Proactive role of citric acid and tyrosine in nicotine addiction and controlling withdrawal symptoms in Wistar rats

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ABSTRACT
Nicotine deaddiction is not an easy job because regular exposure to nicotine leads to the development of neuroadaptation, neuroadaptation increases the number of binding sites on the nicotinic cholinergic receptors in the brain, this may be due to nicotine-mediated desensitization of receptors. Therefore the present study evaluate the commonly available substances like CA (Citric acid) and TY (Tyrosine) that can bring down the nicotine levels and maintain the dopamine levels without altering the nicotine intake. Thirty six animals were divided into 6 groups (n=6). Group-1 rats served as control and received only food and water while group (2) animals received only nicotine (600 μg/kg bw) by subcutaneous route for 21 consecutive days. Group (3-4) received both nicotine with CA 100 mg/kg bw oral and nicotine with CA 200mg/kg bw oral respectively for 21 days. Similarly group (5-6) received both nicotine with TY 100 mg/kg bw oral and nicotine with TY 200mg/kg bw oral respectively for 21 days. From 22nd day, animals in groups 3 to 6 alone received respectively CA (100 and 200 mg/kg bw; oral) and TY (100 and 200 mg/kg bw; oral) for another 5 days. On day 26, the animals in all groups were sacrificed and the blood was collected. From the serum, the levels of nicotine and dopamine were estimated. It was observed that the nicotine level was significantly less in CA100 mg and TY 200 mg doses. There was a significant increase in dopamine level in the nicotine treated group-2 when compared to normal control group-1. The same showed a slight decrease in CA and TY treated groups as compared to nicotine-treated group-2. Hence it is concluded that CA and TY have the ability to reduce the effect of nicotine levels and maintain the dopamine levels that can sustain the level of nicotine. This possibly can control the withdrawal symptoms of nicotine addiction and curtail craving for nicotine in the long run and most probably might lead to the cessation of smoking or tobacco consumption.

INTRODUCTION
Every year prevalence and complication of nicotine addiction and nicotine induced complications including cancer increasing all around the world. The effect of nicotine on the weaker sections of society is huge. People start using nicotine contained substances for a passion and get used to it. When they know about the adverse effects they try to stop and may not be able to stop. This is because they get addicted A study says that there are about 45 million Americans smoke tobacco. About 70% of them...
say they wanted to quit and only 40% quit only for a day. Few highly addicted smokers take serious attempts to quit but they could only quit for just few hours. Every attempt of quitting smoking becomes a failed attempt for an addicted smoker (Shiffman et al., 2006).

A brain imaging study says that the nicotine increases the activity in prefrontal cortex, thalamus and visual system through corticobasal ganglithalamic brain circuits (Arthur and Brody, 2006). Nicotine stimulates the nicotinic receptors and releases variety of neurotransmitters in the brain, it causes the release of dopamine in the mesolimbic area, the corpus striatum and the frontal cortex. Dopamine signals a pleasurable experience (Dani and Harris, 2005; Nestler, 2005; Wang and Sun, 2005).

Unresponsiveness of receptor is believed to play a role in tolerance and dependence (Dani and Harris, 2005). When the nicotinic cholinergic receptors becomes responsive and shows symptoms of craving and withdrawal, the person finds that he need more nicotine to get the desired effect. This is addiction, hence difficult to correct the damage. (Govind et al., 2009) In spite of the complication we have only few drugs to solve this issue. Obviously we have adverse side effects following those drugs. Along with the nicotine replacement therapy there are some drugs that can help people to quit smoking. Zyban, bupropion the generic form is usually prescribed as an anti-depressant medication and it is taught to affect chemicals in the brain that leads to craving. Chantix also called as varenicline is one of the newest medications (Grunberg et al., 1983). It works by interfering with nicotine receptors in the brain and help people to quit smoking. In our present study we have studied the commonly available substances like CA (Citric acid) and TY (Tyrosine) that can bring down the nicotine levels and maintain the dopamine levels without altering the nicotine intake. (Fix et al., 1983) CA is weak acid and it is present in citrus fruits, few studies from late 1982, 1983 says that there is an effect of urine and blood alkalinity on smoking cessation. From these studies we have developed an idea of testing some untouched alkaliizing agents to bring down the nicotine addiction. Searching for a compound that can increase the dopamine levels we came up with the second substance tyrosine. Certain drugs affecting the autonomic nervous system and also the TY in the presence of tyrosine hydroxylase produces dopa and dopamine in brain (Cherek et al., 1982). With these review of literature we took forward our present study to evaluate the substance that can make the person quit smoking without withdrawal symptoms and studied the effect of CA& TY on nicotine and dopamine levels.

