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Effect of nicotine on external morphology, liver and body weight of *Oryctolagus cuniculus*

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Abstract
Effect of nicotine on external morphology, liver and body weight of rabbit (*Oryctolagus cuniculus*) was investigated. Animals were divided into controlled and treated group. One group was kept on normal diet and other was treated with nicotine by 4 different methods i.e. tea, smoke, intra muscular injection and vegetable sprinkle. In between each treatment animals have two weeks of washout time. All animals were kept at 22 ^{0}C and 30-40% humidity. Body weight was recorded before and after each treatment and noted huge weight loss (1185 grams) in nicotine treated group. Nicotine also affected the hair coat and loss of hair found and patchy skin was seen compared to controlled animals. At the end of liver color and weight was recorded which was light brown in treated and dark maroon in controlled group. Liver weight almost 20% was lost in nicotine treated group. Eyes were affected too showing muscular and nervous deteriorating. These results shows deteriorating effect of the nicotine on morphology weight of the animals and their organs most importantly on liver.

Keywords: Rabbits, nicotine, morphology, liver, weight

1. Introduction
Oryctolagus cuniculus, (Linnaeus, 1758) also called a European, an old world, or a domestic rabbit, is the only species in its genus. Domesticated *O. cuniculus* may be found worldwide [1, 2]. A male rabbit is called a buck, and a female rabbit is called a doe. *O. cuniculus* are altricial, the young being born blind and furless, in a fur-lined nest in the warren, and they are fully dependent upon their mother [3]. Home range size varies with population bulk and food abundance, but is usually under 50 acres and often as minute as one or two acres. Male home ranges are on average two times as large as those of females [1]. *O. cuniculus* are mainly crepuscular, being most active around dawn and dusk, although they are not uncommonly seen active during the day [4]. A doe marks the nest with urine and soft mucus covered pellets covered with secretion from the anal gland [5]. These fecal droppings helps repelling outsiders and to detract others from invading the site [6].

Nicotine is an alkaloid used by millions of people worldwide through cigarette smoking and has adverse effects on every organ and particularly on the endocrine system. The use of tobacco is still raising worldwide [7]. Although around 4000 components occur in the cigarette, nicotine is the alkaloid most dynamic in the tobacco. Nicotine is not a direct cause of most tobacco-related diseases, but it is highly addictive [8]. Nicotine is an amine order of pyridine and pyrolidine rings [9]. It has been revealed that nicotine can cross the biological membranes including the blood brain barrier [10]. The acts of nicotine have been extensively examined in human, in animal, and in a variety of cell systems [11]. The predominant effects of nicotine in the whole intact animal or human consist of an increase in pulse rate, blood pressure, raise in plasma free fatty acids and lung injury. Liver is considered to be the chief site of nicotine, metabolism also occurs in the lung and kidney [12]. Both animal data and human studies have shown a link between fatty liver and oxidative stress and lipid peroxidation [13].

Nicotine is extracted from the dry leaves of *Nicotiana tobacum*. Nicotine expenditure results in changes in cardiovascular, neural and endocrine functions through effects on the central and peripheral nervous systems [14]. Nicotine increases the plasma levels of ACTH, epinephrine and cortisol hormones, dose dependently [15]. Through its effects on the nicotinic receptors of acetylcholine in the pancreatic beta-cells, it boosts the segregation and reconstruction of inositol phospholipids and therefore recovers the calcium sensitivity of these cells [16]. It also affects the liver through epinephrine secretion which guides to an increase in the release of glucose from the liver to the blood [14].

The life threatening sickness smoking creates in humans are due to the injurious tars and carbon monoxide found in cigarettes. Circulatory disease such as cardiovascular, heart disease, myocardial infraction and stroke, lung cancer, or chronic obstructive lung is attributable to the consumption of tobacco [17]. When a person smokes it takes less than 20 seconds for the drug to reach the brain. With each puff of a cigarette, nicotine is pulled in lungs where it is absorbed into the blood, nicotine is shaped like acetylcholine. Neurons have special spaces called receptors, into which specific neurotransmitters can fit, like a key fitting into a lock. Nicotine locks into acetylcholine receptors in different parts of the brain. Nicotine raises the heart rate and breathing rate, and causes more glucose, or blood sugar, to be released into the blood. This might be why smokers feel more alert after smoking a cigarette [18].

