FLUORIDE
Quarterly reports

Mitigating Properties of *Carissa carandus* Fruit Pulp on the Pancreatic Histopathologies of Carbofuran Treated Mice

Unique digital address (Digital object identifier [DOI] equivalent):
https://www.fluorideresearch.online/epub/files/243.pdf

Arifa HAIDER†[1], Syeda Nadia AHMAD†[2], Madiha IJAZ[1], Saira SIDDIQUE[1], Sadia Suleman[1], Mehwish NASIR[1], Atif KAMRAN[1], Muhammad Ali KANWAL[1], Iram INAYAT[1], Khawaja Raees AHMAD[3*]
†Authors having an equal contribution.

[1]Department of Zoology, University of Sargodha, Sargodha, Pakistan
[2]Department of Zoology, University of Chakwal, Chakwal, Pakistan
[3]Ex-Professor, Department of Zoology, University of Sargodha, Sargodha, Pakistan

***Corresponding author:**
Khawaja Raees Ahmad

[3*]Ex-Professor, Department of Zoology, University of Sargodha, Sargodha, Pakistan

40100, Punjab, Pakistan

Phone: (+92) 334 7511223

E-mail:k.r.ahmad@gmail.com

Accepted: 2023 Sept 27
Epub as e243: 2023 Oct 8

ABSTRACT

Purpose: The present research work was designed to explore the mitigating properties of *Carissa carandus* fruit pulp extract on the pancreatic histopathologies of Carbofuran treated mice.

Methods: There were 4 experimental groups: 1) Vehicle Control group (VC); treated with 0.1mL of 5% DMSO solution in corn oil for first 3 days and normal water for next five days. 2): Carbofuran group (CF); received 0.1mL of 5% DMSO+5mg/kg Carbofuran solution in corn oil for 3 days followed by normal water for rest of 5 days. 3): *Carissa carandus* group (CC); received 0.1mL of 5% DMSO solution in corn oil for 1-3 days, not treated with any dose on day 4 and 0.1mL of *Carissa carandus* solution was given for remaining 4 days. 4) Carbofuran+ *Carissa carandus* group (CF+CC); treated with 0.1mL of 5% DMSO+5mg/kg Carbofuran solution in corn oil till day 3, no treatment on day 4 and 0.1mL of *Carissa carandus* solution from day 5 to 8. Animals were dissected on the 9th day of study to recover intact pancreas for histopathological and micrometric studies.

Results: All histopathological and micrometric results proved that carbofuran is highly toxic for endocrine and exocrine pancreas. Prominent histopathological landmarks like lipid droplets in various areas inside the islets of Langerhans, acinar fat cells and lipid steatosis in exocrine tissue clearly illustrated degenerative effect of carbofuran. The relative area occupied by endocrine and exocrine cells per unit area (116 μm^2) was significantly ($p \leq 0.05$) lower for CF as compared to VC. Similarly significant ($p \leq 0.05$) increase in mean width of inter acinar spaces was obvious in CF (5.5±0.4µm) as compared to VC (1.7±0.2µm). All these histological and micrometric deteriorations of carbofuran treatments were noticeably reversed in CF+CC group.

Conclusions: These findings clearly indicate that carbofuran is a potent toxicant for endocrine and exocrine pancreas and *Carissa carandus* fruit pulp extract possess rescuing and regenerative potentials against such pancreatoxic effects of carbofuran in male mice.

Key-words: *Carbofuran; Carissa carandus; Koranda; Furadan; Pancreas; alpha cells; beta cells: delta cells*

INTRODUCTION

Carbofuran is derivative of carbamate insecticide commonly known as furadan[1] having anti acetyl cholinesterase activity[2]. Carbofuran exposure induced toxicity in heart, brain and liver, [3] kidney toxicity in rat sperm toxicity in mouse, [4]acute pancreas toxicity in human[5]. It has been previously reported that carbofuran induced oxidative stress in brain by crossing blood brain barrier and disrupting sodium potassium pumps in neuronal membrane of rats[6]. Moreover, it changed the lipid profile of lactate dehydrogenase, catalase, malondialdehyde, antioxidants superoxide dismutase and cholesterol, induced oxidative stress in heart of rats[3]. It also induced hematological alteration such as white blood cells, platelets distribution and liver injuries in a rat model[7].

