ANTI-OXIDANT, IN-VITRO AND IN-VIVO ANTIDIABETIC POTENTIAL OF *COPRINUS ATRIMENTERIA* IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT; Diabetes mellitus (DM) is becoming a global public health issue. Mushrooms have been used as a neutraceutical as well as for medicinal puposes. In the present study *Coprinus atrimenteria* was evaluated against the in-vitro anti-oxidant and anti-diabetic assay using DPPH, ABTS, H_2O_2 and α-amylase, β-glucosidase inhibitory effects respectively. Maximum efficacy was observed in ABTS activity (60%) followed by H_2O_2 and DPPH as compared with control (Ascorbic Acid). Similarly the methanol extract showed a significant inhibitory effects of α-amylase (65.89%) and β-glucosidase (59.89%) accordingly as compare with standard anti-diabetic drug Glucophage (86.34%). The mushrooms *C. atrameneria* showed a significant increase in body weight in comparison with diabetic control and a remarkable decrease in random blood glucose level (RBG). Similarly the Lipid Profiles (TC, LDL, HDL, TG) of albino rats were analyzed and found significant as compare with normal control. These results suggest that *C. atramenteria* mushroom species could be hopefully used as an antioxidant and as alternative medicine for the treatment of diabetes mellitus.

Key Words; *Coprinnus atrementeria* mushroom, anti-oxidant, In-vitro, In-vivo-anti-diabetic potentials.

1. INTRODUCTION

Diabetes mellitus is considered a serious disease, characterize by a high level of glucose in blood and finally leads to disturbance of all type of metabolic and damages of macro and micro-vascular systems of the body. Hyperglycemic conditions are created due to impair insulin secretions, insulin actions or both. As a result various complications may be developed such as kidney failure, blindness, neuropathic conditions, brain stroke, and myocardial diffraction. Two types of disease are reported, one is hereditary insulin dependents type 1 diabetes mellitus, which are mainly developed by the β-cells of pancreas destructions and the others most common type 2 diabetes mellitus [1]. There are two thousands edible or therapeutic mushrooms have been

recognized, which are broadly used as a good and positive effect on human health. There active and positive characteristics are due to the biological active substance, that mushrooms prepare during growth and development, contains polysaccharides. Biologically the active polysaccharides founds in mushrooms are the homo polysaccharides glucans, which are the basic part of cell wall. The pancreas secreted enzymes are not hydrolyzing the bonds [5]. From different studies, it has been documented that fungal polysaccharides minimize the disease symptom. The anti-diabetic characteristics in rodents studies had formerly noted for the following mushrooms cultures; *Agaricusbrasiliensis, Agrocybechaxingu, Catathelasmaventricosum, Grifolafrondosa, Phellinuslinteus, Pleurotuseryngii, Pleurotusflorida, Pleurotussajor-caju, Tremellafuciformis, Ganodermalucidum* [12, 13]. The possible mode of actions of dried powder of the fungus *coprinus atrementeria* on healthy volunteers and the people's sufferings from diabetes DM type2 was investigated. When a patients suffering from diabetes, taking a drug for a month, the glucose level was found decrease in blood serum and the level of insulin was increased. The possible mechanism of the hypoglycemic actions is related with an increase in glucosidase assay and motivations of insulin secretions, resulting the increasing of glucose utilizations by the side tissue and glycogen preparations [20].

2. MATERIALS AND METHODS

The *Coprinus atrementeria* were collected in mid June 2019 from the surrounding area of District Bannu KPK, Pakistan, and was identified by a well-known mycologist Assistant Prof, Dr. Sehroon Khan, University of Agriculture Peshawar KPK Pakistan.

3. Crude Extracts preparation

Fresh C. *atramenteria* were thoroughly washed with distilled water, cut into small pieces, dried and grinded to powder form. 5g of the dried sample were soaked into various organic solvents from polar to non-polar (Methanol, n-hexane, distilled water) for 24 hours, the mixtures were filtered and crude extract was obtained [20].

4. In-vitro anti- oxidant activity of selected mushroom.

4.1. DPPH radical scavenging activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a free radical and receives a hydrogen radical or an electron to become a stable molecule. The efficacy of antioxidants on DPPH radical is due to their hydrogen donating capability. The IC_{50} of scavenging assay was

probably based on the proportion of DPPH radical scavenged by the equation giving below:

Scavenging effect (%) = [(control absorbance–sample absorbance) / (control absorbance)] ×100.

