

# Neuroprotective Effect of Sacha Inchi Oil (*Plukenetia volubilis* L.) in an Experimental Model of Epilepsy

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## ABSTRACT

**Background:** Experimental studies have demonstrated that fatty acids such as omega 3 and 6 have anti-inflammatory, anticonvulsant, cardio protective and anti-tumor effects. **Objective:** The main objective was to determine the protective effect of sacha inchi oil on pentylenetetrazole-induced seizures in albino mice. **Material and Methods:** Thirty male Balb/C albino mice of 8 weeks of age were purchased from the National Institute of Health (Lima-Peru). The animals were divided into 5 groups of 6 animals each one. Group I: Control; PTZ (pentylenetetrazole 80 mg / kg, s.c.). II: PTZ + Diazepam (1 mg / kg; s.c.). Group III, IV, V: PTZ + sachá inchi oil at single doses of 250, 500, 1000 mg / kg respectively orally. Sacha inchi oil was administered 30 minutes before induction of seizures by PTZ. Next, various parameters such as latency, seizure frequency, duration and score were scored according to Racine scale. The program Graph Pad Prism v.4 was used for statistical analysis. **Results:** As result, the experimental group treated with sachá inchi oil at maximum dose of 1000 mg/kg showed better results in response to PTZ induced seizure; low levels of seizures, frequency and duration ( $P < 0.001$ ; ANOVA,  $P < 0.001$  Post-hoc Dunnett test). **Conclusion:** It is concluded that Sacha inchi oil presented anticonvulsant effect at the highest doses tested being very similar to Diazepam. The main mechanism could be by reducing free radical and improving GABA levels in the brain. **Key words:** Sacha inchi, *Plukenetia volubilis*, Convulsion, Epilepsy, Pentylenetetrazole, Omega.

## INTRODUCTION

The modern concept of epilepsy proposed by the International League Against Epilepsy involves a chronic disorder of the brain characterized by an enduring disposition towards recurrent unprovoked seizures. The diagnosis requires at least 2 seizures occurring greater than 24 hours apart or one seizure with a relevant abnormal electroencephalographic pattern or brain scan suggesting a high probability of a second seizure.<sup>1</sup> Approximately, more than 50 million worldwide have been diagnosed with epilepsy, representing a serious problem of public health for all ages, genders and social groups. Medical reports have shown an incidence rate of epilepsy of 50 per 100 000 habitants in developed countries and 100 per 100 000 in developing countries.<sup>2</sup>

Some classic anticonvulsant drugs have shown their efficacy in the prophylaxis of convulsions (symptomatic acute), but not of unprovoked seizures (epileptic). Pathophysiology of epilepsy is known for disorders of neural depolarization membrane, neural morphology and ionic environment. These alterations cause an imbalance of excitatory neurotransmitters (glutamate and aspartate) and inhibitory (gamma amino butyric acid known as GABA).<sup>3</sup>

One of the experimental models to induce seizures is pentylenetetrazole (PTZ), this is a chemical reagent which produces generalized discharges

and acts as a noncompetitive antagonist of GABA<sub>A</sub> receptors, also induces oxidative stress in the brain altering the normal metabolism phospholipids and proteins, oxidative stress is directly associated as one of the factors that causes epilepsy.<sup>4</sup>

Otherwise, evidences about possible anticonvulsant properties for n-3 PUFA, including  $\alpha$ -linolenic, eicosapentaenoic, and docosahexaenoic acids, has been involving to cell cultures and ex vivo preparations. The main explanation is due to n-3 PUFA confer protection against seizures by increasing the threshold for action potentials and by extending the refractory period in neurons. This mechanism appears to result from a partial inhibition of sodium and calcium voltage-gated channels.<sup>5</sup>

*Plukenetia volubilis* L. (*P. volubilis*), which belongs to the family Euphorbiaceae has been used historically in the Amazon population as a food source and cosmetics, also is known as Sacha inchi.<sup>6</sup> Sacha inchi oil derived from the seeds and obtained by cold pressing presents triglycerides, tocopherols and polyphenols mainly ( $\gamma$ -tocopherol). It is one of the best sources of omega-3 (45-55%) and having more unsaturated content fatty acid (35-65%).<sup>7</sup>

Various studies related to polyunsaturated fatty acids (PUFAs) with neuroprotective effect,<sup>8</sup> therefore, this research has as main objective: To evaluate the anticonvulsant effect of Sacha inchi oil (*Plukenetia volubilis* L.) on PTZ-induced seizures in albino mice.