The pancreas is an endocrine gland mainly consist of exocrine acinar cells that secrete pancreatic juice and endocrine islets of Langerhans mainly comprise alpha, beta and delta cells that secrete hormones glucagon, insulin and somatostatin respectively [8--11]. Any drastic changes in exocrine pancreas will lead to disturbance in the digestive system. Moreover, damage of endocrine pancreas will be the ultimate cause of hormonal and insulin imbalance leading towards diabetic condition[12, 13].

Several studies have shown the phytochemical potential of natural medicinal plants to impede the toxicological exposure of insecticides[14]. *Carissa carandus* (commonly known as Karonda) is considered one of the natural phytochemicals containing carisol, epimer of α-amyrin, carissic acid, linalool, β-caryophyllene, ascorbic acid, carissone, carindone and β-sitosterol in its fruit extract as well studies had shown anti-inflammatory activity of karonda in rats[15]. Researchers have proven analgesic potential[16], hepato-protective ability[17], renal protective activity[18], antiulcer potential[19], cardio-protective potential[20] and anti-cancer potential[21]. Studies also revealed that flavonoid and methanol content of koranda extract had shown mitigation potential in term of hypoglycemic as well as anti-hyperglycemic effects on pancreas of rats[22]. Therefore, aims of the present study were to provide information about the ameliorative potential of *Carissa carandus* fruit pulp extract against pancreas histopathologies develop by carbofuran exposure in albino mice.

MATERIAL AND METHODS

The present study was conducted on 40 male albino mice (*Mus musculus*) reared in animal house of The Department of Zoology, University of Sargodha. Mice were divided into four groups in separate cages. Room temperature was regularly monitored and kept at 20-23°C. Water and feed with appropriate amount of essential nutrients was provided daily. For the experiment, 20 healthy mice weighing between 28-30gm were used.

Preparation of required dose of insecticide

The standard solution was 5mg/kg, it was prepared by

For 1000g animal desired dose is = 5mg

For each 1g animal desired dose is= 5mg/1000g

For 30g animal desired dose is= 5mg/1000g*30g

= 0.15mg/30g

The dose of 0.1mL of carbofuran solution was given to the animals. So the dose of each 0.1mL of 5mg/kg of carbofuran solution must contain 0.15mg of carbofuran.

Preparation of koranda fruit pulp extract

Koranda fruits were collected and seeds were separated from fruits. Fruits were crushed in sterilized electric blender in order to obtain the koranda fruit pulp extract. This fruit extract were stored in freezer at -21°C.

Figure 1: Shows {A: *Carissa carandus* is ever green shrub consist of root, stem, flower and red to dark

purple fruit; B: *Carissa carandus* fruits; C: Ripe Karanda fruit; D: *Carissa carandus* flowers}

Corn oil and DMSO

5% DMSO was mixed with corn oil in proportion of 0.5mL DMSO and 9.5mL corn oil.

Dose Groups

Forty animals were divided into four groups and each group contains 10 animals.

- ***Vehicle Control/VC:*** the animals received 0.1mL corn oil with 5% DMSO through gavage on days 1-3 and normal water on days 5-8.
- ***Carbofuran/CF:*** was administered 0.1mL solution of carbofuran in corn oil and 5% DMSO on days 1- 3 through gavage and normal water on days 5-8.
- ***Carissa carandus*/CC:** the animals received 0.1mL corn oil with 5% DMSO on days 1-3 and 0.1mL gavage of CC solution on days 5-8.
- ***Carbofuran+Carissa carandus/CF+CC:*** the animals received 0.1mL solution of carbofuran in corn oil and 5% DMSO on days 1-3 & 0.1mL CC solution on days 5-8 through gavage.

Excision of pancreas

Each animal was dissected at 9th day. All the animals were weighed before dissections. In order to obtain the pancreas cervical dislocation was performed. With help of scissor and forceps entire pancreas was removed from right side of animal body. After excision pancreas was put into (0.9%) saline solution and then transfer into formalin fixative (60% alcohol+30% formalin+10% glacial acetic acid) for 48hours.

Histological observation

After removal from fixative solution pancreas were dehydrated in different groups of ethanol (50%, 70%, 90% and 100%) and finally treated with xylene for clearance. After that, the processed pancreas embedded into wax for blocks. Rotary microtome (ERMA TOKYO 422) used in order to obtain the 2-3 micron section. Sectioned slides were stained with help of Eosin and Haematoxylin.