4.2. The ABTS activity

The ABTS are easily soluble in organic solvents and it can be used successfully for analyzing and evaluate the antioxidant potentials in both lipophilic and hydrophilic samples. In this activity, the solutions of ABTS, blue and green decolonization's is carried out due to giving of H-atom by the antioxidant present in the solutions at 734nm. The ABTS scavenging activity was brought by small and minor changes.

4.3. Hydrogen peroxide-scavenging assay (H_2O_2)

Although H_2O_2 oxidizing agent, while on the other hand it has the potential to neutralize the different enzymes directly, by the oxidation of most important thiol (-SH) groups. It can enter the cell by crossing the cell membrane rapidly. It can possibly react with Cu^{2+} and Fe^{2+} ions to produce an OH (hydroxyl radicals) and it might cause some lethal functions.

5.α-amylase inhibitory assay

The (500 µl) aqueous extracts dilution and 500 µl sodium phosphate buffer (pH 6.8 with 0.005 mol·l-1 NaCl) contains a hog pancreatic α-amylase were placed at incubator at 25 °C for 10 minutes. Then, 0.02 mol·l-1 sodium phosphate buffer solution was mixed to a reaction mixture. Then, the mixtures was placed for 10 minutes in an incubator at 25 °C and stopped with 1.0 ml of dinitrosalicylic acid. The mixture was placed at incubator in a boiling water bath for 5 min, and cool down to a room temperature. By the additions of 10ml of distilled water to the reactions mixtures and their absorbance was calculated at 540 nm in a ultra-visible spectrophotometer [21].

6. α-Glucosidase inhibitory assay

A100 µl of α-glucosidase solution and 50 µl of aqueous extracts was placed at incubator at 25 °C for 10 min. Then, added 50µl of 5 mmol·l-1 pnitrophenyl-α-D- glucopyranoside

solution in 0.1 mol·l-1 phosphate buffer (pH 6.9) to the reaction mixture. The mixture was placed in an incubator at 25 °C for 5 min, and their absorbance was analyzed at 405 nm in the spectro-photometer [22].

7. In-vivo Anti-diabetic activity.

7.1. Inductions of Diabetes mellitus and Experimental Design

We purchased a Swiss male albino rates from NIH (National institute of health) Pakistan. Proceeding to the beginning of experiments, the rats were placed in a good and fresh ventilated animal house at $25C^0$ room temperature with a sound food and water and libitum for a period of one week. For the proper arrangement and improvement of diabetic model, the rates were placed into five groups. Each group consists of five rates. After fasting throughout the night, the fresh readymade solution of alloxane monohydrate (150 mg/kg body weight in normal saline) was injected into intraperitoneal from group II to group V. The group 1^{st} containing rates were placed as a normal group that did not get any type of chemicals. The group VI and VII was also being placed normally and only treated by these extract. According to the protocol prepared, their blood containing glucose contents was to be analyzed by glucometer (Clever Check, Germany), after 48 hours.