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## MATERIALS AND METHODS

### Animals

A total of thirty male albinos Balb / C mice (20-30 g), were purchased from the National Institute of Health (Lima-Peru). Mice were placed in a Plexiglas's cage with access to food pellets and water *ad libitum*, housed in an animal room with controlled temperature (22-24 °C) and 12/12 h light / dark. All mice were divided into five groups of six animals each one, acclimated to the laboratory 15 days prior to the experiments. The experimental protocol was carried out in accordance with the guidelines established by the European Union Animal Care (CCE Council 86/609).

### Collection of plant material

Sacha inchi seeds were collected from Pucallpa, Ucayali, Peru in June 2018. Next, 3 Kg of sample was stored in kraft paper being transported to the laboratory. Botanical identification of plant material was classified and voucher specimen (13-2014-USM-MHN) was deposited at the National Herbarium of the Universidad Nacional Mayor de San Marcos, Lima, Peru.

### Oil preparation and physicochemical analysis

The extraction is performed by cold pressing method. The obtained oil was stored at room temperature in sealed in amber bottle, physicochemical tests were performed according to AOAC.<sup>8</sup> Analysis by gas chromatography with flame ionization detector (GC-FID) was used in order to determine the content of polyunsaturated fatty acids presents in sachu inchi oil (Figure 1).<sup>9</sup>

### Drugs and chemicals

Pentylentetrazole (PTZ), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Trolox, (Sigma-Aldrich, St. Louis, MO, USA) and diazepam (Medifarma Pharmaceuticals Co.). All other chemicals used were of analytical grade.

### DPPH antioxidant activity

The antioxidant activity of Sacha inchi oil and the positive control (Trolox) was estimated based on the Brand-Williams method *et al.*<sup>10</sup> Aliquots (100 uL) at various concentrations of oil and Trolox were mixed with 900 uL of DPPH solution diluted with dichloromethane. The solution was stirred and stored at room temperature for 30 minutes in the dark. The absorbance of the reaction solution was measured by spectrophotometric method at 517 nm. The percent antioxidant activity

was calculated according to the equation: % Antioxidant activity = [(ABS control - ABS sample) / ABS control] × 100.

Where: ABS control: control absorbance; ABS sample: sample absorbance

### Anticonvulsant effect

Mice were randomly divided into five groups (n = 6) and were treated according to the experimental protocol: Group I: Control; PTZ (pentylentetrazole 80 mg / kg, s.c.). II: PTZ + Diazepam (1 mg / kg; s.c.). Group III, IV, V: PTZ + sachu inchi oil at single doses of 250, 500, 1000 mg / kg respectively orally. Treatments were administered 30 minutes before administration of PTZ (80 mg / kg, ip). Diazepam (1 mg / kg, ip) was administered 15 minutes, respectively, before injection of PTZ. Animals were placed in a Plexiglas's cage (20 x 20 x 20 cm) and observed for 30 minutes to determine the onset latency of seizures, frequency of seizures, mortality and duration of a seizure. Animals that survived after that period of time were considered protected. In addition, each attack was classified according to Racine scale modified as follows: 1-mouth and facial movements; 2-head nodding; 3-legs movement; 4-lift; 5-stretch and fall.<sup>11,12</sup>

### Biochemical parameters

Two biochemical parameters were determined by spectrophotometry in order to determine the effect of sachu inchi oil on GABA neurotransmitter and MDA as antioxidant marker *in vivo*.

### GABA estimate by spectrophotometry

GABA (gamma aminobutyric acid) from different experimental groups was determined and isolated immediately for transferring to a homogenization tube containing 5.0 mL of 0.01 M hydrochloric acid. The brain homogenate was transferred to a bottle containing 8.0 ml of alcohol all frozen and held for 1 h at 0 °C. The contents were centrifuged for 10 min at 5000 g, the supernatant was collected in petridish. The precipitate was washed with 5 mL of 70% alcohol for three times and the washings were combined with the supernatant. Then the samples were evaporated to dryness at 70 °C in a water bath. Dried samples were added 1 mL of water and 2 mL of chloroform and centrifuged at 2000 g, the upper phase containing GABA (2.0 mL) was separated and 10 mL of this was applied as spots on Whatman (N° 41). The mobile phase consisted of n-butanol (50 mL) acetic acid (12 mL) and water (60 mL).