Nicotine also attaches to neurons that release a neurotransmitter called dopamine which stimulates the brain's pleasure. Smoking cigarettes causes a flood of dopamine in the brain. It's this flood of dopamine that gives intense feelings of pleasure [19].

This drug is so addictive that smoking related deaths double the total amount of deaths caused by alcohol, illicit drugs, fires, car accidents, homicides, suicides, and AIDS [20].

2. Materials and methods

2.1 Rabbits (*Oryctolagus cuniculus)*

Male rabbits, *Oryctolagus cuniculus* (Linnaeus, 1758) 10 weeks of age, 2 kg weight, bought from a pet shop, Manshera bazaar, Pakistan, were divided in two groups, i.e., controlled and nicotine treated. Animals were housed in individual cages. (3 × 3 feet). In cage "A" controlled *O. cuniculus* were kept and in cage "B" nicotine treaded *O. cuniculus* were kept. Animals were placed in 22 ℃ (71.6 °F) room temperature and 35 to 40% humidity. Nicotine was administered in different ways such as in vegetables, as tea, in injections and via cigarette smoke.

2.2 Nicotine administration by tea

Nicotine was directed by giving 1000 ml tea solution (Figure 2) to *O. cuniculus* every day by mixing 600 ml water with 50 gm tea (Lipton Yellow Label, Karachi Pakistan), 50 gm sugar and boiling the solution for 10 min at 100 °C on gas burner. Milk (350 gm) was then added and let it to be boiled for more 5 min. Tea solution was left to be cooled down, and then given to *O. cuniculus*. It was given to treated animals every morning at 9:00 am for 30 d. (Fig: 1) Simultaneously, both groups were given normal vegetables tomatoes, cabbages, carrots, cauliflower and potatoes. Effect of nicotine on morphology of *O. cuniculus* was checked and pictures were taken by 14 mega pixel camera (Kodak, Toronto Canada). *Oryctolagus cuniculus* were were kept on regular food and water to washout the previous tobacco tea treatment for 2 weeks (14 d), therefore they could come to their normal position for next nicotine management. *O. cuniculus*, became so addicted to tea solution that they jumped inside the cage and tried to break the cage by seeing the tea.

2.3 Nicotine administration in vegetables

Similarly, orally nicotine was feed to *O. cuniculus* by mixing nicotine with vegetables (Figure 3 a, b) at the ratio of 50 mg/kg per day for each animal. In vegetables mostly used tomatoes, cabbages, carrots, cauliflower and potatoes. Vegetables were washed properly to avoid any type of contamination. Dried tobacco was extracted from cigarette every day, and was sprinkled on the vegetables. Animals were allowed to eat vegetables for half hour 9:30 am to 10:00 am for 30 d. Effect of nicotine administration on morphology of *O. cuniculus* was again analyzed. O*ryctolagus cuniculus* were than treated normally for 2 weeks (14 d) (Fig: 2).

2.4. Nicotine administration by cigarette smoke

A rate of 4 cigarettes per day was given. Cigarettes were first kindled and then fixed in the cage, during cigarette smoking the whole cage was covered with plastic sheet in order to retain the smoke for long time inside the cages. Approximately, 20 min from 9 pm to 9:20 pm for 32 d.

2.5. Injecting syringe

Injection was given through syringe into the animal bodies, at the ratio of 1 ml, 2 syringes was injected per week in 32 days. The syringe was injected in hind leg muscles. Totally 15 syringes were injected during the whole experimental period. Pure tobacco juice was extracted from tobacco leaves by grinding them, and then mixed with water in order to dilute the solution, after that this solution was injected to animals.

