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Serotonin Gene Polymorphisms Association with Immune Response, Intestinal Inflammation, and Anxiety Behaviour in Rats Exposed to Sodium Arsenite

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Abstract

Sodium arsenite (SA) is one of the major environmental toxicants and one of the causes of colitis. The potential impact of genetic variation in the serotonin gene exon on behaviours, such as anxiety and immune responses, was explored to investigate the development of intestinal mucosal inflammation, which causes colon pathophysiology in rats exposed to SA. Three groups of 10 rats were employed to achieve our experiment goal: Group 1 served as the control group, Group 2 was administered SA at a dose of 5 mg/kg, and Group 3 were administered SA at a dose of 30 mg/kg for 60 days. Gene expressions for serotonin, cytokine profiles, oxidative stress, and anxious behaviour were estimated. Results indicated a significant increase ($p < 0.05$) in the serotonin, pro-inflammatory cytokines, and oxidative stress (reactive oxygen species, hydrogen peroxide, and nitric oxide) levels and caspase-1 activity in rats exposed to SA, while the level of IL-10 showed a significant decrease in these rats compared with the control group. Rats that had been exposed to SA showed anxiety-like behaviours when subjected to open field testing, as opposed to those in the control group. PCR-single-strand conformation polymorphism (SSCP) analysis showed a multitude of single nucleotide polymorphisms (SNPs) and haplotypes within the serotonin exon area. These genetic variations were correlated with alterations in the serotonin gene polymorphism, which are usually responsible for colitis development. This study demonstrates that genetic variants in the serotonin gene exon are associated with elevated colonic serotonin levels, which subsequently resulted in increased pro-inflammatory cytokines, elevated oxidative stress, increased Caspase-1 activity, decreased anti-inflammatory cytokines, and anxiety-like behaviors in rats exposed to sodium arsenite.

Keywords: Anxiety behaviour, Intestinal Inflammation, Serotonin gene, Sodium arsenite, Open field test, Oxidative stress

1. Introduction

Inorganic sodium arsenite (SA) is one of the important environmental toxicants worldwide. One major cause for human intoxication caused by this element is the consumption of polluted drinking water, which often contains high levels of inorganic arsenic, particularly in groundwater used as drinking water (Bhowmick *et al*., 2018). SA, a form of arsenic, is quickly absorbed by the gastrointestinal system and tends to accumulate in various body fluids and tissues. Arsenite toxicity usually induces changes in various bodily markers and behaviours; several researchers have confirmed that exposure to elevated SA levels in drinking water causes detrimental effects on human health (Prakash and Verma, 2021). These effects encompass a range of physiological disorders, including oxidative stress, gastrointestinal disorders, and immune dysfunction (Jing *et al.,* 2012; Acharyya *et al.,* 2015; Campbell and Colgan, 2019). Gastrointestinal reactive oxygen species (ROS) can be generated from endogenous sources, such as the mitochondrial respiratory system, or through the involvement of the intestinal microbiome (Maraldi *et al*., 2021).

Arsenic toxicity in the gastrointestinal tract causes damage to the epithelial mucosa, especially to enterochromaffin cells (EC), leading to symptoms such as ulcerative colitis, nausea, abdominal pain, and irritable bowel syndrome (Prakash and Verma, 2021). Endocrine enterochromaffin cells are distributed throughout the gut mucosa and are responsible for the secretion of serotonin; the gastrointestinal tract produces, stores, and releases approximately 95% of the body's serotonin. A lack of serotonin is related to a high incidence of gastrointestinal diseases, and it is also involved in processes such as absorption and assimilation, as well as influences mucus and fluid secretion and ion transport within the gastrointestinal system (Hatamnejad *et al*., 2022). Given arsenic's impact on the gastrointestinal tract, its influence on enterochromaffin cells and serotonin regulation is particularly relevant for understanding gut-related disorders. Serotonin (5-HT), an intestinal serotonin, plays a decisive role in regulating different gastrointestinal functions, including gut motility, sensation, increased peristalsis and immune response regulation (Prakash and

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Verma, 2021). Furthermore, pancreatic enzyme secretion is under the regulatory influence of intestinal serotonin. The impact of 5-HT is accomplished through epithelial 5-HTR2 receptors close to the intestinal mucosa (Banskota *et al*., 2019).

