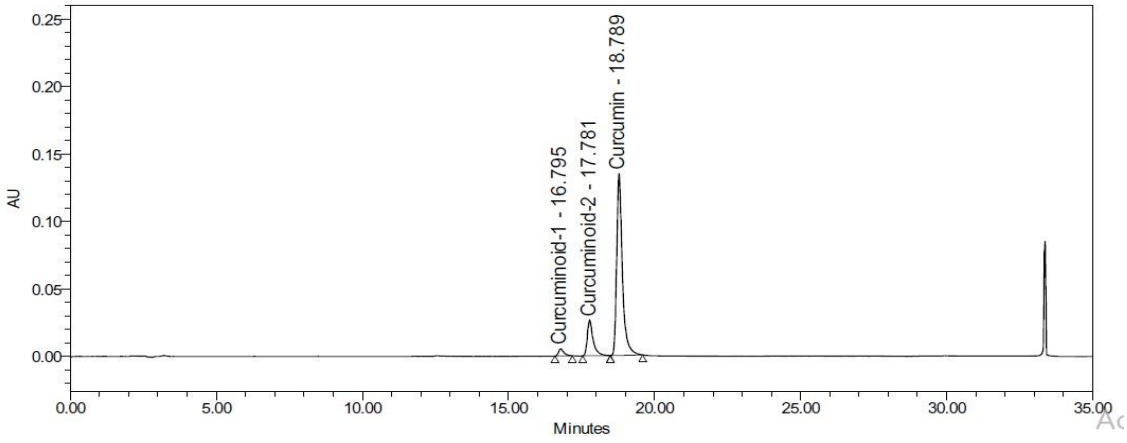


Large Intestine in Male and Female

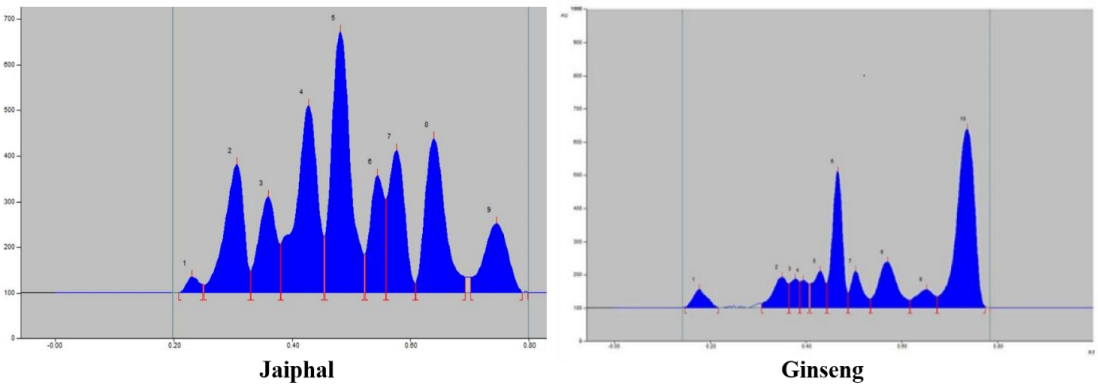
3) Standardization

a) By HPLC

The following graph represents the Standardization chromatogram by using the HPLC method.



b) by HPTLC



Jaiphal Ginseng

Figure 1: The above images represent the chromatogram of Nutmeg and ginseng extract by performing the HPTLC method.

DISCUSSION AND CONCLUSION

Oxidative stress is the result of an imbalance in the body between the oxidizing system, consisting mainly of free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS), and antioxidant systems. Oxidative stress is likely to be involved in the age-related development of cancer. The reactive species produced in oxidative stress can cause direct damage to the DNA and are therefore mutagenic, and it may also suppress apoptosis and promote proliferation, invasiveness, and metastasis.[8] Reactive oxygen species are a common type of free radical and cause oxidative stress. The imbalance of ROS decreases the antioxidant activity of molecules. The ROS has a beneficial and deleterious role.[9] At low concentrations of ROS, it produces beneficial effects and when there is overproduction it can damage or inhibit the normal function of lipids proteins and DNA. Due to the intracellular reduction of O2 to ROS, it becomes toxic to cells and tissue.[10] The ROS is produced from endogenous and exogenous substances. The endogenous production of ROS is from mitochondria, Cytochrome P450, peroxisomes, inflammatory cell activation. The mitochondria generate hydrogen peroxide and use 90% of the cellular oxygen.[11] During the process of reducing oxygen for the production of water, superoxide (O2), hydrogen peroxide (h2O2), and hydrogen radical (OH) are produced.[12] All these free radicals are toxic to the cells and destroy cells. The cell's destruction in turn causes free radical generation. The other endogenous sources of free radicals are neutrophils, eosinophils, macrophages.[13] The activated macrophages increase the oxygen uptake and give rise to a variety of Reactive oxygen species. The liver macrophages participate in free radical-induced hepatoxicity and cancers.[14]