Effects of nicotine on weight of *O. cuniculus* were checked. Weight was recorded with the help of electronic balance on the monthly basis after every nicotine administration, which gave the exact weight fluctuation of the nicotine effected and controlled *O. cuniculus*.

3. Results

3.1 Effect of nicotine on eyes structure

Nicotine affected the eyes. It was observed that the eyes of the controlled *O. cuniculus* were in normal position whereas; the eyes of the nicotine effected *O. cuniculus* were ejected out of their sockets due to weakness.

3.2 Effect of nicotine on hair coat

At the end of second nicotine administration in form of sprinkling tobacco on vegetables, it was noted that, hair of the nicotine treated *O. cuniculus* began to fall from their body, showing clear patches of pink body skin.

3.3 Effect of nicotine on weight of the rabbits

Weights of the *O. cuniculus* were taken on the monthly basis during the experimental work by electronic balance. The weights of the both groups before the start of the experiment were 2 Kg (2000 gram). Weight of *O. cuniculus* was recorded after first month of nicotine management (June) in form of tea solution, it was seen that controlled group did not lost any weight, but treated group lost 200 gram. After the end of the second month of nicotine management (July) in form of sprinkled tobacco on vegetables again weights were checked, results showed that controlled group gained 18 gram while, treated group lost again 500 gram. After the third nicotine management August in form of cigarette smoke, when weights were analyzed, the results showed that controlled group gained no weight but treated group lost 485 grms. Finally in last month of nicotine administration by injections nicotine effected *O. cuniculus* were much weakened and were 815 grams but controlled group was healthy, 2022 grams. (Graph:1, Table 1)

3.4 Effect of nicotine on liver colour

O. cuniculus were dissected to analyze the livers. Nicotine greatly effects the color of liver.

Controlled group was normal dark maroon, it was because the controlled group metabolic activities and other function were normal and well developed and food given to them was healthy and nicotine free. While the color of the nicotine affected *O. cuniculus* was changed from normal maroon color to light brown with disturbed gall bladder (Fig: 3).

3.5 Effect of nicotine on weight of liver

At the end of experiment just after the dissection, weights of both controlled and treated liver were measured. Weight of the controlled liver was found 51.3 gm while that of the treated one was found 40.6gm (Table: 2, 3, 4).

4. Discussion

Nicotine is an alkaloid used by millions of people worldwide and notorious for deteriorating effects on many organs particularly on liver, heart, lungs, pancreas and endocrine system. Nicotine is one of the major substance of tobacco leaves and millions of consume it through cigarette smoke and also by insecticide. Cigarette smoke has massive negative health consequences worldwide, and the use of tobacco is still rising globally [7]. During the present study from June to October 2011 the effect of nicotine on the physical appearance of eyes, hair and body weight of the *O. cuniculus* and also the color and structure of the

liver was noted. Results showed that nicotine had worse effects on the physiology and anatomy of *O. cuniculus*.

Study was conducted to determine the effects of nicotine and its metabolite cotinine on the hemoglobin of the rabbit liver. Nicotine was administrated by drinking water 10 mg/ 200 ml per day. At the end of the experiment study concluded that the level of hemoglobin decreased in the liver and there color was not red purple. This effect was may be, due to change in the chemical nature of the liver and hepatocytes cells were effected and also haemogolobine level was disturbed due to which they loss there maroon color [21].

Another work on nicotine and its effects on hair loss was done. Hundreds of studies on nicotine revealed the harmful effects of nicotine on hair loss. Many harmful effects are closely associated to scalp and hair loss. Healthy scalp produces healthy hair shafts. Hair shafts arise from hair follicles. Hair follicles are tiny tubular cavities, at the base of hair follicles there exists a bulb like structure called dermal papillae that multiplies itself gradually to produce hair shaft. Hair follicles are surrounded with hundreds of thousands tiny blood capillaries which carry blood from arteries to hair follicles. Supply of oxygen and nutrients are carried out by blood capillaries. Therefore, nutrients and oxygen transference through blood capillaries is the only fundamental source of follicular nourishment that makes growth of healthy hair possible [22].

