Effect of Piperine Supplementation on Monensin Induced Stress using Invitro Culture Method

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***Abstract*— Piperine which may act as antioxidant to prevent the cells against the formation of free radicals which develops in the cells when they are treated with polyether Ionophore Monensin i*nvitro* culture method. Monensin also increases the intracellular calcium ion concentration in the cell membrane and function just like a hydrogen peroxide which also possess this property. Yeast extract peptone dextrose media were Effect of Monensin and Piperine were studied by MTT assay, Lipid peroxidation, Catalase and Glutathione activity.**

Index Terms—piperine, vitro culture

I. Introduction

Piperine is one of most and pungent alkaloid present in a popular spice namely black pepper (*Piper nigrum*) and some herbs of leaf moss. It has a long range of biological effects. It is most used in medicinal purposes to treat certain diseases, including irregular heartbeats, Angina and neurasthenia. Piperine has also anti-inflammatory, antiarthritic, antidepression like activity cognitive enhancing effect, and also a blood pressure lowering effect (Wattanathorn,et al., 2008). Piperine supplementation decreases the effect of Monensina Carboxyl Ionophore. Monensin is one of a carboxyl polyether ionophore which increases Mitochondrial (ROS) Reactive oxygen species production by increases cell cycle arrest apoptosis through regulation of cell cycle and apoptosis. In case of mammalian fibroblasts cells monensin induced toxicity was noticed to develop an ionic improper balance that highly affected the shape size and function of mitochondria. Disruption or damaging of mitochondrial functions leads to generation of reactive oxygen species.

II. Objectives

1) To analyze the effect of Piperine and Monensin supplementation on Yeast cell viability.
2) To analyse the effect of supplementation of Piperine on Monensin induced stress as assessed by lipid peroxidation and antioxidants.

III. Review of Literature

Piperine is one of the most spice used for various for treatment of diseases. In India and China it is used as a traditional medicine belonging to family Piperaceae called as king of spices in India. Piperine [$C_{17}H_{19}NO_3$].

1) Piperine a bio enhancer: Piperine has shown different numerous biological phenomena including anti- inflammatory activity, enhance fertility, antioxidant activity, antidepressant activity, antiplatelet activity.

2) Antimicrobial activity of piperine: Piperine has shown the antimicrobial activity prevents body against the attack of microbes Khan and Siddiqui 2007 reported that aqueous decoction of *Pipernigrum* have very high antibacterial activity. Silver particles from this show good antibacterial activity against the pathogens that destroys crops .They also concluded that silver nanoparticles isolate from this plant play important role in crop improvement and protection.

3) *Antidepressant activity of piperine:* Piperine has also shown antidepressant activity in the mice to reduce the stress in the mice. Antidepressants are the drugs used to control depression helps the body to control stress Song et al., 2007 reported the antidepressant effect of piperine in mice suffering from chronic mild stress condition.

4) Anti-inflammatory activity of piperine: Inflammation is a type of biological response which show by body after the attack of pathogens which is characterised by redness, swelling and pain in the localised area. Sarvesh et al., 2007 reported that piperine prevents the attachment of neutrophils with endothelial monolayer. Endothelial monolayer cells blocks the tumour necrosis factor –α, which induces the expression of cell adhesion molecules. They also observed that pre-treatment of endothelial cell with piperine decline the phosphorylation and slow down the IkB-α through by reducing the function of tumour necrosis factor-α induced IkB Kinase activity.

5) Antiplatelet effect of piperine: Platelets are the cells found in circulatory system of animals helps in blood clotting mechanism. Park et al., 2007 separated four amides or piperine, pipernolanine, piperictadecalide and piperlongunine from the black pepper.

6) Anti thyroid activity of piperine: Thyroid is a endocrine gland present in the neck region helps to control basal metabolic rate, tissue regeneration growth and development excess or low concentration of thyroid hormones leads to malfunctioning of the gland. Kumar et al., 2006 analyzed the effect of piperine with carbimazole.

