Nicotine: A Critical Review on its role in Stress

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Abstract - Nicotine, an alkaloid which is extracted mostly from tobaccoleaves. Nicotine is having a great character in neurodegenerative kind of disorders. Some of researchers suggested that short-term nicotine usage can help in improve memory by decreasing oxidative stress from the brain. So this review aimed whether nicotine is having an effect on initiating ROS or not and its antioxidant characters in human body. The different cells are treated with nicotine, and then the oxygen levels will be measured which will help us to decide the effects of nicotine on stress. As our research says by blocking Fenton's or Iron reaction in our body, it is possible for nicotine to break down the mechanism of initiation of free radicals in our body. Also through review we experienced that a depletion in the levels of active oxygen may be occurring for blocking of Iron reaction (Fenton's reaction) chelating with metal. The construction of metal chelating power of nicotine were experimented, observed and the observation said nicotine can bind with metals that it can do metal chelation. The results also saying that nicotine helped in a decline in the level of GSH ieGlutathione in the presence of tertiary butylhydro peroxide ; a study also has been done on the experiments which were conducted to make us confirm the job of nicotine in glutathione level, lungs cell or alveoli cell. These findings tell us nicotine in both electronic cigarettes and conventional may prevent the symptoms of other neurodegenerative disorders which is caused by reducing oxidative stress. But this common smoke actually contains more harmful substances that can cause cancer. These are not exactly the case or issue if goes with electronic cigarettes, which contains nicotine ; the important discussion is that it does not have any toxic contents .

Keywords: Nicotine, Oxidative stress, Reactive Oxygen Species, Effect on memory, antioxidants, E-cigarettes.

I.INTRODUCTION

Medical specialty of nicotinesays nicotine is an alkaloid, which is easily be entered to thelungs and easily cross through the cell line Blood Brain Barrier ie it will immidiately try to enter the brain and chemically binds with the receptors of nicotine ienAChRs [Whiteaker P, 2004, Science, 306][GardierA. M, 2005] . Encouragement of these nicotine binded with receptors tries to release different neurotransmitter ,which may include dopamine. But let us see when it enters into lungs. If nicotine absorbs and enters to thebiologicalmembrane then it totalydepends upon pH value that has been experimented. Generally,nicotineis being very poor basehaving pKa value 8.0[Hanne MorcK Nielsen, 2002].If the medium is acidic this always becomes to being ionizedandit cannot quickly penetrate the membranes of our body.Smoke from cooked tobacco contains acid therefore it cannot penetrate the buccal membrane [Hanne MorcK Nielsen, 2002] . But Smoke from E-cigarettes [USAToday, July2013] or air treated cigarettes contains an excess of alkaline whose pH value isapprox 6.5 having a great amount of the nonionized nicotines which tries penetrate the biological membrane[Hanne MorcK Nielsen, 2002].Reserchers suggest that pH of cigarette smokes is alkaline; the most of nicotines are ionised , thus facilitating rapid lung absorption. First it tries to enter the alveoli cell and airways of lungs then it suddenly enters into the blood and then our brain[Gori G. B, Benowitz N. L, 1986].

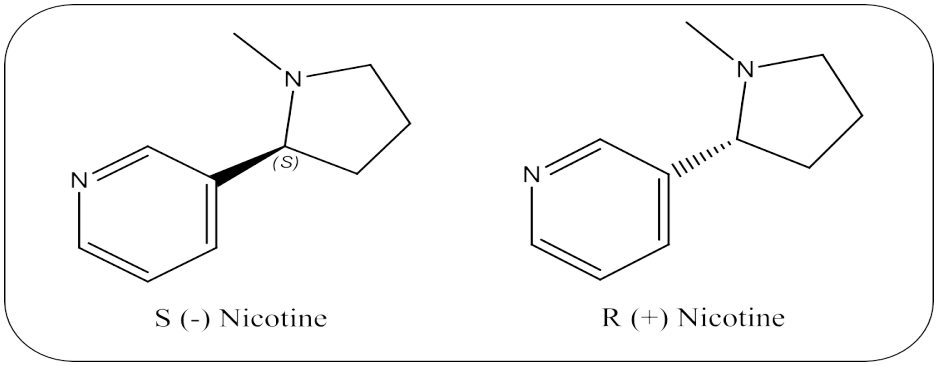

Figure-A (Structure of nicotine with its Enantiomers)