Photomicrography

These slides were finally observed under trinocular research microscope (Labomed CXR2) for histopathological observations mechanically fitted with Sony Cybershot (Model: DSC-W35) 7.2 megapixel digital camera.

Micrometry

The measurement of mean Cross Sectional area (CSA) of individual acinar cells, relative abundance of beta cells, alpha cells & delta cells, number of endocrine cells/unit area, relative area occupied by endocrine cells and width of inter acinar spaces were obtained from randomly selected (400X) sections of pancreas in calibrated scale of CoralDRAW11. The calibrated values were put in the following formula.

CSA = (Length x Width/4) x 3.14

Analysis of data and statistical application

The micrometric data was analyzed by applying to ANOVA and Tukey multiple Range test (TMRT) in SPSS Software.

RESULT

Micrometric Results

Relative abundance of Alpha, Beta and Delta cells/unit area (µm^2)

Statistical analysis of data for relative abundance occupied by Alpha cells, beta cells and delta cells through one way ANOVA revealed that the overall abundance of alpha, beta cell and delta cells decreased in the CF group {(1.9 ±0. 1)$^{\alpha}$, (3.3±0.2)$^{\beta}$,(0.6±0.1)$^{\epsilon}$} and increased in CC groups{(3.0±0.0)$^{\alpha}$, (6.6±0.2)$^{\beta}$, (2.8 ±0.1)$^{\epsilon}$} as compared to VC group {(3.9 ±0.1)α, (5.9±0.2)$^{\beta}$, (2.7±0.1)$^{\epsilon}$}. Abundance rate was also high in the CF+CC mention as {(3.7±0.1)$^{\alpha}$, (7.6±0.3)$^{\beta}$, (2.5 ±0.1)$^{\epsilon}$}. However, selective degeneration is more in delta cell of CF group whereas selective regeneration was abundant in beta cells of CF+CC group (Figure 2).

This micrometric results table shows that the relative area occupied by endocrine and exocrine cells/unit area (116 µm^2) was significantly ($p \leq 0.05$) lower in CF as compared to VC. Similarly, significant ($p \leq 0.05$) extension in mean width of inter acinar spaces was obvious in CF (5.5±0.4µm) as compared to VC

(1.7±0.2µm). Mean CSA of Endocrine cells has shown very significant differences (p≤0.05) between CF (17.1 ± 0.5µm^2) and VC (28.6 ± 0.6µm^2) groups. Mean CSA of acinar cells have shown significant decreased value for CF (42.0 ± 5.7µm^2) as compared to VC (77.8± 3.0µm^2) (Table 1).

Biochemistry Results

Biochemistry results revealed that glucose level significantly increased in koranda group (242.3±13.3 mg/dl). A Cholesterol level significantly increases in CF+CC (179.3±41.3 mg/dl). Triglycerides level significantly increased in koranda group (103±19.2 mg/dl). An HDL level significantly increased in carbofuran group (41 ± 0.5 mg/dl). LDL and VLDL level increased in CF+CC group respectively (93 ± 22.1mg/dl ; 19± 2.9 mg/dl) (Table 2).

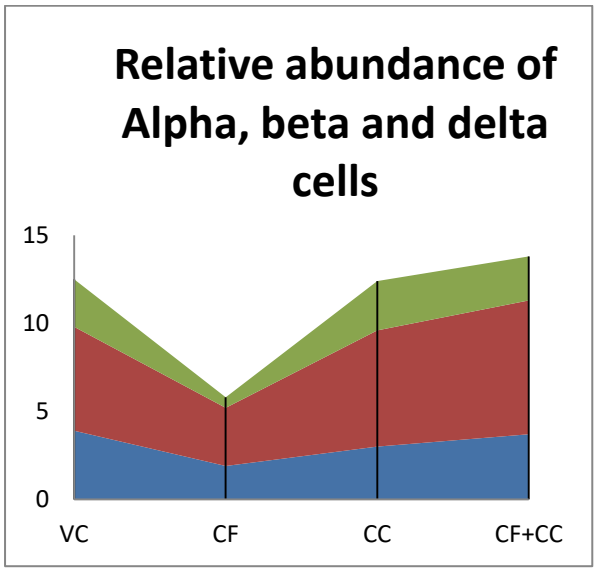

Figure 2. Area graph showing the comparison of relative abundance occupied by **Alpha** (Blue), **Beta** (Red) and **Delta** (Green) cells among groups. (VC. Control, CF. carbofuran, CC. *Carissa carandus*, CF+CC. Carbofuran+ *Carissa carandus*)