**Aims and Objectives**

1. To study the substance that can make the person quit smoking without withdrawal symptoms.
2. To study the substance that can deaddict.
3. To study the effect of CA on nicotine levels.
4. To study the effect of TY on nicotine levels.
5. To study the effect of CA on dopamine levels.
6. To study the effect of TY on dopamine levels.

![Figure 1: Effect of CA & TY on Serum Nicotine level in different groups](image)

Serum nicotine level in Control, Nicotine group (N), Nicotine and Citric Acid 100 mg/kg and 200 mg/kg (N+CA100 and N+CA200), Nicotine and Tyrcone 100 mg/kg and 200 mg/kg (N+T100 and N+T200). Values are mean ± SE (n = 6 each). The ‘F’ and ‘P’ values are by one way ANOVA with Bonferroni multiple comparison test.

aSignificantly different from control group.
bSignificantly different from nicotine group.

Serum Dopamine level in Control, Nicotine group (N), Nicotine and Citric Acid 100 mg/kg and 200 mg/kg (N+CA100 and N+CA200), Nicotine and Tyrcone 100 mg/kg and 200 mg/kg (N+T100 and N+T200). Values are mean ± SE (n = 6 each). The ‘F’ and ‘P’ values are by one way ANOVA with Bonferroni multiple comparison test.

aSignificantly different from control group.
Table 1: Effect of CA & TY on Serum Nicotine level in different groups

| Group Name   | N  | Missing | Men   | StdDev | SEM  |
|--------------|----|---------|-------|--------|------|
| Control      | 6  | 0       | 4.290 | 2.776  | 1.133|
| Nicotine     | 6  | 0       | 83.033| 4.344  | 1.774|
| N+CA100      | 6  | 0       | 25.067| 4.846  | 3.465|
| N+CA200      | 6  | 0       | 38.803| 8.489  | 3.465|
| N+T100       | 6  | 0       | 34.967| 8.489  | 3.465|
| N=T200       | 6  | 0       | 29.783| 20.811 | 8.496|
| Source of Variation | DF | SS     | MS    | F       | P    |
| Between Groups | 5  | 203083.615 | 4061.723 | 43.680 | <0.001|
| Residual     | 30 | 2789.659 | 92.989|        |      |
| Total        | 35 | 23098.274|        |         |      |

Comparison | Diff of Means | t    | p    | P<0.050
Nicotine vs. Control | 78.743 | 14.144 | <0.001 | Yes
Nicotine vs. N+CA200 | 57.967 | 10.412 | <0.001 | Yes
Nicotine vs. N+T200 | 53.250 | 9.565  | <0.001 | Yes
Nicotine vs. N+T100 | 48.067 | 8.634  | <0.001 | Yes
Nicotine vs. N+CA200 | 44.230 | 7.944  | <0.001 | Yes
Nicotine vs. control | 34.513 | 6.199  | <0.001 | Yes
N+CA100 vs. N+CA100 | 30.677 | 5.510  | <0.001 | Yes
N+CA100 vs. N+T100 | 9.020  | 1.620  | 1.000  | Do Not Test
N+CA100 vs. N+T100 | 3.837  | 0.689  | 1.000  | Do Not Test
N+T100 vs. control | 30.677 | 5.510  | <0.001 | Yes
N+T100 vs. N+CA100 | 9.900  | 1.778  | 1.000  | Do Not Test
N+T100 vs. N+T200 | 5.183  | 0.931  | 1.000  | Do Not Test
N+T200 vs. control | 25.493 | 4.579  | 0.001  | Yes
N+T200 vs. N+CA100 | 4.717  | 0.847  | 1.000  | Do Not Test
N+CA200 vs. control | 20.777 | 3.732  | 0.012  | Do Not Test

*Significantly different from nicotine group.