Figure 1. Nicotine

We have already discussed Nicotine is extracted from tobaco leaves. Its chemical properties says, it is a 3^0 amine. This is consist of pyridine ring and pyrrolidine ring in its structure. This is a chiral compound [Armstrong, 1998]. 3-(1-methyl-2-pyrrolidinyl)pyridine is the IUPAC name of nicotine. This exists as 2enantiomers, available in the tobaccos as dextrorotatory(R), levorotatory(S)withS or levorotatoryis the major product ie greater than 99% [Armstrong, 1998]. Its physical properties say that it is a light yellow colour and an oily liquid. Its molecular weight is 162.23. Nicotine is very much investigated in those days because of its presence in tobaco products which is harmful for our body. Actually this alkaloid can be used as a medicine for quiting smoking.

There should be always a balance between oxidants and the antioxidants which is an obvious challenge for any system (living system) and irregular rising in balance can lead to the initiation of (ROS) reactive oxygen species within human body which creates stress in the body which is called oxidative stress due to oxygen containing species [Bagchi D, 1995] . Influences of ROS are constructed from many internal and external sources both and also It has been believed that this will be the cause of so many problems in our body which does not have permanent solution. [Stohs S. J, 1995]

ROS are chemically reactive molecules containing oxygen for example (Peroxide,superoxide, hydroxyl radical) The primary ROS in human body is Superoxide anion [Fridovich I, 1986] and is experimented being produced in the whole mechanism of a metabolic process or else through some of activities of oxygen by the burning radiation. Then a second ROS is produced by the reaction of the primary ROS with other molecules by metal catalyzed process. [Arch. Biochem, 1986]

Antioxidant is any substance that acts to prevent the oxidants from oxidation of another chemical. Antioxidant is having very important role in protecting the human cellular system from Reactive Oxygen Species [Valco M, 2006]. Antioxidant can slow down or remove oxidative destruction from a target molecule by blocking ROS series in our body [Moncol J, 2006].

II.EXPERIMENT

2.1Culture of cell lines

2.1.1 Culture of BBBcells -

The cells were raised on EBM media. The whole media was prepared by mixing adequate quantity of penicillinstreptomycins(5 ml) with FBS(5 ml), IGF(125µl), EGF (125µl) and hydrocortisone(50 µl), hepes buffer (5 ml of 1 Molar). The Cells are enlarged in the same way as other cell lines will enlarged cultured. Next culturing of CHO and HepG2 will be followed.

2.1.2 Culturing CHO and HepG2 -

HepG2 ,CHO and cells enlarged in DEMM 2: 2 media . The whole media prepared with approx (10-12) % (FBS)ie fetus bovine serum, approx (2-3%) pencilin, and 6-7ml L-gultamine for 550ml of solution . The entire cells present in liquid nitrogen are taken in 75cm of 2 separate flask, that should contain from before 10-20 ml quantity of whole solution . These filled flasks will be applied to CO2 of (5-6)% at atleast 37^{o} C. Next step is to chane the media in every 2-3 days until every flask is not reaching atleast 85-90% of congestion. If for atlest one time the flask will full, next is to treat the cells with 1-2ml of Trypsin EDTA which will be infused for 2 minutes. In the last step , the reaction of this trypsin will be blocked with the help of the soy trypsin inhibitor.

2.1.3 (A549) Alveolarcellline -

These are adhesive cells which grow like monolayer type in our human body. This cell was also being cultured. Let us discuss about the experimental procedures

III. EXPERIMENT

3.1 Assay of (ROS) ReactiveOxygenSpecies -

We already have discussed in 4 cell lines 1. CHO 2. HepG2 3. BBB 4. A549 ROS will be measured . ROS in cells was tested on a resource plate with the help of plate reader Fluo-star with a proper wavelength of 490 nm and a wavelength having 520-530 nm in the presence of the reagent (DCHF-DA) 2 ',7'-dichloro-dihydro-fluorescin diacetate which is one type of fluorescence dye . It is possible to convert into DCF, a high and stable fluorescent dye compound in the availability of ROS. The above 4 different cell lines were taken for experiment to determine the job of nicotine in the generation of ROS.