7) Anti-hypertensive effect of piperine: Hypertension is increase in the blood flow inside the blood vessels which causes the death of an organism. It is a silent killer disease. Intravenous supplementation of Piperine 1-10 mg/kg reduce in

accurate arterial pressure in anesthetized rats, another dose was given 30g /kg did not show in any changes in arterial pressure.

8) Anti-asthmatic effect of piperine: Asthma is a type of respiratory disorder in which there is increase in the goblet cells resulting into secretion of more mucous which cause difficulty in breathing.

9) Anti apoptosis effect of piperine: Choi et al., in 2013 reported that apoptosis is a morphological process to control cellular self-destruction of cells. It is important process maintain the population of cells. Piperine at the concentrations of (10-100mM) prevents the organ of corti -1 cells of cochlea a hearing organ in ear from cisplantin inducing apoptosis by the increase the enzyme activity of the enzyme oxygenase -1 the main pathway responsible for this is C-JUN terminal kinase.

10) Anti-diarrheal activity of piperine: Diarrhoea is gastrointestinal disorder in which there increase in bowel moments losing the water balance in the body. Sham Kumar et al., 2012 demonstrated that liquid piperine extract at a dose of 75, 150, 300mg/kg showed anti –diarrheal, antimotility and antisecretary property in mice.

11) Digestive activity of piperine: Digestive system play important in absorption and assimilation of food particles by killing the microorganisms at the time of digestion. Srinivasan et al., 2007 demonstrated that dietary piperine increases the digestion physiology by stimulating the activity of pancreatic enzymes and slow down the food transit time in the alimentary canal.

12) Piperin effects on metabolism: A bioavailabilityenhancer: Piperine supplementation helps in the absorption of various drugs and nutrient substances from the alimentary canal by different process changes the membrane architecture and increases the penetration level of absorption.

13) *Anticancer activity of piperine:* Cancer is uncontrolled or abnormal growth of cells which develops into mass like structure called tumour. Samykutty et al., 2013 evaluated the anticancer activity of piperine in prostrate cancer cells lines. Piperine induces apoptosis mechanism of cell by the activation of caspase 3-enzyme a family of enzymes which helps in apoptosis mechanism in the cell. These enzymes causes the breakdown of PARP-1 proteins in many prostrate cancer cells like PC-3,DU 145,And LNCAP, Prostrate cancer cells.

14) Piperine as an antioxidant: Antioxidant molecules prevents the oxidation in cells which further leads to development of free radicles. In a previous study, Kumar et al., in 2004 studied the effect of that piperine supplementation in rat feed with diet rich in fats.

15) Oxidative stress: During the process of oxidative stress there is release of free radicals which damages the biomolecules of the cells lipids, proteins and DNA. Pathogenesis of various diseases in the body including hypertension, diabetes mellitus, and atherosclerosis etc.

16) Types of reactive species: There are two types of reactive species existing in nature ROS Reactive oxygen species and RNS Reactive Nitrogen species. One of ROS namely superoxide is produced mostly by electron reduction of oxygen by different oxidases.

17) Sources of ROS generation in yeast cells: Environment is one of main factor which generates the reactive oxygen species production and other reactions of aerobic process. It also play important role in generation of ROS production in yeast.

18) Yeast culture and its importance: Yeast are unicellular microorganisms their cellular organisation is same as higher organisms having well developed nucleus which contain the genetic material found inside it this property classifies the yeast into eukaryotic single cell organism.

19) Yeast model organism: Yeast (*Sacromycescerevassie*) a single cell organism grows very fast. It can be quickly replicated and easy to manipulate genetically. Yeast cells duplicate and divides its own cell is almost 90 min as compared to human's cells which needs 24 hours for growing and divides itself.

20) Monensin anionphore: Monensin is a vetenary drug used in veterinary science for different purposes. Monensin produced by *Streptomyces cinnamonensin* is mostly widely used ionophorein vetenary medicine according to food and drug administration mostly used for coccides diseases in poultry birds.