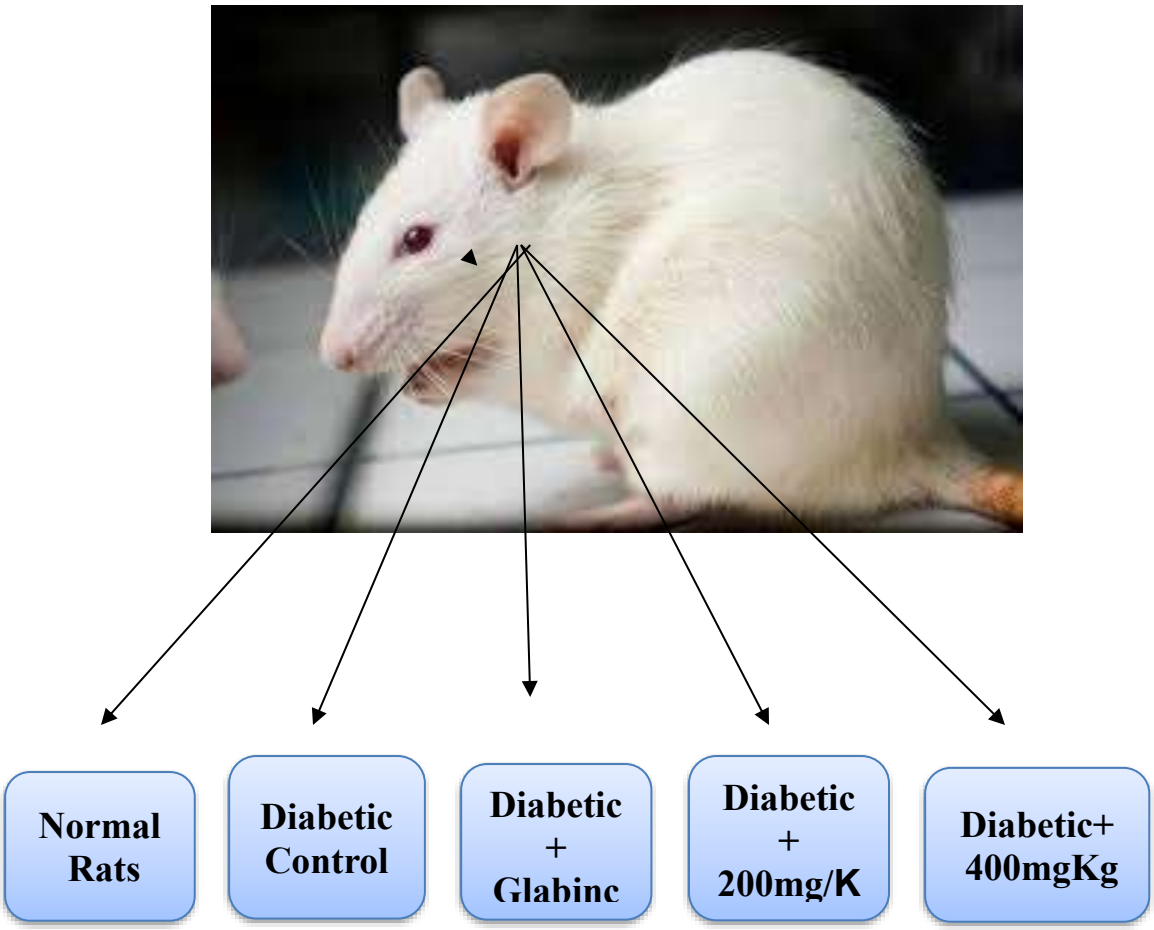

Fig 1. Schematic representation of experimental design

8. In-vivo anti-diabetic activity of selected mushroom biochemical profile

The following Morphological and biochemical parameters of the selected rats were determined and compared with control.

i. Body weigh

ii. Fasting Blood Glucose (FBS)

iii. Total cholesterol (TC)

9. Statistical analysis

The results of the experiments were measured as mean ± standard Deviation (SD) of three replicates. Data were collected and statistically analyzed by using analysis of Variance (ANOVA), a tool in (SPSS 14.0). The significant level was set at $p < 0.05$.

10. Results and Discussions

Antioxidant activity of *C.atramentreia*

The Various concentrations (10mg/ml, 50mg/ml and 100mg/ml) of three different solvent extracts of *C.atrementeria* confirmed various percent inhibitions. Interestingly, the free radical scavenging bio-assay of each extract was a dose dependent. The highest scavenging activity was shown by ABTS activity (52.50%).