Next the cells were seeded by applying corresponding technique for 12 hours. Next half of a day will be watched out of extraction of cells. Then the reagent was changed to DCFH-DA reagent at concentration 50 µM which is made up of serumfree reagent . For one hr it will be left and media will replaced by different possible concentration of nicotine 1µM to 1000µM. The injection of cells will be done with nicotine atleast for two to three hour and the measurement of fluorescence will be noted .

3.2 Assay of Viability of Cell .

One performance has been performed with cell line A549 in the presence of one of the popular dye iecalcein AM, which is hydrophobic in nature , of course this should penetrate easily to living cells which are stable . By the application of esterase this can be converted to calcein, which is hydrophilic in nature. This will be acting as a very strong kind of fluorescent . operations of cells are performed using proper technique. After the cells being seeded the plates will be incubatedatleast for half of a day. Next step is to treat with nicotine in different concentration like 2mM-6mM atleast and these were prepared by the media which is serum-free. whole day will be left for observation. This will be filled with 2-3 µM concentration of taken dye. After half an hour observation next step is to read the plates using appropriate spectral studies of same wavelength used in case of assay of ROS.

3.3 Ability of metal chelation .

This will be done by one of the popular methodDinisetal. The Solution of 30µl from the sample and water of approx 0.40ml will be mixed with Fecl2 (0.07ml) . Next step is to stirred properly and vigorously atleast for 2 minute, and reaction mixture will be added to 0.2 to 0.5 ml of ferrozine (5mM). For ferrozine we can follow its contents from given reference . This solution is will be applied for atleast for 10 to 15 mins and the color will be observed at 560-570 nm . Then the formula for calculating chelation of Fe^{+2} .

3.4 Glutathione(GSH)Measurement.

Winter et.al method introduced byRP-HPLC will help to determine the GSH standard. One of the modern and wellknown liquid chromography named HPLC having column of 100 x 5.6 mm will be packed . Then HPLC contained peak will be measured. Shimadzu model of Chromatopac will be used for quantitation . One of the

phase (mobile phase) is made up of 70% of acetonitrile and 30% of water , and the pH will be adjusted with the help of Ortho phosphoric acid and acetic acid. GSH Output will be extracted from the columns in which flow rate having 1-1.5ml per minute. The incubator will fill up by seeded A549 cells and separated in 4 flasks. The treatementof second and fourth groups will be done with nicotine . The 1st one will be the control to compare with others and the 3rd one will be reacted in the presence of 0.5 mM of t-BHP. Next step is final step where the cells were homogenate were made up of in (SBB)ie Serine Borate Buffer (Corresponding quantity of HCl, boric acid, L-serine, 7.5 of pH, DETAPAC). The required quantity (Approx 50 to 100 µl) of these solution which was homogenated will be mixed to 300µl of water and NPM of 700µl. The outgoing agent like NPM acts with sulfhydryl group to form emissions of light.The observation which was made for atleast for 5 to 10 minutes this is needed to mix 2N of HCl with the mixture for which reaction will stop.Above sample was filtered with a 0.50µl of acrodisc followed by injection into the coloumn of HPLC. Then formed adduct detected with the fluorescent detector.

3.5 Protein Calculation .

The technique was used to calculate protein levels was Bradford using Bio-Rad. The corresponding method will be followed to determine protein level . When the sample is absorbed , then next is to measure the standard solution and absorbance. Then the standard curve will help the unknown concentrations of protein to derive .

IV.STATICAL ANALYSIS

Thedatacollcted from above experiments can be analysed through statistical analysis. The data can be analysed through variance calculation method. Where we will get the result as p is less than 5 we will consider it as significant group. P is experimental group which will be compared with the control group.