21) *Antibacterial activity of monensin:* Monensin due to its ionophoric nature shows very large amount of antimicrobial activity fluctuates the pH balance in the cells by altering the sodium potassium balance within the cells create very treat alterations in the cellular structures which ultimately leads to death of an organism (Russell et.al.,1987).

IV. Materials and Methods

The Yeast cells from glycerol stock were first revived on Yeast extract peptone dextrose media under specific growth conditions at temperature 30°C for 48 h.

1) Cadmium chloride and piperine: Cadmium chloride stock and piperine stock solution was prepared using ethanol.

2) Yeast culture and growth condition: For antioxidant and MTT assay, cells were grown in Yeast extract peptone dextrose media containing 1 % yeast extract, 2 % peptone and 2 % glucose on an orbital shaker at 30 °C and 160 rpm.

3) Agar culture: To check the purity of yeast cells, these cells were grown in yeast peptone dextrose adenine media containing 1 % yeast extract, 2 % peptone, 2 % glucose and 1.5 % agar in a bacteriological incubator at 30°C.

4) MTT Assay: The effect of cadmium and piperine on cell viability, was performed by MTT assay at 600nm. These cells were washed once with distilled water and pH was adjusted to

7.4. Cells with 20 µl were inoculated in test tube containing 3 ml Yeast extract peptone dextrose media supplemented with piperine and cadmium chloride. Monensin (10µm), Piperine (120µm), Monensin (10µm) and piperine (120µm). Cells of 1 ml suspension from different group were harvested through centrifugation at 8000 rpm for 10 min. Cells were suspended in PBS. 120µl of cell suspension and 80µl of MTT was added and incubated for 2-3 hours in orbital shaker. Afterwards cells were harvested and suspended in 100µl of DMSO followed by incubation for 10min. Finally, the cells were against centrifuge at 8000 rpm for 10 min. Supernatant was collected at 540 nm.

Fig. 4. Representative of Yeast culture media

Fig. 5. Yeast growth in YPDA media

5) Estimation of protein: Biuret reaction of protein with alkaline cupric tartrate also enhanced the colour development and were measured at 660 nm. Stock protein standard (BSA) (1mg/ml) was prepared by dissolving 10 mg Bovine serum albumin in 10 ml of water. Working standard (100 µg/ml was prepared by dissolving 1 ml of stock in 9 ml of water. Different concentration of working BSA standard was prepared by as per following Table-1.

TABLE-I
DIFFERENT CONCENTRATION OF BSA WORKING STANDARD SAMPLES

S.No.	Volume of BSA working standard	Distilled water	Concentration (µg/ml)	OD660nm
1.	0.2	0.8	20 µg/ml	0.068
2.	0.4	0.6	40 µg/ml	0.137
3.	0.6	0.4	60 µg/ml	0.190
4.	0.8	0.2	80 µg/ml	0.251
5.	1	0.0	100 µg/ml	0.351

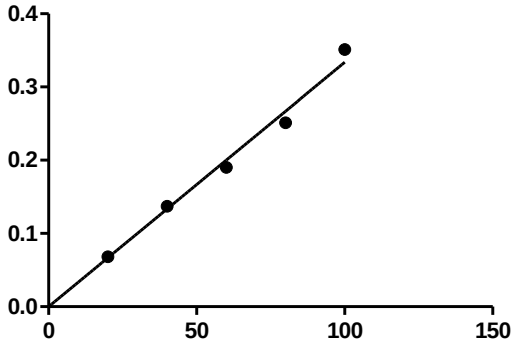

Fig. 6. Standard graph for protein estimation

5 ml of lowery reagent was added to 1 ml of each standard and mixed thoroughly. Tubes were kept for 5 min in room temperature and there after 0.5 ml of 1 N folin reagent was added into each tube. Tubes are incubated for 30 min and absorbance was taken at 660 nm.