Recent reports also suggest that serotonin has immunomodulatory capabilities that can impact the severity and development of inflammation within the intestines, especially in inflammatory bowel diseases (Coates *et al*., 2017; Ala, 2022). Changes in the serotonin amount secreted by the EC stimulate the nervous system and induce both pathophysiological and physiological responses, such as irritable bowel syndrome (IBS), which has been associated with an imbalance of intestinal microbiota, abnormal intestinal motility, mucosal inflammation, and psychosocial factors (Kendig and Grider, 2015; Esteban-Zubero *et al*., 2017). Moreover, several studies have found that serotonin plays a chief role in activating immune cells to generate proinflammatory cytokines. These findings confirmed that the severity of heavy metals toxic-induced colitis connected with deficiency of IL-10 is raised when combined with serotonin which promotes impacts of serotonin reuptake transporters deficiency. Therefore, serotonin is considered a critical signalling molecule in the colitis pathogenesis (Kwon *et al*., 2019).

Some reports showed that arsenic plays a role in inducing injurious impacts on the immune system of both humans and lab animals; arsenic causes immune suppression and alters the activation and proliferation of macrophages and Th1/Th2 lymphocytes and secretion of cytokines IL-2, IL-10, IL-5, IL-4, IFN-γ and TNF-α (Dangleben *et al*., 2013). Ferrario *et al*. (2016) demonstrated that lymphocytes in individuals exposed to arsenic-contaminated water showed a lower proliferation rate than those of individuals unexposed in the same regions. Moreover, the researchers found that arsenic levels positively correlated with oxidative stresses such as malondialdehyde (MDA), and negatively correlated with superoxide dismutase (SOD) and vitamin E levels in the serum, indicating that chronic exposure stimulates lymphocyte oxidative harm (Dangleben *et al*., 2016 ; Ferrario *et al*., 2016).

Sodium arsenite has genotoxic, carcinogenic, and mutagenic impacts (Ozturk *et al*., 2022). It can also trigger epigenetic disorders via mRNA dysregulations and alterations in histone modifications and global methylation. Moreover, arsenic affects the oxidation processes of proteins and enzymes and, therefore, impacts DNA by producing cross-links between proteins and DNA. Heavy metals' genotoxic ability originates from their capacity to produce reactive oxygen species (ROS), which hinders DNA repair capacity. Due to their high affinity for the sulfhydryl group, these metals can cause negative effects such as genotoxic impacts and mutations in organisms, ultimately leading to the inactivation of essential enzymes. This damages the repair systems by either reducing the expression of DNA repair enzymes or inhibiting ligation (Litwin *et al*., 2013).

Bjørklund *et al.* (2020) mentioned the occurrence of behavioural changes, such as anxiety and memory impairments, resulting from heavy metal poisoning. The open field tests are a widely used ethological estimation tool to evaluate rat anxiety-like behaviour. During this experimental procedure, rodents can freely navigate an unobstructed enclosure enclosed by vertical barriers. In this scenario, rodents demonstrate a propensity to avoid the central areas of the arena and instead choose to stay in the peripheral sections (Kuniishi *et al.,* 2017). This study focuses on investigating the possibility that changes in the level and gene polymorphism of serotonin in the intestine play a significant role in behavioural disorders and immune response activation; this inflammation of the intestinal mucosa contributes to the pathophysiology of colitis and irritable bowel syndrome (IBS) in rats exposed to sodium arsenite.