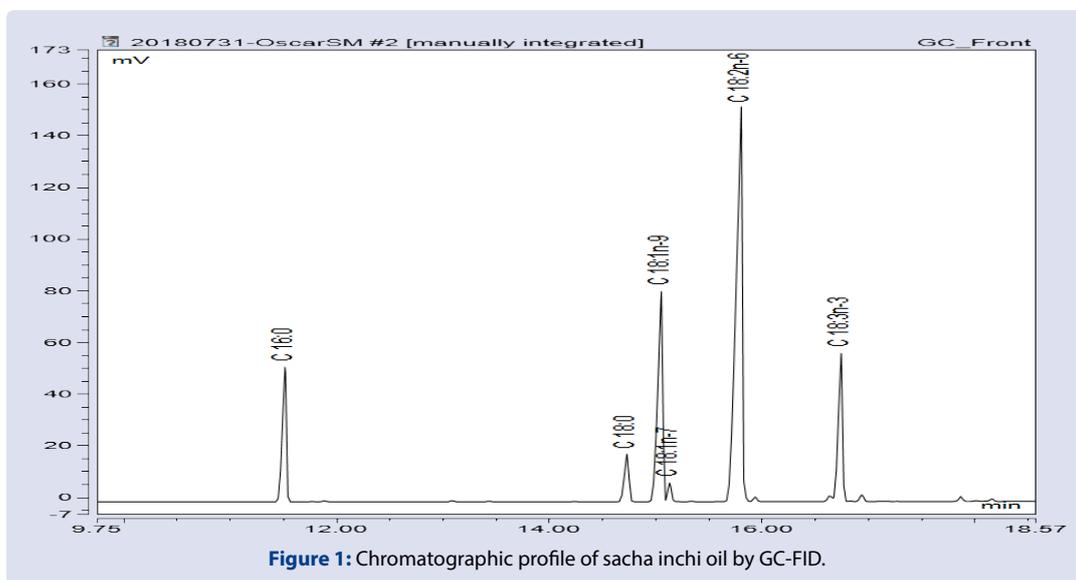


Figure 1: Chromatographic profile of sachu inchi oil by GC-FID.

The paper chromatogram was developed with an ascending technique. The paper was dried in hot air and then spread with a solution of ninhydrin 0.5% in 96% ethanol. The paper was dried for 1 hour at 90 °C. The blue color spot developed paper was sheeted and heated with 2 mL of ninhydrin solution in water bath for 5 minutes. water (5.0 mL) was added to the solution and maintained for 1 h. The supernatant (2.0 mL) was decanted and the absorbance was measured at 570 nm by spectrophotometry. GABA standard was used to extrapolate the absorbances of the samples.<sup>13</sup>

### Estimation of lipid peroxidation

Lipid peroxidation was detected by measuring malondialdehyde (MDA) determined by the method of Buege and Aust.<sup>14</sup>

### Statistical evaluation

Statistical analyzes were performed using Graph pad prism v4. The parameters measured are expressed as mean  $\pm$  standard error of the mean, percentage of antioxidant activity. Linear regression was used to obtain IC<sub>50</sub>. The anticonvulsant effect was analyzed using one-way ANOVA followed by Dunnett's test. The results were presented as mean  $\pm$  SD (n = 6). Values with *P* < 0.05 were considered statistically significant.

## RESULTS

### Chemical characteristics of Sacha inchi oil

The chemical characteristics of sacha inchi oil are described in Table 1 such as values of refractive index, density, free fatty acids, iodine index, saponification value, peroxide value and conjugated dienes of crude oil.

In the analysis by gas chromatography for the presence of palmitic acid it was determined: (C16: 0) 10.67  $\pm$  0.06%, stearic acid (C18: 0):4.36  $\pm$  0.01%, Oleic acid (C18: 1): 21.10  $\pm$  0.02%, Linoleic acid (C18: 2):50.36  $\pm$  0.01%,  $\alpha$ -linolenic acid (C18: 3): 12.20  $\pm$  0.02%, Vaccenic acid (C18: 7): 1.31  $\pm$  0.004%.