One Way Analysis of Variance; Dependent Variable: Nicotine; Normality Test (Shapiro-Wilk): Failed (p < 0.050); Equal Variance Test (Brown-Forsythe): Passed (P = 0.604); The differences in the mean values among the treatment groups would be expected by chance; there is a statistically significant difference (P < 0.001).

Power of performed with alpha = 0.050: 0.721

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparisons for factor: Group

One Way Analysis of Variance. Dependent Variable: Dopamine; Normality Test (Shapiro-Wilk): Failed (p < 0.050); Equal Variance Test (Brown-Forsythe): Passed (P = 0.604); The differences in the mean values among the treatment groups than would be expected by chance; there is a statistically significant difference (P = 0.011).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Bonferronitmt-test):

Comparisons for factor- Group wise
Table 2: Effect of CA & TY on Serum Dopamine level in different groups

| Group Name     | N | Missing | Men  | StdDev | SEM  |
|----------------|---|---------|------|--------|------|
| Control        | 6 | 0       | 0.126| 0.00925| 0.00377|
| Nicotine       | 6 | 0       | 0.227| 0.0376 | 0.0154 |
| N+CA100        | 6 | 0       | 0.207| 0.514  | 0.0210 |
| N+CA200        | 6 | 0       | 0.180| 0.0473 | 0.0193 |
| N+T100         | 6 | 0       | 0.187| 0.0593 | 0.0242 |
| N+T200         | 6 | 0       | 0.186| 0.0405 | 0.0166 |
| Source of Variation | |       |      |        |      |
| Between Groups | 5 | 0.0347  | 0.00695| 3.610  | 0.011 |
| Residual       | 30| 0.0577  | 0.00192|        |      |
| Total          | 35| 0.0925  |        |        |      |

Comparison | Diff of Means | t | p | P<0.050
---|---|---|---|---
Nicotine vs. Control | 0.101 | 3.995 | 0.006 | Yes
Nicotine vs. N+CA200 | 0.0467 | 1.843 | 1.000 | No
Nicotine vs N+T200 | 0.0412 | 1.625 | 1.000 | Do Not Test
Nicotine vs N+T100 | 0.0397 | 1.566 | 1.000 | Do Not Test
Nicotine vs N+CA100 | 0.0197 | 0.777 | 1.000 | Do Not Test
Nicotine vs control | 0.0815 | 3.218 | 0.046 | Yes
N+CA 100 vs. N+CA200 | 0.0270 | 1.066 | 1.000 | Do Not Test
N+CA 100 vs. N+T200 | 0.0215 | 0.849 | 1.000 | Do Not Test
N+CA 100 vs. N+T100 | 0.0200 | 0.790 | 1.000 | Do Not Test
N+T100 vs. control | 0.0615 | 2.428 | 0.321 | No
N+T100 vs. N+CA200 | 0.00700 | 0.276 | 1.000 | Do Not Test
N+T100 vs. N+T200 | 0.00150 | 0.0592 | 1.000 | Do Not Test
N+T200 vs. Control | 0.0600 | 2.369 | 0.367 | Do Not Test
N+T200 vs. N+CA200 | 0.00550 | 0.217 | 1.000 | Do Not Test
N+CA200 vs. control | 0.0545 | 2.152 | 0.594 | Do Not Test

**Figures 2:** Effect of CA & TY on Serum Dopamine level in different groups

**MATERIALS AND METHODS**

After the institutional animal ethical committee clearance at Saveetha University, 36 animals were taken. The animals were divided into 6 groups.