Histological Results

Histological section of mice pancreas in the vehicle control group showed normal, healthy and round shaped islets of Langerhans. Evenly distributed endocrine cells in islets and rich network of blood vessel with the close acquaintance around the islets of Langerhans was detected. Similarly, healthy acinar cells with prominent peripheral nuclei were also observed Fig: 3 (A)

Carissa carandus treated group showed more prominent features as compared to control group as the islets of Langerhans become larger and healthier, showing the hypertrophy as well as hyperplasia in islets of Langerhans as well localized hyperplasia in individual endocrine cells. There was an increase in eosinophilic cytoplast in acinar cells, so they become larger in size. Fig: 3 (B).

Mice pancreas treated with carbofuran showed the landmarks of lipid accumulation (lipid droplets formation in various spaces) inside islets of Langerhans that resulted in shrinkage of endocrine cells. Exocrine degeneration by prominent lipid steatosis was observed (as indicted by the accumulation of lipid) in acinar tissues. Both exocrine as well as endocrine pancreas steatosis were observed in histopathological result depicting the destructive nature of carbofuran insecticide. Fig: 3 (C1) (C2)

Histological sections of the Carbofuran+*Carissa carandus* group indicated both degenerative as well as rehabilitative landmarks in term of lipid accumulation inside the islets and islets emergence in the endocrine area respectively. Acinar fat cells depict carbofuran degenerative effect and developing acinar indicates the rehabilitative power of *Carissa carandus* inside the exocrine tissue. Regenerative signs were observed in both exocrine as well as endocrine depicting mitigating potential of *Carissa carandus* against carbofuran intoxication Fig: 3 (D).

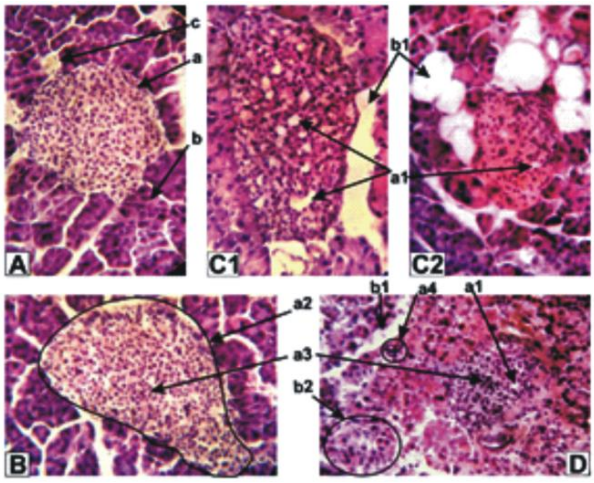

Figure 3: Histological section of albino mice pancreas. at 400x: A: (CON), B: (CC), C1: (CF), C2: (CF), D: (CF+CC); a: Islet of langerhans, b:Healthy single acinus, c: Blood vessel, a1: Fat accumulation inside the Islet, a2: Hypertrophy (hyperplasia) of the Islet, a3:

localized hyperplasia of endocrine cells, a4: emerging Islet, b1: lipid droplets in acinar cells, b2: regenerating (developing) acinar cells.

Table 1. Micrometric results of various parameters of exocrine and endocrine Pancreas (CSA= cross sectional area)

Micrometric Parameters	Groups			
	VC	CF	CC	CF+CC
ŧRelative area occupied by endocrine cells (116 um^2)	22.8± 1.9^{b}	77.7± 5.0^{a}	99.7± 7.3^{b}	92.3 ± 6.3^{b}
ŧMean Width of inter acinus spaces (µm)***(mm)***§§	1.7± 0.2^{a}	5.5± 0.4^{c}	3.5± 0.2^{b}	1.7± 0.3^{a}
ŧCSA of endocrine cell (um^2)**	28.6± 0.6^{b}	17.1± 0.5^{a}	24.8± 2.6^{b}	23.7± 2.7^{ab}
ŧCSA of acinar cell(um^2)***	77.8± 3.0^{b}	42.0± 5.7^{a}	99.3± 3.34^{c}	92.9± 5.7^{bc}
ŧRelative area occupied by exocrine cells (116 um^2)	844.2 ± 38.8^{b}	190.4 ±35.2^{a}	860.9 ±38.8^{b}	853.3± 84.5^{b}

ŧ: analyzed by ANOVA, *** highly significant, **: very significant, *: significant, no star: no significant difference. ^{abc} the mean values in a row not sharing a common superscript differ significantly (p≤0.05) with each other.