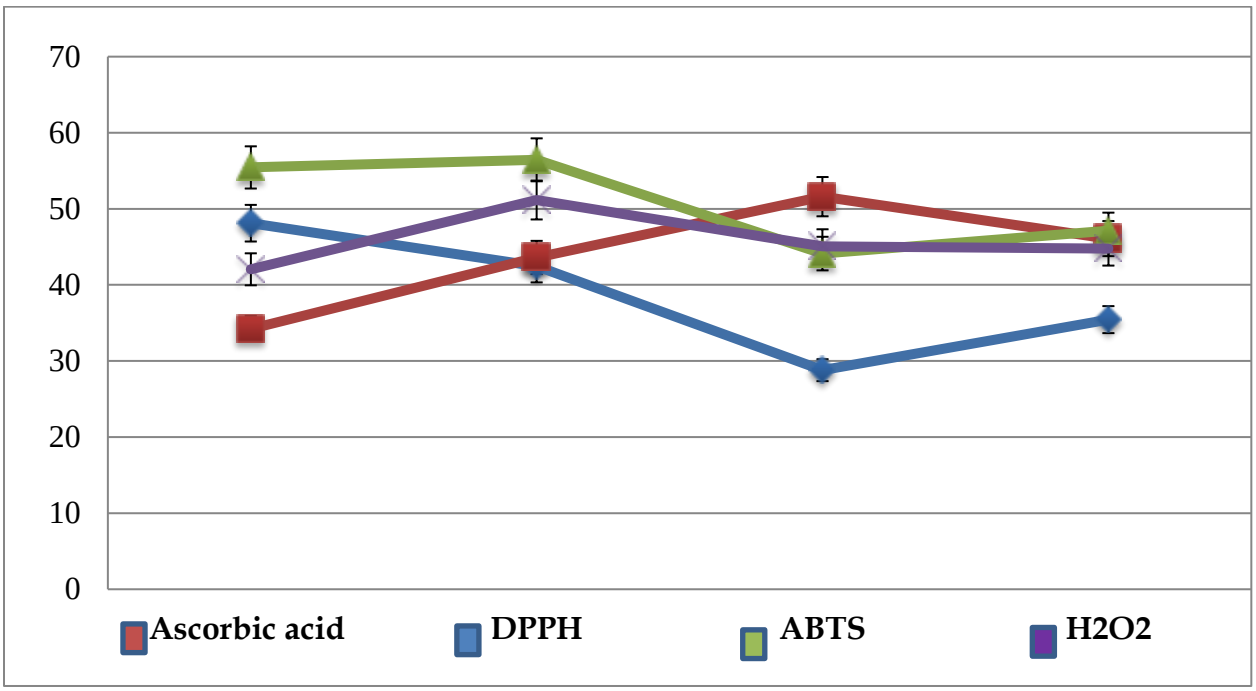

Fig 2. % inhibition of C.atrameteria Using DPPH, ABTS and H_2O_2 as compared with standard drug Ascorbic Acid

The *in-vitro* α–amylase inhibitory assay of methanolic extracts of *coprinus atrementeria* was analyzed. This experimental result showed that percentage inhibitory effects of α- amylase was a dose dependent. The extract showed a greater inhibitory effect from4.13±0.08 to 11.22±0.04% with an IC50 value of 0.75µg concentration. For amylase inhibitory effect gulcopgahe was used as a standard drug. The Glucophage at the concentration 04 µg/ml to 10 µg/ml proved a prominent inhibitory effect with an IC50 at a concentration of 0.31µg/ml. Our experimental work proved that the methanolic extracts of mushroom have a greater α-amylase inhibitory action (IC50 = 0.75 µg dry extract) because of their capacity to binds with proteins molecules.

Table 1. ***In-vitro*** **anti diabetic studies of α-amylase, and α.glucosidase inhibition activity of various fraction of** ***C. atramenteria***

Sample fraction of (*C.atramenteria*)	Con.ug/ml	% inhibition of α.amylase	% inhibition of α.glucosidase
Methanol extract	10	36.33	37.43
	50	49.78	51.77
	100	65.89	58.88
Hexane Extract	10	22.77	34.33
	50	28.21	47.77
	100	35.88	57.88
Aqueous Extract	10	35.55	36.33
	50	44.44	47.77
	100	54.38	59.89
Glucophage (control)	10	55.86	57.43
	50	65.66	68.78
	100	85.87	86.88

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

In-vitro **α–glucosidase inhibitory assay**

In-vitro study the α-glucosidase inhibition assay of methanolic extracts of *C.atrementeria* was analyzed. The experimental results showed that there was increase in a dose dependent manner in percent inhibition against enzyme glucosidase. The methanolic extracts (2-10μg/ml) of mushroom have a potent glucosidase inhibitory assay in a dose dependent manner. In this activity the, extracts showed a remarkable inhibition from 3.24±0.05 to 12.88±0.12 %with an IC_{50} value of 0.80 μg dry extracts (Table 2).Glucophage was used as a drug for amylase inhibition. Glucophage at a conc. of (2-10μg/ml) proved a remarkable inhibition of α-glucosidase from 17.70±0.09 to34.93±0.12% with an IC_{50} value 0.46μg dry extracts. The α–amylase inhibitory assay was observed in between mushroom extract and standard drug as shown in fig. our present study, the methanol extract of mushroom proved a remarkable and greater α- glycosidase inhibitory assay (IC_{50} = 0.80 μg dry extracts), which showed the presence

of polyphenols and flavonoids because polyphenols not only the capability to reduce the oxidative pressure, but also stop the carbohydrates hydrolyzing enzyme due to their capability of their protein molecules [23].According to the previous study our results showed that a relationship developed between a flavonoids and total phenols contents positively and their capability to stop the intestinal α-glucosidase and pancreas α-amylase [24]. .