Group 1 received only sterile water

Group 2 received nicotine at a dose of 600 micrograms/kg bw subcutaneously for a period of 21 days

Group 3 received nicotine for 21 days (fixed dose) and Citric Acid 100mg/kg bw for 21+5 days

Group 4 received nicotine for 21 days (fixed dose) and Citric Acid 200mg/kg bw for 21+5 days

Group 5 received nicotine for 21 days and Tyrosine 100mg/kg bw for 21+5 days

Group 6 received nicotine for 21 days and Tyrosine 200mg/kg bw for 21+5 days

The study was conducted for 25 days. On the 26th day all the animals were euthanized, blood was collected; serum separated and sent for nicotine levels estimation and dopamine level estimation.
RESULTS

By analyzing the obtained data using one way analysis of variance, the nicotine levels have a significant change whereas the dopamine levels are not significant. This suggest that the nicotine levels are decreased greatly when compared with the control and other test groups and dopamine levels are almost same, which means the dopamine values are almost same as the nicotine. The comparison of nicotine levels was done, the nicotine, control, CA100, CA200, TY100 and TY200 were compared their mean and standard error of mean represented in Figure 1 and in Table 1. The nicotine values of the groups showed statistically significant difference from the control group. There is significant change in the nicotine levels between control and nicotine groups. The nicotine levels of CA100, CA200, TY100 and TY200 showed significant difference with the control as well as with the nicotine treated group. The correlation of dopamine levels among all the groups show non-significant, the nicotine, control, CA100, CA200, TY100 and TY200 were compared and their mean and standard error of mean are represented in Table 2 and in Figure 2. The dopamine levels were low in the control group and it is very high in only nicotine treated group. The dopamine levels in CA100, CA200, TY100 and TY200 groups were also very close to the dopamine levels of the only nicotine group. This shows there is no change in the dopamine levels.

DISCUSSION

Nicotine is a component from tobacco, nicotine is the reason for tobacco addiction. There are various components in the tobacco that may cause various health related illness, but nicotine causes addiction as well. Unfortunately there are very few medication to nicotine deadication. Few investigators have studied about the alkalizing agents on smoking cessation. Urine was alkalized by sodium bicarbonate oral administration. The result was little change or no change in the number of cigarettes smoked. After sodium bicarbonate there was only 10% to 15% change and cannot expect to have high significance overall effects. Few studies on varenclines states that varencline effectively blocks the nicotine response in vivo and in vitro as well, demonstrating the appropriate antagonist profile. These attributes are desirable from the perspective of controlling nicotine dependence and reducing the potential for side effects mediated via overactivation of nicotinic receptors. Side effects form overexposure to nicotine and other nicotinic agonists include nausea, vomiting and seizures (Cherek et al., 1982).

A novel series of compounds from which varencline was identified for cessation of smoking. The study says neuronal nicotinic acetylcholine receptors mediate the dependence – producing effects of nicotine. Nicotinic receptors partial agonists to inhibit dopaminergic activation produced by smoking while simultaneously providing relief from the carving and withdrawal syndrome that accompanies cessation attempts (Yann et al., 2011). There are many studies showing the effects of nicotine but there only few studies that have worked on nicotine deadication. Hence we have taken up this study to identify substance that can reduce the effects of nicotine and to maintain the dopamine levels. The first compound is CA (Gabutti et al., 2004). Earlier studies on CA states that it is a week organic acid found in all the citrus fruits like lemon, grapefruit and orange. The rice source of CA is lemon and lime juice (Penniston et al., 2008).

Another study says that citrate decreases lipid peroxidation and down regulates inflammation by reducing polymorphonuclear cell degranulation and attenuating the release of myeloperoxidase, elastase, interleukin and platelet factor 4 (Gritters et al., 2006) Another study says that citric acid has been proven to reduce the hepatocellular injury evoked in rats by carbon tetrachloride (Tiranathanagul et al., 2011).