2. Material and methods

2.1. Ethical approval

The study protocol received approval from the Department of Biology, College of Sciences, University of Babylon (Protocol No. 1312/10-3-2023). The experiments were conducted in adherence to approved guidelines and ethical standards outlined by the National Committee for Research Ethics in Science and Technology (NETNT).

2.2. Experimental animals

This experiment used 30 male Wistar rats (weighing 250 ± 10 g). The rats were obtained from the animal facility at the College of Science, University of Thi Qar. The rats were kept under standard conditions of humidity (50 ± 10%) and temperature (24 °C ± 2) with a 12/12 h dark-light cycle, provided in food and water.

2.3. Experimental design

The rats were randomly divided into three groups; each group contained ten rats. In Group 1, the control group, the rats were administered 1.0 mL of distilled water orally via gavage for 60 days. In Group 2, the sodium arsenite group (SA 5 mg), the rats were administered a low dose (5 mg/kg) of $NaAsO_2$ orally via gavage for 60 days. In Group 3, the sodium arsenite group (SA 30 mg), the rats were administered a low dose (30 mg/kg) of $NaAsO_2$ orally via gavage for 60 days.

2.4. Preparation of sodium arsenite ($NaAsO_2$)

SA was acquired from the laboratory of the Department of Chemistry at the College of Science, University of Babylon, Iraq. Aqueous solutions of low and high doses of SA were prepared by mixing 5 and 30 mg/kg of SA with distilled water, respectively. Animals in groups 2, 3 were orally fed daily with 1.0 ml of either the low or high dose of the SA aqueous solution, respectively. The aqueous extract of sodium arsenate (stock) was stored at room temperature because it is classified as a stable solution. The predetermined lethal dose (LD50) of SA is about 41 mg/kg (Bashir *et al*., 2006).

2.5. Tissue samples

Approximately 50–100 mg of intestinal tissues, specifically from the colon, was rinsed using a phosphate-buffered saline (PBS) solution. The pH range of the PBS was 7.0 to 7.4, and its concentration was 0.01 M. The rinse stage aimed to cleanse with phosphate-buffered saline (PBS) to eliminate residual blood, followed by immediately freezing in liquid nitrogen for further usage. The tissue sample was homogenised in a phosphate-buffered saline (PBS) solution using a glass homogeniser

(Model USCG-P150N, China). The homogenates were centrifuged at 5000 g at a temperature of 4°C for 10 minutes. The resulting supernatants were preserved immediately by freezing them until they could be analysed for serotonin, cytokines, and reactive oxygen species (ROS).

2.6. Biochemical parameters

Estimation of serotonin, cytokine profiles, and Caspase-1 activity

Serotonin levels, pro-inflammatory cytokines (interferon INF-γ, tumour necrosis factor-alpha TNF-α, IL-1β, and IL-12 levels), anti-inflammatory cytokine (IL-10) levels, and caspase-1 activity were measured in intestinal tissue supernatant by using special kits of the enzyme-linked immunosorbent assay (ELISA).

2.7. Estimation of oxidative stress markers (µmol/g)

Reactive oxygen species

According to the manufacturer's instructions, reactive oxygen species (ROS) levels were measured in colonic tissue supernatant using the fluorescence method of the ELISA kit principle (MyBioSource company, United States) at excitation wavelengths 500 nm and emission wavelengths 525 nm.

2.7.1. Hydrogen peroxide and nitric oxide levels

According to the manufacturer's instructions, hydrogen peroxide (H_2O_2) and nitric oxide (NO) levels were measured in colonic tissue supernatant using a colorimetric assay ELISA kit (Elabscience, China). The NO and H_2O_2 levels can be calculated by measuring the OD values at 550 nm and 405 nm, respectively.