Table 2: *C. comatus* methanol activity on body weight (gm) of diabetic and normal rats

Groups, n=6	1st Day	7th Day	14th Day	21th Day	% variations
Normal	175	176	176	180	8.20
Diabetic control	162	161	156	151	7.04
Diabetic+Glabinclamide10mg/Kg	164	161	159	154	5.02
200mg/Kg CCME	166	169	171	175	8.85
400mg/Kg CCME	167	170	175	177	8.20

All the values are expressed as a means (+ - sem) in triplicate against Diabetic control

P* < 0.05, P <0.01 P*** < 0.001.**

***C.comatus effects* on body weight in alloxan induced and normal rats**

All the rats were observed regularly on 1st, 7th, 14th and 21th day of treatment. The mean body weight of normal control group was recorded from 1st day (200g), 7th day (205g), 14th day (208g) and 21th day (212g), it indicates that the normal control rates maintained their body weight during the experimental durations. diabetic control 1st day (185g),7th day (183g),14th day (180g) and 21th day (174g), it showed the progressive decrease in the body weights due to the tissue damage and fast oxidations proteins and fats, they did not maintains their body weights with respect to normal groups of rates.

Table4. 3: *C. atrementeria* effect on blood glucose level on selected rats.

Group. n =3.	1st Day	7th Day	14th Day	21th Day	Variations
Normal Control	80	82	85	87	4.67
Diabetic Control	210	220	238	250	10.77
Diabetic + Glabinclamide 40mg/kg	240	180	144	99	4.1
Diabetic + CAME (200 mg/kg)	240	222	210	205	2.66
Diabetic + CAME (400 mg/kg)	215	210	198	195	5.60

All the values are expressed as a means (+ - sem) in triplicate against Diabetic control

P* < 0.05, P <0.01 P*** < 0.001.**

The *Coprinus atrementeria* methanolic extract (200 mg/ml, 400 mg/ml), while alloxane 40 mg/kg were used for antidiabetic activity in diabetic rates and glabinclamide were used as a standard for hypoglycemic agents. Normal control on 1st day was 80% and 21th day 87%, Diabetic control on 1st day210% and 21th day 250%, Diabetic and glabinclamide at 40mg/ml on 1st day240% and 21th 99%, at 200 mg/kg CAME on 1st day 240%, 205% and 21th day 205% and 400 mg/kg extract on 1st day 215% and 21th day 195% mg/kg respectively. From the above mention data, it is clear that *A.campestress* methanolic extract did not show any remarkable change in the reduction of fasting blood glucose as compared to the diabetic and normal control groups of alloxan induced diabetic rates.

Table 4: *C.comatus* effect on serum lipid profile of normal and diabetic rats

No. of rats in each group=3	TC (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	T.G (mg/dl)
Normal Control	169.06	58.23	51.04	102.37
Diabetic Control	208.65	102.39	60.76	143.23
Diabetic + G.B (40mg/kg)	186.38	76.18	53.62	126.38
Diabetic + CCME (200 mg/kg)	195.54	96.81	58.47	123.87
Diabetic+CCME(400mg/kg	188.80	68.50	64.99	122.06

All the values are expressed as a means (+ - sem) in triplicate against Diabetic control

P* < 0.05, P <0.01 P*** < 0.001.**

Effects *of Coprinus comatus* methanolic and aqueous extract on lipid profile in alloxane induced diabetic rates

Coprinus.comatus methanolic extract showed a significant increase in Total cholesterol level (169.06% to 188.80%), triglycerides (80.00% to `160.16%) the LDL (22.16% to 60.07%) respectively and decreased the level of HDL (39.83% to 20.16%). The groups treated with *Coprinus.comatus* methanolic extract *(*CCME) and *Coprinus.comatus* aqueous extract *(*CCAE) at the dose of 200 and 400mg/kg for 21 days treatment prevented the diabetic condition in dose dependent manner and a remarkable reduction of serum cholesterol, TG, LDL, and significant increase in HDL were observed. The results were presented in the (Table no 4).