Table 2. Level of glucose, cholesterol, triglycerides, HDL, LDL and VLDL in VC, CF, CC and CF+CC treated groups

Biochemical Parameters	Mean + SEM			
	VC	CF	CC	CF+CC
ŧGlucose (mg/dl)***	84 ±3.6^{a}	242.3±13.3^{c}	178±19.0^{b}	181±19.2^{bc}
ŧ Cholesterol (mg/dl)	92±6.5^{a}	115±5.2^{a}	140±13.5^{a}	179.3±41.3^{a}
ŧ Triglycerides (mg/dl)	99±15.9^{a}	103±19.2^{a}	80 ±7.9^{a}	81 ±23.5^{a}
ŧ HDL (mg/dl)	24± 1.5^{ab}	19 ± 0.8^{a}	41 ± 0.5^{b}	26 ± 9.0^{ab}
ŧ L DL (mg/dl)	66± 13.6^{a}	82± 8.4^{a}	83± 13.7^{a}	93 ± 22.1^{a}
ŧ VLDL (mg/dl)	20 ± 3.2^{a}	20± 3.6^{a}	16± 1.5^{a}	19± 2.9^{a}

ŧ: analyzed by ANOVA, *** highly significant, **: very significant,*: significant, no star: no significant difference. ^{abc} The mean values in a row not sharing a common superscript differ significantly (p≤0.05) with each other.

DISCUSSION

Insecticidal toxicity of endocrine glands is a hot issue of research nowadays[23]. Carbofuran is derivative of carbamates insecticides that causes various drastic toxicities in liver, kidney, ovary, thyroid gland and blood, also act as endocrine disruptor[24].

The pancreas is an endocrine organ consisting exocrine acinar cells that secrete digestive enzyme and endocrine islets of Langerhans mainly comprised of alpha, beta and delta cells that secrete hormones glucagon, insulin and somatostatin respectively[25]. Results reported here have been obtained from the research work conducted on the pancreatic pathology of carbofuran exposure. Any drastic changes in exocrine pancreas become cause of digestive system damage as well toxicity in endocrine pancreas leading towards hormonal and insulin imbalance that is ultimate cause of diabetes[26].

Result of present research demonstrated that fatty acinar cells or lipid droplets in acinar cells in carbofuran treated groups seems to be well in line with the above said lipid deposition in exocrine pancreas on ethanol exposure[27]. High fat diet became the cause of lipid droplets in acinar cells and intralobular fat accumulation in exocrine pancreas[28,29]. Endocrine lipid

accumulation, fatty acinar cells and pancreatic steatosis indicate toxicity related to Carbofuran histopathologies. Whereas the micrometric results further associate these histopathological findings as comparative decreased in abundance of alpha cells, beta cells and delta cells. It has been reported in literature that carbofuran induce oxidative stress in many organisms[30]. The increase deposition of fat in exocrine and endocrine pancreas may be a secondary effect of carbofuran mediated through increased number of insulin receptors on the exocrine pancreas as well as beta cells of pancreas.

In this context it seems that increased number of receptors on above mention two types of cells may lead to lipogenesis and depositions of lipid droplets that consequently lead to pancreatic steatosis at this case at the expense of all the functional exocrine tissue and the beta cells of endocrine pancreas. This logic appears more appropriate if we see it in connection with micrometric results showing a smaller number of exocrine cells per unit area and endocrine cells per unit area. However, results obtained from endocrine pancreatic steatosis of carbofuran exposure, are not analogous to any of the prevailing information in the reported literature as it is firstly reported work. It is reported in literature that lipid accumulation becomes a cause of inflammation, oxidative stress, diabetes and pancreas pathogenesis leading to diabetic conditions in some animal models [31,32].