2.8. Behavioural study

The open field test

The open field test is a method used to assess locomotor activity, exploratory behaviour, and anxiety levels. The open field comprises a rectangular enclosure measuring 70 × 70 cm, including walls 30 cm in height. Thick paper was placed on the enclosure's floor, upon which lines were drawn to partition the floor into sixteen individual squares. Isolated, dimly illuminated room was used to conduct the research. During the experiment, a rat was placed separately in one of the box's four corners and given 10 minutes to investigate the equipment. Following the conclusion of the experimental trials, the rat was reintroduced to its designated housing enclosure. At the same time, the open field apparatus underwent a thorough cleansing procedure employing a 70% ethyl alcohol solution to ensure optimal sanitation before further testing sessions. Exploratory behaviours and anxiety levels were assessed by measuring the frequency of entrances to the middle square and the time spent in this area. An elevated occurrence of these behaviours implies heightened locomotor activity and exploratory tendencies, as well as a reduced fear level (Habib *et al*., 2015; CHabuk *et al*., 2019). The behavioural parameters were recorded on video and automatically analysed using the software SMARTV3.0.06 (Panlab, Spain).

2.9. Genomic DNA extraction

A DNA Mini Kit (Favorgen, Taiwan) was used to extract genomic DNA from 1.5 mL tissue samples. The purity of the isolated genomic DNA was confirmed to be at a 260: 280 ratio using a nanodrop spectrophotometric method. The established standard procedures determined the integrity of the recovered genomic DNA using agarose gel electrophoresis.

PCR experiment

Gene-specific primers (forward 5-TCTGCCCGATTTTCAAAG-3; reverse 5-GTGAGAGACTCCAAGCTGAAA-3) were used to amplify distinct fragments within the rs8154473 region, including the third exon of the SLC6A4 gene of the rat[21]. The polymerase chain reaction (PCR) experiment was performed using a lyophilised PCR Accu Power Pre Mix (Promega Co., USA), with a final volume of 20 mL for the amplified fragment (initial denaturation 95°C for 5 min, 62°C annealing, and 72°C extension). Following the completion of the PCR test, electrophoresis on agarose gels verified that the resulting PCR products exhibited the anticipated size of 548 base pairs. Per the specified protocols, all PCR products were submitted to a Sanger dideoxy-sequencing facility (Macrogen Inc., South Korea) to verify the electrophoretic genotypes acquired for all samples. BioEdit Suite, version 7.0, correlated the observed variance with the relevant DNA sequences.

2.10. . Statistical analysis

The results were presented as the mean value accompanied by the standard error (SE). The statistical analysis was conducted using a one-way analysis of variance (ANOVA) and the Duncan post-hoc and correlation tests after data were tested for normality and homogeneity of variances. The software SPSS version 25 was employed for this analysis, under the significance value of $P < 0.05$.

3. Results

3.1. Colonic serotonin levels

Figure 1 depicts the serotonin levels in the colons of rats subjected to different doses of SA (5 and 30 mg/kg) over 60 days. Rats that were administered SA demonstrated a statistically significant ($P < 0.05$) elevation in serotonin levels within the colon, as observed in the 5- and 30-mg/kg dosage groups compared to the control group. However, a statistically significant rise ($P < 0.05$) in serotonin levels was seen in rats belonging to Group 3 compared to those in Group 2.

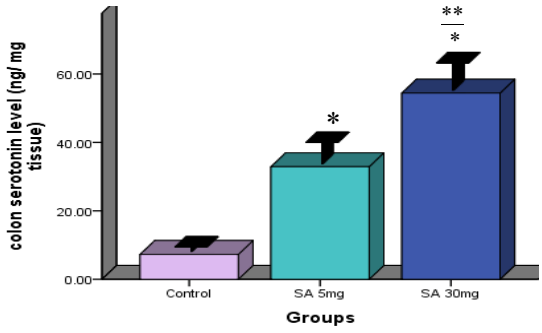

Figure 1. Levels of Serotonin in the colon tissue of rats exposed to SA for 60 days. Each data represents mean±SE from ten rats. *P < 0.05 compared with control group, **P < 0.05 compared with SA group 3