Considering the impact of oxidative stress and its correlation with cancer, there is a great need to develop a potential solution to the same. To the needful required to tackle the increasing prevalence and considering its impact on the life of people RV new vision has developed Turmeric and Cinnamon chewable tablet for reducing oxidative stress. The current study aims to evaluate the antioxidant activity of Turmeric and Cinnamon chewable tablets by in-vitro antioxidant activity. Also, it aims at the toxicity testing of Turmeric and Cinnamon chewable tablets in animal models and further the standardization of the same by using HPLC. The possible mechanism through which the formulation acts may be represented as Curcumin induces Glutathione-S-transferase and Inhibit the free radical generation and act as a free radical scavenger and antioxidant, thus inhibiting lipid peroxidation.[15] Curcumin acts by various mechanisms and the exact mechanism is not

known. Curcumin primarily exerts its therapeutic effects by inhibiting the degradation of IκBα and subsequent inactivation of NF-κB, thus initiating a cascade of downstream inflammatory and immunogenic events. Curcumin's inhibition of NF-κB activation, in turn, leads directly to the inhibition of expression of several proinflammatory cytokines (e.g., TNF, IL-1, IL-2, IL-6, IL-8, and IL-12) and downregulation of the mRNA expression of several pro-inflammatory enzymes (e.g., COX, LOX, MMPs, and NOS). Also, curcumin's immunogenic response is further enhanced by its ability to inhibit TLRs.[16]

ACKNOWLEDGEMENTS

The authors would like to acknowledge all the team of researchers which was involved in performing the tests and all the back office team that contributed to the successful completion of the research work.

REFERENCES

1. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative Stress: Harms and Benefits for Human Health. Oxidative Medicine and Cellular Longevity, 2017, 8416763.
2. Sato H, Shibata M, Shimizu T, Shibata S, Toriumi H, Ebine T, Kuroi T, Iwashita T, Funakubo M, Kayama Y, Akazawa C. Differential cellular localization of antioxidant enzymes in the trigeminal ganglion. Neuroscience, 2013; 17, 248: 345-58.
3. Navarro-Yepes J, Zavala-Flores L, Anandhan A, Wang F, Skotak M, Chandra N, Li M, Pappa A, Martinez-Fong D, Del Razo LM, Quintanilla-Vega B. Antioxidant gene therapy against neuronal cell death. Pharmacology & Therapeutics, 2014; 1, 142(2): 206-30.
4. Rajendran P, Nandakumar N, Rengarajan T, Palaniswami R, Gnanadhas EN, Lakshminarasaiah U, Gopas J, Nishigaki I. Antioxidants and human diseases. Clinica chimica acta, 2014; 25; 436: 332-47.
5. Wu JQ, Kosten TR, Zhang XY. Free radicals, antioxidant defense systems, and schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 2013; 1; 46: 200-6.
6. Taniyama Y, Griendling KK. Reactive oxygen species in the vasculature: molecular and cellular mechanisms. Hypertension, 2003; 1, 42(6): 1075-81.
7. Bhaumik S, Chattopadhyay S, Ghosal S. Effect of shilajit on mouse peritoneal macrophages. Phytotherapy Research, 1993; 7(6): 425-7.

8. Handa O, Naito Y, Yoshikawa T. Redox biology and gastric carcinogenesis: the role of Helicobacter pylori. Redox Report, 2011; 1, 16(1): 1-7.
9. Valko M, Rhodes C, Moncol J, Izakovic MM, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-biological interactions, 2006; 10; 160(1): 1-40.
10. Wickens AP. Ageing and the free radical theory. Respiration physiology, 2001; 15, 128(3): 379-91.
11. Inoue M, Sato EF, Nishikawa M, Park AM, Kira Y, Imada I, Utsumi K. Mitochondrial generation of reactive oxygen species and its role in aerobic life. Current medicinal chemistry, 2003; 1, 10(23): 2495-505.
12. Conner EM, Grisham MB. Inflammation, free radicals, and antioxidants. Nutrition, 1996; 1; 12(4): 274-7.
13. Roberts RA, Ganey PE, Ju C, Kamendulis LM, Rusyn I, Klaunig JE. Role of the Kupffer cell in mediating hepatic toxicity and carcinogenesis. Toxicological Sciences., 2007; 1, 96(1): 2-15.
14. Mateos R, Bravo L. Chromatographic and electrophoretic methods for the analysis of biomarkers of oxidative damage to macromolecules (DNA, lipids, and proteins). Journal of separation science, 2007; 30(2): 175-91.
15. Catanzaro M, Corsini E, Rosini M, Racchi M, Lanni C. Immunomodulators inspired by nature: a review on curcumin and Echinacea. Molecules, 2018; 23(11): 2778.
16. Jagetia GC, Aggarwal BB. “Spicing up” of the immune system by curcumin. Journal of clinical immunology, 2007; 27(1): 19-35.