### DPPH radical scavenging

The antioxidant activity on DPPH free radical four different concentrations (100.0; 500.0; 1000.0 and 2000.0  $\mu$ g / mL) with their respective percentages were determined and placed in Table 2. The 50% inhibitory concentration (IC<sub>50</sub>) was 1350.50  $\pm$  0.56  $\mu$ g / mL. Moreover, the extract inhibited the radical DPPH in a dose dependent. The extract reduced the DPPH to 75.50% for 2000  $\mu$ g / mL.

### Anticonvulsant effect

In the anticonvulsant effect, these parameters showed a significant decrease, as the onset of seizures, seizure duration, scoring and seizure frequency (*P* < 0.01) similar to diazepam group (Figure 2). Treatment with Sacha inchi oil (500 and 1000 mg / kg, po) and diazepam (1 mg / kg, ip) showed significant protection against PTZ seizures (Table 3) induced.

## DISCUSSION

The scientific literature has shown several components from *P. volubilis* seeds. especially high content of fatty acids such as  $\alpha$ -linolenic acid ( $\alpha$ -Ln) ( $\omega$ 3, 12.8-16.0 g / 100 g seed), followed by linoleic fatty acid (L) ( $\omega$ 6, 12.4-14.1 g / 100 g seed). The  $\omega$ 6 /  $\omega$ 3 ratio within the range of 0.83-1.09.<sup>15</sup> The  $\gamma$  and  $\delta$  tocopherols were the most important tocopherols, while the most representative phytosterols have been the  $\beta$ -sitosterol and stigmasterol. Furthermore, Sacha inchi oil contains unsaturated fatty acids such as linolenic acid (omega 3) (C18: 3), linoleic acid (omega 6) (C18: 2) and oleic acid (omega 9) (C18: 1). Also, it has been reported palmitic acid (C16: 0)

**Table 1: Chemical characteristics of sacha inchi oil.**

Physicochemical characteristics	Sacha Inchi*
Refractive index	1.478 $\pm$ 0.00
Density at 25 ° C	0.920 $\pm$ 0.02
Free fatty acids (% Oleic acid)	0.200 $\pm$ 0.02
Iodine index (g of I/100g)	93.80 $\pm$ 0.40
Peroxide values mEqO <sub>2</sub> /Kg	0.890 $\pm$ 0.05
]Saponification index (mg/KOH/g)	185.50 $\pm$ 0.20

\*Average of three repetitions  $\pm$  standard deviation.

**Table 2: Antioxidant activity of Sacha inchi oil against DPPH radical.**

Concentration $\mu$ g / mL	% Antioxidant Activity	
	Sacha inchi oil	Trolox
2000.0	75.50 $\pm$ 2.1	97.30 $\pm$ 1.0
1000.0	43.25 $\pm$ 1.0	95.20 $\pm$ 2.0
500.0	19.75 $\pm$ 1.1	96.61 $\pm$ 1.0
100.0	9.80 $\pm$ 0.6	35.50 $\pm$ 0.5

\*Average of three repetitions  $\pm$  standard deviation.

**Table 3: Protection of sacha inchi oil on PTZ-induced seizures.**

Indicator	n = 6				
	PTZ	DZP	250	500	1000
Alive	0/6	6/6	3/6	5/6	6/6
Protection (%)	0	100	50	83	100