6) Preparation of cells homogenate: Cell suspension of 10 ml was centrifuged at 8000 rpm for 10 min and cell pellet was lysed in cell homogenate buffer through centrifuging at 3000 rpm for 10 min. Supernatant was collected for further use

7) Preparation of post nuclear supernatant (PNS): The cells supernatant collected in first step was centrifuged at 2500 g for 10 min at 4°C in cold centrifuge to get the nuclear pellet and supernatant. The supernatant was removed and stored at -20 °C for future use. Lipid peroxidation and catalase activity was analysed.

8) Preparation of post mitochindrial supernatant (PMS): Supernatant obtained was again centrifuge for 10 min at 10,000 rpm at 4 °C to get mitochondrial pellet and post mitochondrial supernatant, was stored at -20°C for future use.

9) Catalase assay: This method determines the dichromate was dissolved in acetic acid reduced to chromic acetate after reacting with H_2O_2 in hot condition. This reaction was allowed to proceed at time interval to split the H_2O_2. Reaction was stopped at different time interval 0, 30, 45 and 90 second by addition of dichromate / acetic acid mixture. Which was measured at 610nm.

10) Lipid peroxidation: To analyse the effect of cadmium and piperine, 2 ml of TBA-HCl and 1 ml of supernatant was added and placed in water bath for 15 min. After cooling, precipitate was removed and centrifuged at 1000rpm for 10 min. The absorbance was measured at 535nm.

11) Estimation of total reduced glutathione: PMS of 2 ml, 2ml of sodium phosphate dibasic and 1ml of DTNB was added from each group. Absorbance was measured at 412nm.

12) Statistical analysis: The data were analysed using Graph Pad prism. The differences between means were analyzed by one-way ANOVA followed by tuky test. Differences were considered to be significant at $P < 0.05$.

V. RESULT

1) Effect of monensin and piperine supplementation on cell viability: Reduction of MTT to insoluble purple formazon and widely used to determine the cell viability. Results show that monensin significantly (P<0.05) decreased the cell viability as compared to control and piperine. Piperine also show negative effect on cell viability.

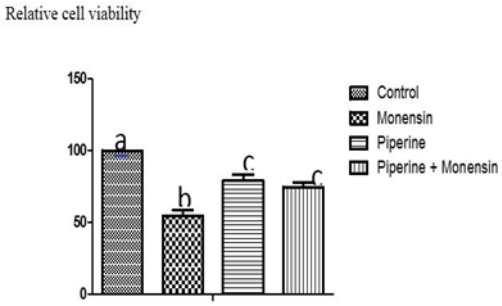

Fig. 7. Relative cell viability after the treatment of the yeast cells with monensin, piperine and monensin and piperine. Value are Mean ± SEM. Value with different superscript differ significantly.

Fig. 8. Formazon crystal used for measuring the absorbance.

2) Effect of monensin and piperine supplementation on lipid peroxidation and antioxidant:
In the present study, effect of monensin and piperine supplementation on yeast cells oxidative stress was analysed through measuring lipid peroxidation and measuring total reduced glutathione and catalase activity.

3) Effect of monensin and piperine supplementation on lipid peroxidation: Effect of monensin, piperine, piperine along with monensin on lipid peroxidation was analysed and presented in the following figure, shows the trends of lipid peroxidation in different group. There was a significant decrease (P<0.05) in lipid peroxidation in all the groups in relation to control**.**

4) Effect of monensin and piperine supplementation on catalse activity: Effect of monensin, piperine, piperine along with cadmium on catalase activity was analysed and presented in the following figure, shows the trends of catalase activity in different group. A Significant (P<0.05) decrease in catalase was observed in relation to control in all the treatment group. Although in piperine catalase activity are significantly (P<0.05) higher than monensin and in both piperine andmonensin.