DISCUSSION

In the human body the, hydroxyl (OH) radicals produced, play a key roles in tissue wounding at the site of swelling in disease caused by the oxidative stress. The hydroxyl radicals (OH) produced in free solution, were detect their ability to demolish 2-deoxy-2-ribose into small pieces that form a pink chromogenic when giving a heat with TBA at low PH. The ferric-EDTA were placed in an incubator with hydrogen peroxide and ascorbic acid at pH 7.4. By additions of methanolic extract to the reactions mixture was found that they remove the hydroxyl (OH) radicals from the carbohydrates and prevent its degradations. The methanol extract of C.*atrementeria* proved a great potential of hydroxyl (OH) from the reaction mixture. The IC50

antioxident value of methanolic and aqueous mushroom extracts was founded to be (hydroxyl radical) 291,441 µg / ml and hydrogen peroxide 476,371µg / ml respectively. In-vitro activity test play a major role in analyzing the antidiabetic activity of drugs as a preliminary screening tool, where the initial screening of a large number of potent therapeutic candidates may be necessary [24].This can be carried out by delaying the up taking of glucose throughout the stoppage of carbohydrates hydrolyzing enzymes, α-glucosidase and α-amylase ,which are finding at the sites of the small intestines brush, and helps for the breakdown of oligosaccharides ; disaccharides in monosaccharide's suit for assimilation [25].A various studies have been documented on the inhibitory assay studies of α-amylase and α-glucosidase in various therapeutic mushroom and plants. In current study, the *in -vitro* antidiabetic studies showed a remarkable inhibition of α-amylase and α-glucosidase assay. In intestinal system the, digestive enzymes α-amylase play a major role in carbohydrates digestions. These antidiabetic medicinal approach decrease the postprandial glucose level in blood by inhibiting the enzyme α-amylase. These may be an important plan blood glucose management [26]. The percent inhibition at 2, 4, 8, and 10 µg/mL concentrations of *C. atrementeria*on α-amylase and alpha-glucosidase proved a concentrations dependens reduction in percent inhibition. Antidiabetic (α-amylase) IC50 was found to 0.75 µg/ml and (α-glucosidase) 0.80 µg/correspondingly. (Table 3 and 4). Therefore, the antidiabetic effect of C. *atrementeria* could be attribute to its inhibitory effects against α-amylase and α-glucosidase that delay the process of digestion. The inhibitory activity of the *Coprinus comatus* an *Agaricus bisporus* were more prominent than the others, we further focused the *in-vivo* hypoglycemic characterizations of the said mushrooms. After 21 day treatment, the results indicates that the extract treated antidiabetic rats progressively decrease glucose level in alloxane induced diabetic rats during experimental days. It suggests the hypoglycemic potentials of the sample mushroom. Failure of the glucose utilizations for the productions of energy occurs due to the diabetes mellitus and consequently decreases the protein storage, by tissue, resulting in progressive loss of the body weight. The present investigations revealed that extract trade diabetic rats remarkably reversed the loss of body weights in alloxane mediated diabetic rats. After alloxane induction in the body produce free radicals and leads to a tissue injury, especially beta cell of the pancreases Hallowell, Getteridge, 1985. Our study

demonstrates that the persistent hyperglycemic conditions in alloxane induced diabetic rates are due to the damages of beta cells of pancreases.

CONCLUSION

Various fractions of crude extracts of *C.comatus* was analyzed for anti-oxidant potential using DPPH, ABTS and H_2O_2 , In-vitro α-glucosidase α-amylase and In Vivo antidiabetic activities in alloxan induced diabetic rats. In this study, the minimizations of carbohydrates hydrolyzing enzymes by the C. *atrementeria* mushroom are dose dependent. Therefore the anti-diabetic effects of C. *atrementeria* mushrooms might be attribute to its inhibitory assay of hydrolysable enzymes thereby delaying the digestions of carbohydrates to prevent rising of glucose in blood.

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