Certain phytochemicals with antioxidant properties improves the tissues potential to cope up with oxidative stress of intoxication[33, 34]. To our knowledge, our research study is the first report to investigate the mitigation phytochemical potential of *Carissa carandus* against carbofuran induced pancreatoxcity in mice. The unique findings of the present research work indicating hypertrophy of the Islet and localized hyperplasia of endocrine cells in *Carissa carandus* treated group. Post treatment of *Carissa carandus* in carbofuran treated animal groups shows regenerating acinar cells in exocrine pancreas and the signs of appearance of new islets also indicate proliferations and migration of the pancreatic cells from the preexists islets to new places along the blood vessels, Moreover, regeneration occurs in alpha, beta and delta cells, but high rate of selective regeneration occur exclusively in beta cells indicating *Carissa carandus* anti-diabetic effect. It has been reported in recent study that *Carissa carandus* fruit extract regenerate beta cells in wistar rats depicting anti diabetic effect[35]. *Carissa carandus* fruit extract has unique phytochemical components that explicit mitigation potential against histological pancreatoxcity by carbofuran. No oil causes rejuvenation of the endocrine capacity of the pancreas after pathological destruction of bifenthrin[36]. It is reported in literature that koranda fruits were used to make jellies and jams, but polyphenolic, flavonoid and flavanone phytochemical components of *Carissa carandus* fruit explicit anti-diabetic potential in Swiss albino rats[37]. Results of the present study revealed that Carbofuran induces following toxic effect for both endocrine (fat accumulation in islets of Langerhans, endocrine steatosis) as well as exocrine (fatty acinar cells and lipid droplets in exocrine pancreas) pancreas and koranda show anti diabetic effect.

CONCLUSIONS

Carbofuran exposure causes histopathological alterations in the pancreatic tissues. Such destructive changes by carbofuran exposure can be recovered by *Carissa carandus* fruit pulp extract therapy, signifying the enormous potential of *Carissa carandus* mainly for restorations of the endocrine component of pancreas and beta cells.

ACKNOWLEDGEMENTS

We are thankful to the University of Sargodha and University of Chakwal for providing the required facilities for this research work.

FUNDING

Not Applicable.

CONFLICT OF INTERESTS

None

REFERENCES

[1] Purushothaman BP, Kuttan R. Protective effect of curcumin against carbofuran-induced toxicity in Wistar rats. J Environ Pathol Toxicol Oncol. 2017: 36(1). https://doi.org/10.1615/JEnvironPatholToxicolOncol.2017016796

[2]Mishra S, Zhang W, Lin Z, Pang S, Huang Y, Bhatt P, Chen S. Carbofuran toxicity and its microbial degradation in contaminated environments. Chemosphere.2020:127419.https://doi.org/ 10.1016/j.chemosphere.2020.127419

[3]Jaiswal SK, Gupta VK, Ansari MD, Siddiqi NJ, Sharma B. Vitamin C acts as a hepatoprotectant in carbofuran treated rat liver slices in vitro. J Tox Rep. 2017: 265-273. https://doi.org/ 10.1016/j.toxrep.2017.06.001.

[4]Yao L, Liu L, Song S, Kuang H, Xu C. Development of indirect competitive enzyme-linked immunosorbent and immunochromatographic strip assays for carbofuran detection in fruits and vegetables. Food Agric Immunol. 2017; 28(4): 639-651. https://doi.org/ 10.1080/09540105.2017.1309359

[5}Khalaf M, Abbas M, Saleh S. Pancreatic Dysfunction Associated With Severe Acute Anticholinesterase Insecticide Poisoning In Adults. Ain Shams J Forensic Medi Clin Toxicol. 2017; 28(1): 144-148. https://doi.org/ 10.21608/ajfm.2017.18312

[6]Jaiswal SK, Siddiqi NJ, Sharma B. Carbofuran Induced Oxidative Stress Mediated Alterations in Na+-K+-ATPase Activity in Rat Brain: Amelioration by Vitamin E. J Biochem Mol Toxicol. 2014; 28(7): 320-327. https://doi.org/ 10.1002/jbt.21568

[7]Hossen MS, Tanvir EM, Prince MB, Paul S, Saha M, Ali MY, Gan SH, Khalil MI, Karim N. Protective mechanism of turmeric (*Curcuma longa*) on carbofuran-induced hematological and hepatic toxicities in a rat *model.* Pharm Biol. 2017; *55*(1):1937-1945. DOI:10.1080/13880209.2017.1345951