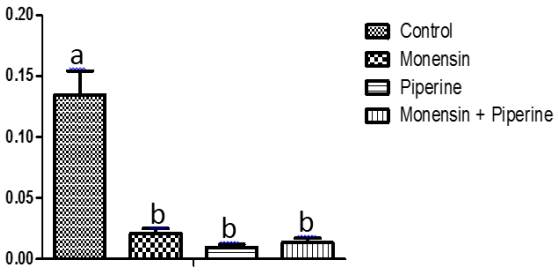

Fig. 9. Lipid peroxidation level after the treatment of the yeast cells with monesin, piperine and monensin + piperine. Value are Mean ± SEM. Value with different superscript differ significantly.

Fig. 10. Lipid peroxidation assay

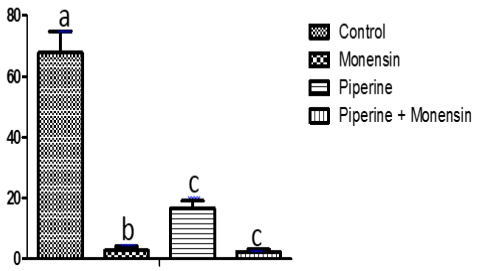

Fig. 11. Catalase activity after the treatment of the yeast cells with monensin, piperine and monensin + piperine. Value are Mean ± SEM. Value with different superscript differ significantly.

Fig. 12. Catalase assay

5) Effect of monesin and piperine supplementation on reduced glutathione level: Effect of monensin, piperine, piperine along with monensin on total reduced glutathione level was analysed and presented in the following figure, shows the trends of reduced glutathione in different group. A significant ($P<0.05$) decrease in total reduced glutathione was observed in relation to control in all the treatment groups. The amount of glutathione is expressed as µm / mg protein.

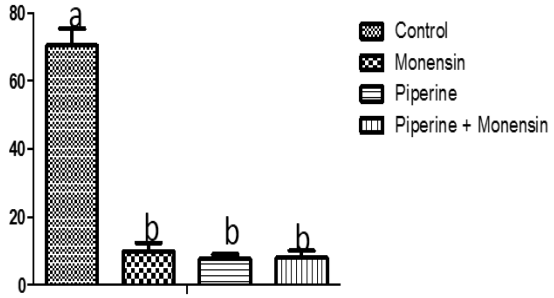

Fig. 13. Total reduced glutathione level after the treatment of the yeast cells with monensin, piperine and monensin + piperine. Value are Mean ± SEM. Value with different superscript differ significantly.

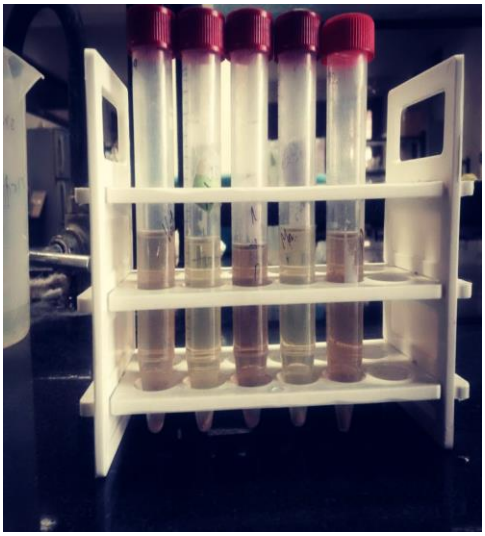

Fig. 14. Catalase assay

VI. Conclusion

Piperine has shown different numerous biological phenomena including anti-inflammatory activity, enhance fertility, antioxidant activity, antitumor, antidepressant, antiplatelet activity.The present results demonstrated that monensin and piperine both severely affected the cell growth and induces oxidati ve stress in yeast cells after 24 h of treatment as indicated by cell viability test and different antioxidant assay. During the oxidative damage, ROS and side chain of polyunsaturated fatty acid react to produce the lipid peroxidation. Thus MDA level in cells imitate the degree of lipid peroxidation and reactive oxidative damage of cells.

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