One study says that the aerosol spray of citric acid stimulates the tracheal sensations produced by cigarette smoke and satisfies smokers desire for cigarette. Smokers rated the respiratory tract sensations produced by citric acid aerosol equal to or better than that of low tar and nicotine cigarettes in terms of liking, similarity to their brand, and reduction in cigarette craving (Rose and Hickman, 1987) Similar study on ca inhaler says that the citric acid aerosol may promote successful smoking reduction or cessation in a subgroup of smokers and relieves withdrawal symptoms like craving for cigarettes (Behm et al., 1993).

Another study supports the citric acid aerosol saying that smoking not only depends on the pharmacological effects of nicotine but also the sensory stimulation from smoke inhalation, particularly the tracheal scratch. They have evaluated a new pocket-sized device for delivering a citric acid aerosol and examined some of the important variables for providing a satisfying substitute for cigarettes. The study concluded by saying that the inhaler may provide a useful tool for smokers while trying to quit smoking (Levin et al., 1990).

The second substance is the TY. A study on TY says that the TY transiently and significantly increases...
the extracellular concentration of striatal dopamine, the peak increase in ECF dopamine occurred 60mins after rats received the highest 200mg/kg TY dose. Another study on TY says that administration of tyrosine (100mg/kg i.p) elevated retinal dihydroxyphenyl acetic acid among light-exposed animals, but failed to affect dopamine release among animals in dark. And it also adds that the physiological stimulus - light exposure can cause catecholaminergic neurons to become tyrosine – dependent, they also suggest that food consumption may affect neurotransmitter release within the retina (Gibson et al., 1983). One more similar study states that the dopamine rise is short lived, suggests that receptor – mediated feedback mechanism responded to the increased dopamine release by diminishing neuronal firing or sensitivity to tyrosine (Acworth et al., 1988). Another study says that older adults showed a dose – dependent increase in plasma tyrosine concentrations and the plasma response was higher than for young adults with the same dose (van de Rest et al., 2017).

A text book of pharmacology about the biosynthesis of dopamine which is from a starting material L-phenylalanine a dietary amino acid and is absorbed from the gut and oxidized in the liver by phenylalanine hydroxylase to form l-tyrosine. The circulated L-tyrosine is then actively transported into the cytoplasm of the noradrenergic neuron. Within the neuronal cytoplasm, L-tyrosine is then hydroxylated by tyrosine hydroxylase to L-dopa. Than L-dopa is converted to dopamine by cytoplasmic L-amino acid decarboxylase. In the dopaminergic neurons, within the CNS, this is the last step in the biosynthetic process, because DA serves as a main neurotransmitter in the CNS. The nicotine levels of only nicotine treated group did not decrease as that of the test groups. This suggests that the CA and TY may be interfering in the nicotine metabolism (Table 1 and Table 2). The dopamine levels are maintained normal in the test groups suggests that the CA and TY have an effect on the production of dopamine.

**CONCLUSIONS**

Our present study concluded that the CA and TY have the ability to reduce the effect of nicotine levels and maintain the dopamine levels, which can sustain the pleasure that of nicotine. Hence during abstinence of nicotine the dopamine levels are maintained which will maintain the pleasurable feeling. This can control the withdrawal symptoms and stops craving for nicotine and finally leads to the cessation of smoking or taking nicotine therapy.

CA and TY decrease the nicotine levels, which means it reduced the effect of nicotine. But it elevated the dopamine levels than controls not to the nicotine group though, suggests that the withdrawal symptoms did not occur yet. Finally CA and TY are maintaining the dopamine levels. Developing this study further may lead us to bring down the nicotine levels as that of the control and increase the dopamine levels as that of the nicotine group which can deaddict the animal without withdrawal symptoms. In abstinence for 5 days there is difference between the controls, only nicotine treated group and the other test groups. Test groups showed significant fall in the nicotine levels and maintained almost normal levels. Nicotine ànicotine increased and dopamine is elevated à pleasant feeling. Test group à nicotine decreased and dopamine levels remains same àpleasant feeling maintained. If pleasant feeling is maintained there is no urge or craving or withdrawal symptoms during abstinence Hence CA and TY is working and aim is fulfilled. Further studies are required to bring it to the human models.

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**Conflict of Interest**

None.

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None.

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