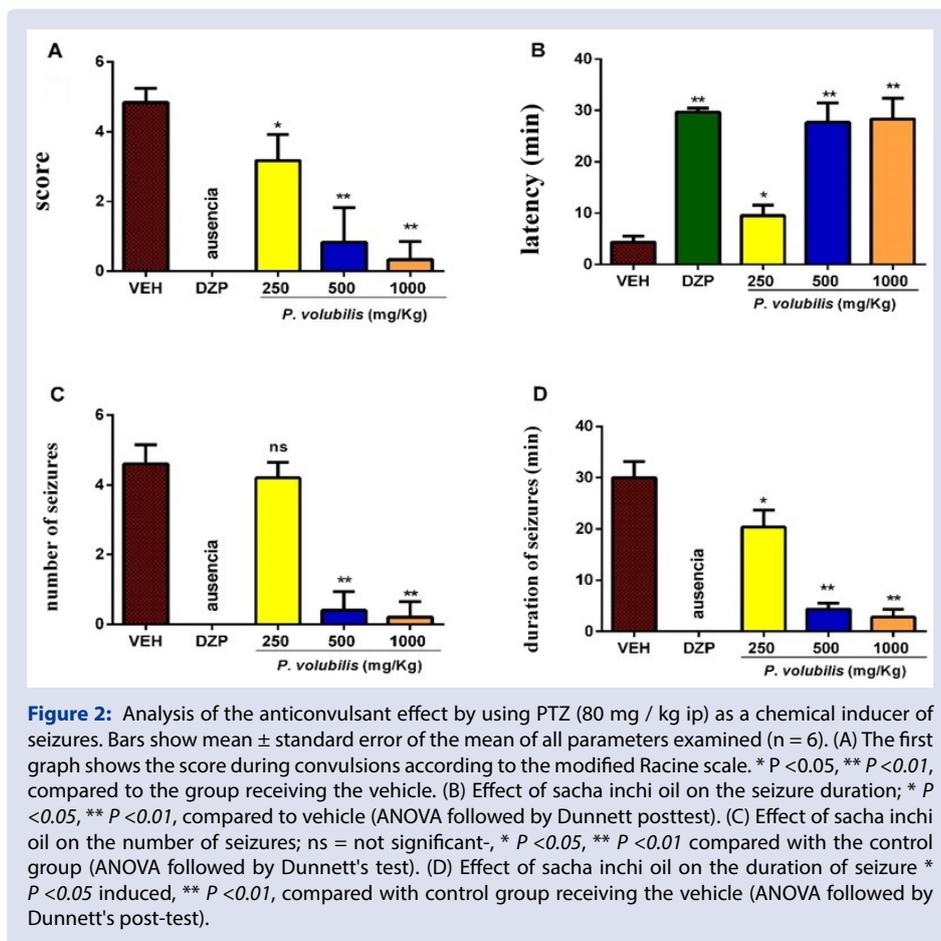
DZP: Diazepam; PTZ: pentylene tetrazole.

and stearic acid (C:18) by gas chromatography and these findings are within the range established and reported by Maurer *et al.*<sup>16</sup>

In regard to oxidative stress, increased formation of free radicals, particularly reactive oxygen species (ROS) produces a high intracellular concentration of Ca<sup>2+</sup>, which is involved in mechanisms of neuronal necrosis or apoptosis system.<sup>17</sup> The relationship between free radicals and seizures might have a multiple mechanism, for example, free radicals may induce seizures by direct inactivation of glutamine synthase, establishing abnormal release of excitatory neurotransmitters such as glutamic acid. Also, the onset of convulsions induced by oxygen in animals correlate with decreased levels of GABA in the cerebral cortex due to inhibition of glutamate decarboxylase by oxygen free radicals.<sup>18-20</sup>

The antioxidant activity demonstrated in this study on DPPH radical, and MDA *in vivo* may be related to the anticonvulsant effect.<sup>19</sup> Seghatoleslam *et al.* studied the administration of ascorbic acid and flavonoids of *N. sativa* in animal studies demonstrating the reduction of neuronal damage triggered by free radicals, which are particularly high in the processes of inflammation and neurodegenerative disorders. Also animals pretreated with ascorbic acid, before induction of seizures by pilocarpine have shown a significant reduction of 60% in the frequency of brain damage hippocampal, the latency to the first seizures, suppression behavioral convulsive episodes, followed by decreased lipid peroxidation (MDA and nitric oxide (NO)).<sup>21,22</sup>

It is known that omega-3 fatty acids can improve GABAergic transmission in animals with epilepsy by stimulating the formation of new hippocampal interneurons or by altering the expression of calcium-binding proteins.<sup>23</sup> On the other hand, polyunsaturated fatty acids, including n-3 (omega-3) fatty acids such as eicopentanoic acid (EPA), docosahexaenoic acid (DHA) and n-6 fatty acid (omega-6) as dihomoc-linolenic acid and arachidonic acid are present in high levels