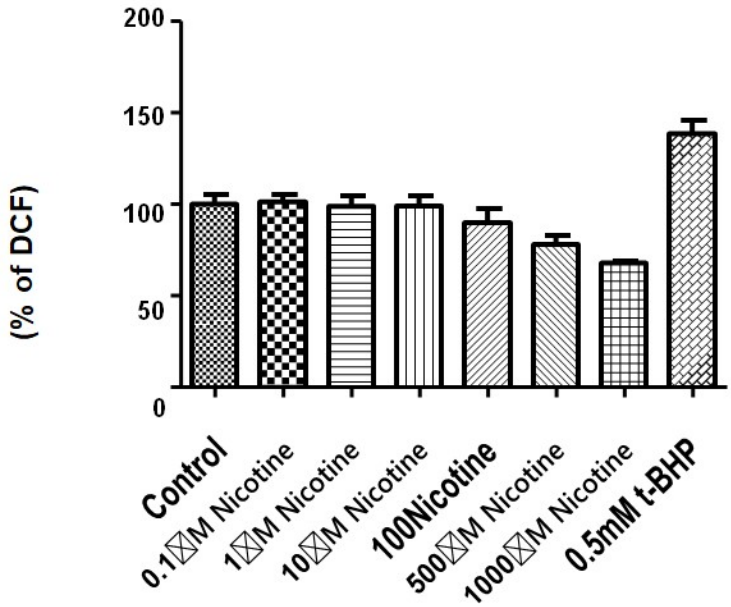

Fig 2. This graph is presenting creation of ROS and how A549 cells are being affected by nicotine. Different conc were used for the experiment. The controller is t-BHP. The difference between the groups are clearly visble.The whole reaction is done in the presence of DCF dye. Here p<5.

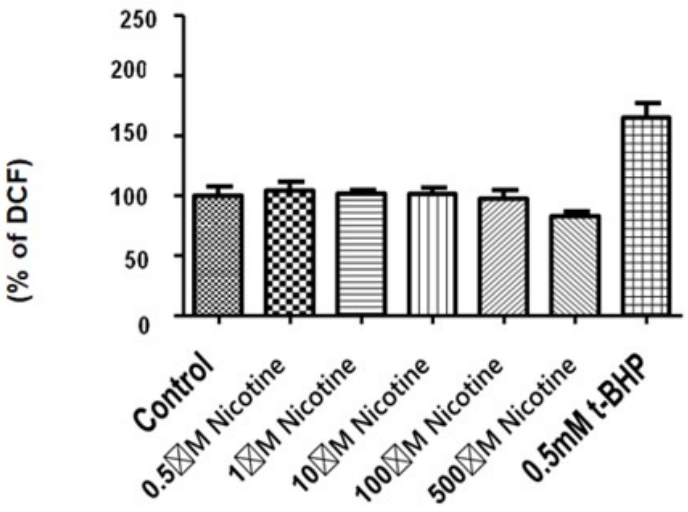

Figure 3 This graph is presenting creation of ROS and how HepG2 cells are being affected by nicotine . Different conc were used for the experiment. The controller is t-BHP. The difference between the groups are clearly visble. Here P<5.

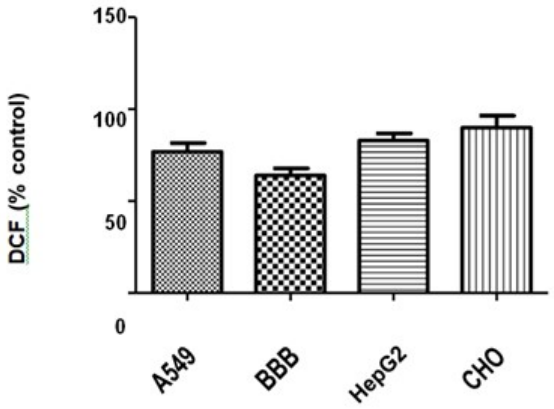

Figure 3:This graph is presenting Testing of ROS in different cell lines. The perecentage of DCF is different means groups are different from each other. And also the percentage is showing the value of flourescence. p<5 here.