Effect of nicotine administration on weight and histology of some vital visceral organs in female albino rats was done. Twenty four female rats with regular oestrous cycle in the same phase of the cycle were divided into two equal groups with each group receiving 0.5 mg/kg nicotine and 0.9% normal saline daily respectively. Six rats from each group were killed by cervical dissociation after 30 and 60 days treatment. The ovary, uterus, brain, kidney, heart, adrenal, pituitary and the liver were removed, weighed and histological study carried out. Nicotine was applied against the rates and their result shows that the weight of the liver did not decreased and it effect on the weight of other organs like ovary, kidney, pituitary and uterus which reduced significantly [23] found effect of nicotine on the weight of male rats. Male rats were treated 14 day period with 0.8 mg/kg nicotine, 10 mg/kg caffeine or a combination of 0.8 mg/kg nicotine and 10 mg/kg caffeine were pretreated (once daily). Rats were treated with nicotine and their results showed that the nicotine effects on their weights and it the end of experiment the rates loss there weights [23].

5. Tables and figures

Fig 1: Nicotine in the form of tea solution to treated *Oryctolagus cuniculus*

Fig 2: Nicotine administration by sprinkling tobacco on vegetables; carrots, potatoes, cabbage and tomatoes

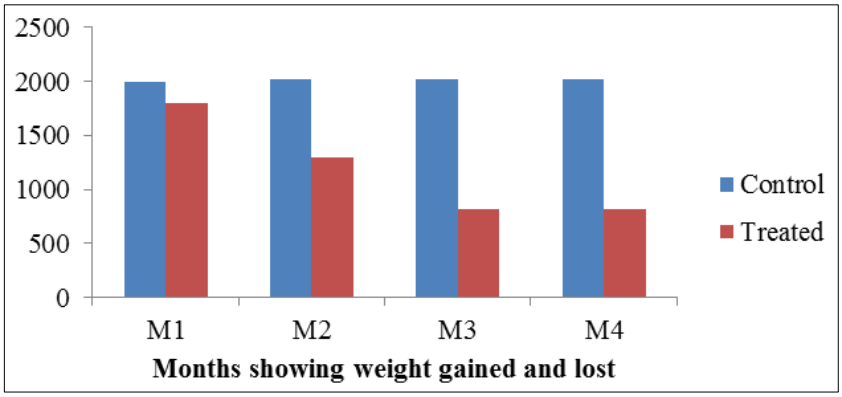

Fig 1: Weigh of controlled and treated during experiment time

Table 1: Anova Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Ntreated - Controlled	-932.000	608.464	304.232	-1900.202	36.202	-3.063	3	.055

Table 2: weight of controlled and treated liver

S. No	Weight of Liver	Weight in gm
1	Controlled	51.3
2	Treated	40.6

Table 3: Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Ntreated	1082.50	4	599.785	299.892
	Controlled	2014.50	4	9.849	4.924

Table 4: Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Ntreated & Controlled	4	-.879	.121

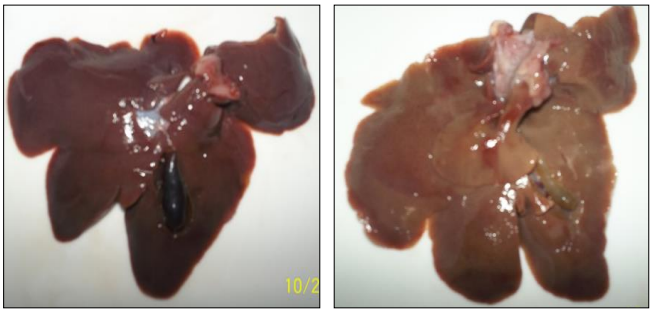

Fig 4: liver of controlled with normal color treated with effected color

6. Conclusions

From the results we concluded that nicotine is a harmful alkaloid which negatively affects the morphology and different organs of the body especially liver, also it plays role in weight and hair loss.

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