**Table 4: Estimate of GABA and MDA in mouse brain.**

Indicator	n = 6				
	PTZ	DZP	250	500	1000
GABA (ng / g tissue)	12 $\pm$ 1.5	87 $\pm$ 1.8 **	35 $\pm$ 2.5 *	62 $\pm$ 1.2 **	73 $\pm$ 1.1 **
(MDA 10 <sup>-6</sup> mol / g brain tissue)	8.3 $\pm$ 0.2	4.4 $\pm$ 0.2*	3.0 $\pm$ 0.2**	1.5 $\pm$ 0.02**	1.1 $\pm$ 0.03*

MDA: malondialdehyde; DZP: Diazepam; PTZ: pentylenetetrazole; \* P < 0.05; \*\* P < 0.01.

in the brain. In humans, the intake of long-chain omega-3 fatty acids, which are commonly found in fish and food supplements such as fish oil, not only contributes to the development of the central nervous system, but can also reduce the risk of certain nervous system diseases in adults, including epilepsy as reported by Bourre.<sup>24</sup>

Although, there is no well-established importance of polyunsaturated fatty acids in neurodegenerative and neurological diseases, it is necessary carry out further studies in different experimental models to validate the use of Sacha Inchi as co-adjuvant in epileptic diseases.

## CONCLUSION

It is concluded that Sacha inchi oil (*P. volubilis* L.) showed protective effect on pentylenetetrazole-induced seizures at tested dosages being the most active dose of 1000 mg / kg, and the mechanism would be via the generation of the neurotransmitter GABA and its antioxidant effect.

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Nil

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## CONFLICTS OF INTEREST

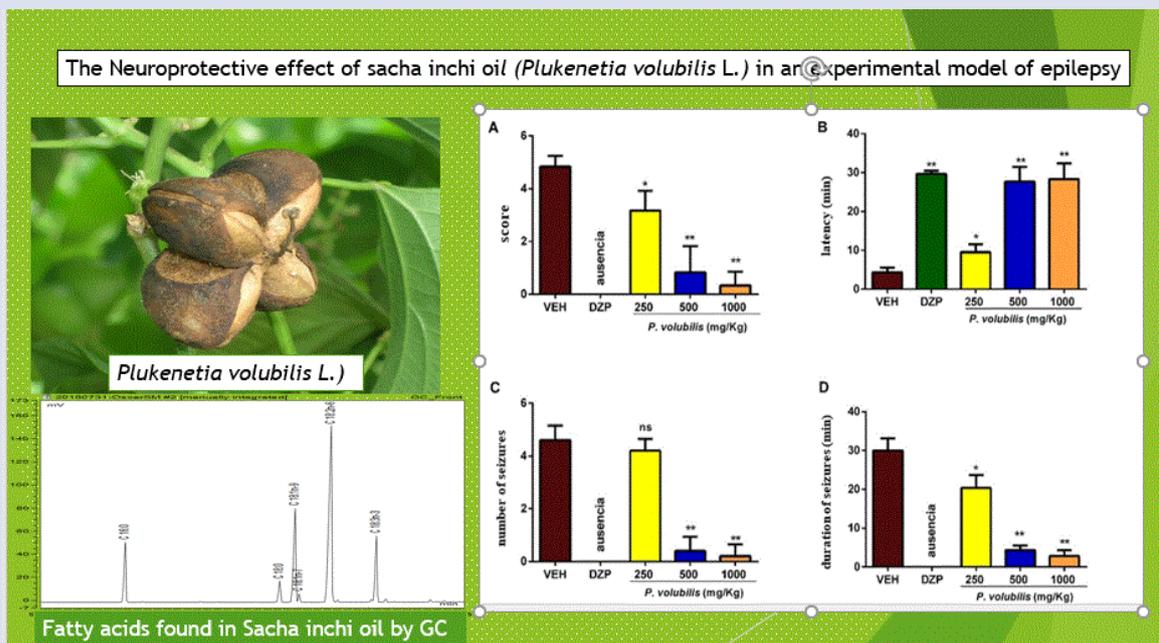
There are no conflicts of interest.

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## GRAPHICAL ABSTRACT



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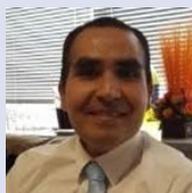
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