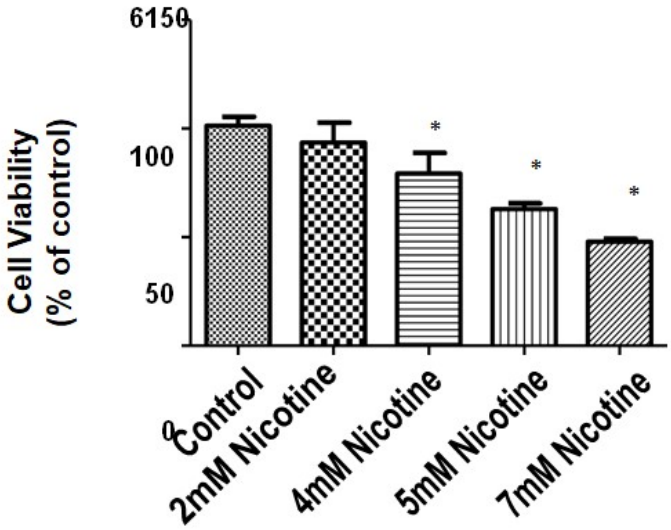

Figure .4This Figure is presenting Cytotoxicity character of nicotine in lungs cell A549 cells. According to the reports collected from the experiments cells will be treated with different conc of nicotine using the dye fluorescence. P< 0. And also groups are different from control group

Figure 5.VI

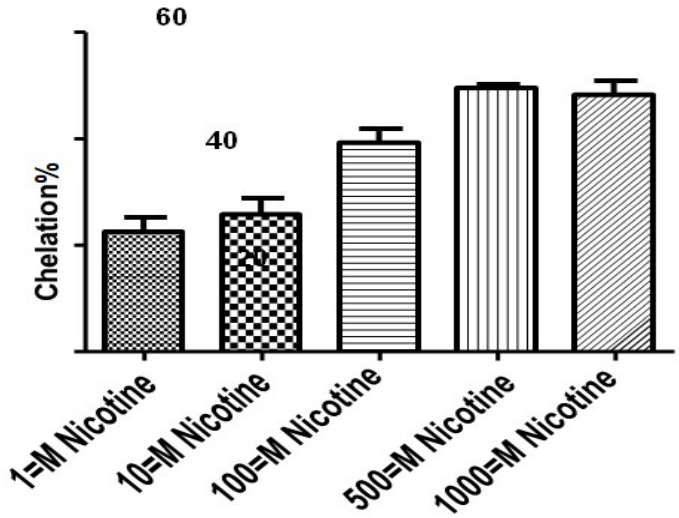

Figure 5.5This graph is representing chelating capabilities of nicotine with metals. All the cells were treated with nicotine as explained before but in various quantity and conc . The graph is clearly explaining that the groups are different from each other.

V. RESULT

ROS was tested in all the cells. As per result the cells were taken didn't show any response towards any concentration of nicotine that means ROS was not being generated by nicotine of any concentration. Four cells were taken as testing group that are CHO,BBB,HePG2.When all the experiments occurs to check the metal chelation property of nicotine then it shown a positive sign that means nicotine is having this character and the experiment was done in the presence of ferrozine. Metal chelation is concentration dependent that means if nicotine conc increases then metal chelation with iron will also increase. It shows antioxidant properties of nicotine.Glutatheone are an important character in deriving the limit of oxidative stress in human body systems. The alveolar cell was first reacted in the presence of nicotine and it was treated with t-BHP. From this experiment whatever the results we got, it is indicating that nicotine didn't show any significant role in glutatheone level in cells. When for further investigation was made then the group was reacted in the presence of tertiary-BHP suddenly shown an increase in the level of glutatheone.

VI.CONCLUSION

From the above findings we got the conclusion that nicotine is having only the effect on lungs which is concentration dependent and it has been observed from all the experiments that nicotine didn't produce the ROS in the human body which is the first cause of oxidative stress. So nicotine is not the cause of any kind of oxidative stress in human body. . Not only it has been observed that nicotine is having no role in generating ROS in a single cellular system but also it does not produce any kind of ROS in BBB,HePG2 and CHO . Nicotine has been seen as inhibiter of GSH depletion that is in the environment of free radical initiater. Thus from here we can also come to a conclusion that it protects cellular system from ROS and hence oxidative stress. This also has been experimented that nicotine has the ability of metal chelation power with iron. This also is proving the protecting ability of nicotine from oxidative stress and hence protecting cell death . It reduces the symptoms of some of the neurodegenerative diseases. All those diseases are being created by smoking is due to the presence of nicotine along with the toxic substances in conventional or common cigarettes which does not happen with electronic cigarettes, which is having only nicotine not any kind of toxic substances. So ordinary cigarettes can be replaced by E-cigarettes for the addicted people.

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