[8]Burke SJ, Karlstad MD, Collier JJ. Pancreatic islet responses to metabolic trauma. Shock (Augusta, Ga.). 2016; 46(3):230. DOI: 10.1097/SHK.0000000000000607

[9]Mussa BM, Sood S, Verberne AJ. Implication of neurohormonal-coupled mechanisms of gastric emptying and pancreatic secretory function in diabetic gastroparesis. World J Gastroenterol. 2018; 24(34): 3821. DOI: 10.3748/wjg.v24.i34.3821

[10]Lu CJ, Fan XY, Guo YF, Cheng ZC, Dong J, Chen JZ, Zhang B. Single-cell analyses identify distinct and intermediate states of zebrafish pancreatic islet development. J Mol Cell Biol. 2019; 11(6), DOI: 435-447.10.1093/jmcb/mjy064

[11]Nasir M, Suleman S, Mumtaz A, Ahmad K, Inayat I, Saddique S Saleem, Ahmad KR. *Nigella sativa* ameliorates the pancreatoxic effects of bifenthrin in mice. Fluorid. 2020: 53(2 Pt 2):302-311.

[12]DeaIglesia-Garcia D, Vallejo-Senra N, Iglesias-Garcia J, López-López A, Nieto L, Domínguez-Muñoz JE. Increased risk of mortality associated with pancreatic exocrine insufficiency in patients with chronic pancreatitis. J Clin Gastroenterol. 2018; 52(8): e63-e72. DOI: 10.1097/MCG.0000000000000917

[13]Rojas J, Bermudez V, Palmar J, Martínez MS, Olivar LC, Nava M, Tomey D, Rojas M, Salazar J, Garicano C, Velasco M. Pancreatic beta cell death: novel potential mechanisms in diabetes therapy. J Diabetes Res. 2018: 9601801. DOI:10.1155/2018/9601801

[14]Ahn K. The worldwide trend of using botanical drugs and strategies for developing global drugs. BMB Rep. 2017; 50(3), 111. DOI:10.5483/BMBRep.2017.50.3.221

[15]Dutta S. Assessment of acute anti-inflammatory effect of *Carissa carandas* leaf extracts in wistar albino rats. J Adv Pharm Educ. 2015; 5*:* 40–3. https://doi.org/ 10.1016/B978-0-323-85591-4.00027-1

[16]Hati M, Jena BK, Kar S, Nayak AK. Evaluation of anti-inflammatory and anti-pyretic activity of *Carissa carandas* L. leaf extract in rats. J Pharm Chem Biol Sci. 2014; 1(1): 18-25.

[17]Hegde K, Joshi AB. Hepatoprotective effect of *Carissa carandas* Linn root extract against CCl4 and paracetamol induced hepatic oxidative stress. IJEB. 2009; 47(8): 660-667.

[18]Saha R, Hossain L, Bose U, Rahman AA. Neuropharmacological and diuretic activities of *Carissa carandas* linn. leaf. Pharmacologyonline. 2010;(2):320-327.

[19]Merai AH, Jadhav AG. Anti-ulcer activity of *Carissa carandas* using root extract in albino rats. World J Pharm Pharm Sci. 2014; 3(4):1314-26.

[20]Shamim S, Ahmad SI. Pharmacodynamic study on acute hypotensive activities of *Carissa carandas* extract in normal rats. Pak J Pharm Sci. 2012; 25(3): 577-582.

[21]Sulaiman SF, Wong ST, Ooi KL, Yusof SR, Muhammad T, Sifzizul T. Anticancer study of *Carissa carandas* extracts. Project Report. 2008: 1-6.

[22]Gaurav S, Navneet N, Sandeep R, Singh P, Amit P, Manisha N, Reni K. Effect of aqueous leaves extract of *Carissa carandas* linn. on blood glucose levels of normoglycemic & alloxan induced diabetic wister rats. Int J Curr Pharm Res. 2010; 2(3): 65-7.

[23]Gerunova LK, Bardina EG, Gerunov TV, Sechkina IV. Pesticides as endocrine disruptors and neurotoxicants. IOP Confe Ser: Earth Environ Sci. 2019:052049. DOI: 10.1088/1755-1315/315/5/052049

[24]Yanti EZ, Hermadi HA, Rejeki PS, Dhamayanti Y, Luqman EM. The Increasing Inflammatory Cells, Degeneration and Duodenal Necrosis on Infant Mice from Carbofuran Exposed Mothers. J Int Dent Medical Res. 2020; 13(2):785-790.

[25]Atkinson MA, Campbell-Thompson M, Kusmartseva I, Kaestner KH. Organisation of the human pancreas in health and in diabetes. Diabetologia.2020; 63(10):1966-1973.DOI:10.1007/s00125-020-05203-7

[26]Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL. Mechanism of Pancreatic B-cell death in type 1 and type 2 diabetes: Many differences, few similarities. Diabetes. 2005; 54(2):97-107. https://doi.org/10.2337/diabetes.54.suppl_2.S97

[27]López JM, Bombi JA, Valderrama R, Giménez A, Parés A, Caballería J, Navarro S. Effects of prolonged ethanol intake and malnutrition on rat pancreas. Gut. 1996; 38(2):285-292. DOI:10.1136/gut.38.2.285

[28]Matsuda A, Makino N, Tozawa T, Shirahata N, Honda T, Ikeda Y, Sato H, Ito M, Kakizaki Y, Akamatsu M, Ueno Y. Pancreatic fat accumulation, fibrosis, and acinar cell injury in the Zucker diabetic fatty rat fed a chronic high-fat diet. Pancreas. 2014; 43(5):735. DOI:10.1097/MPA.0000000000000129

[29]Matheus VA, Monteiro LCS, Oliveira RB, Maschio DA, Collares-Buzato CB. Butyrate reduces high-fat diet-induced metabolic alterations, hepatic steatosis and pancreatic beta cell and intestinal

barrier dysfunctions in prediabetic mice. Exp Biol Med. 2017; 242(12):1214-1226. DOI:10.1177/1535370217708188

[30]Astuti SD, Victory VS, Mahmud AF, Putra AP, Winarni D. The effects of laser diode treatment on liver dysfunction of *Mus musculus* due to carbofuran exposure: An in vivo study. J Adv Vet Anim Res. 2019; 6(4):499. DOI:10.5455/javar.2019.f374

[31]Lee JS, Kim SH, Jun DW, Han JH, Jang EC, Park JY, Son BK, Kim SH, Jo YJ, Park YS, Kim YS. Clinical implications of fatty pancreas: correlations between fatty pancreas and metabolic syndrome. WJG. 2009; 15(15):1869. DOI:10.3748/wjg.15.1869

[32]Guglielmi V, Sbraccia P. Type 2 diabetes: Does pancreatic fat really matter?. *Diabetes Metab* Res Rev. 2018; 34(2):e2955. DOI:10.1002/dmrr.2955

[33]Tanvir EM, Afroz R, Chowdhury MAZ, Khalil MI, Hossain MS, Rahman MA, Rashid MH, Gan SH. Honey has a protective effect against chlorpyrifos-induced toxicity on lipid peroxidation, diagnostic markers and hepatic histoarchitecture. Eur J Integr Med. 2015; 7(5):525-533.DOI: 10.1016/j.eujim.2015.04.004

[34]Paul S, Islam M, Tanvir EM, Ahmed R, Das S, Rumpa NE, Hossen M, Parvez M, Gan SH, Khalil M. Satkara (*Citrus macroptera*) fruit protects against acetaminophen-induced hepatorenal toxicity in rats. Evid Based Complementary Altern Med. 2016: 9470954. DOI:10.1155/2016/9470954

[35]Singh R, Shrivastava M, Sharma P. Antidiabetic effect of *Carissa carandas* in rats and the possible mechanism of its insulin secretagogues activity in isolated pancreatic islets. J Biomed Ther Sci. 2019; 6(1), 1-7.

[36]Itankar PR, Lokhande SJ, Verma PR, Arora SK, Sahu RA, Patil AT. Antidiabetic potential of unripe Carissa carandas Linn. Fruit extract. Int J Curr Pharm. 2011;135(2): 430-433. DOI:10.1016/j.jep.2011.03.036

[37] Nasir M, Suleman S, Mumtaz A, Ahmada K, Inayat I, Saddique S, Saleem B, Ahmad KR. *Nigella sativa* ameliorates the pancreatoxic effects of bifenthrin in mice. Fluoride. 2020; 53(2 Pt 2):302-311.