3.2. Colonic Cytokines profile

The changes in the pro-inflammatory cytokines (TNF-α, IL-1β, IL-12), anti-inflammatory cytokine (IL-10) levels, and caspase-1 activity in the colon tissue of laboratory animals are shown in Table 1. There was a significant increase ($P < 0.05$) in the levels of pro-inflammatory cytokines and caspase-1 activity in groups exposed to SA (5 and 30 mg/kg) for 60 days compared to the control group. Meanwhile, the levels of IL-10 significantly decreased ($P < 0.05$) in rats that received SA compared to those of the control group using a one-way ANOVA with the Duncan post-hoc test.

Table 1. Levels of cytokines profiles and caspase-1 activity in the colon tissue from rats exposed to SA for 60 days (n=10 per group).

Parameters \ Groups	Control group mean±SE	SA 5mg group mean±SE	SA 30mg group mean±SE
IFN- γ (pg/mg tissue)	94.28 ± 2.14	96.7± 3.62	99.29 ± 5.07
TNF-α (pg/mg tissue)	32.87 ±1.46	132.57 ± 4.24*	171.71± 3.03*#
IL-12 (pg/mg tissue)	61.12 ± 3.64	252.41 ± 6.63*	305.62± 8.212*#
IL-1β (pg/mg tissue)	4.19 ± 0.44	81.91 ± 3.08*	157.59 ± 2.33*#
caspase-1 (pg/mg tissue)	5.51 ± 0.45	15.97 ± 0.76*	33.13 ± 2.57*#
IL-10 (pg/mg tissue)	7.80 ± 0.73	4.36 ± 0.55*	3.76 ± 0.49*

* The means are statistically significant compared with the control group at $P < 0.05$.

The means are statistically significant compared with the SA group (5gm/kg) at $P < 0.05$.

3.3. Oxidative stress markers

Table 2 presents the levels of oxidative stress detected in the colon tissue across various experimental groups. The experimental findings demonstrate significant elevations ($P < 0.05$) in the concentrations of ROS, H2O2, and NO in the rats subjected to treatment with SA (5 and 30 mg/kg) in comparison to those of the control group using a one-way ANOVA with the Duncan post-hoc test.

Table 2. Presents the levels of oxidative stress markers in colonic tissue of rats exposed to SA for 60 days (n=10 per group).

Parameters \ Groups	Control group mean±SE	SA 5mg group mean±SE	SA 30mg group mean±SE
ROS µmol/ g tissue	2.29±0.39	24.59 ±1.83*	56.29±4.59*#
H_2O_2 mmol /mg tissue	77.28±2.96	133.23±3.32*	219.74±5.547*#
NO µmol/g tissue	1.68±0.33	11.89±1.54*	39.48±2.94*#

* The means are statistically significant compared with the control group at $P < 0.05$.

The means are statistically significant compared with the SA group (5gm/kg) at $P < 0.05$.

3.4. Correlation between colonic Serotonin with cytokines profile and oxidative stress

The Spearman correlation coefficient suggests a highly significant positive correlation at the 0.01 level between serotonin and pro-inflammatory cytokines (IL-1β, IL-12, and TNF-α), caspase-1 activity, and oxidative stress parameters. There was a negative correlation at the 0.01 level between serotonin, IL-10, and interferon INF-γ, which showed no significant correlation with serotonin. The correlations between the parameters were analysis by Pearson's test.

Table 3. Correlation coefficients for colonic serotonin with cytokines profile and oxidative stress.

		IFN	TNF-α	IL-12	IL-1β	caspase-1	IL-10	ROS	NO	H_2O_2
Serotonin levels	Pearson Correlation	0.244	0.875**	0.862**	0.921**	0.858**	- 0.767**	0.825**	0.903**	0.791**
	Sig. (2-tailed)	0.194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

** Correlation is significant at the 0.01 level (2-tailed).

3.5. The open field test

This test assesses the locomotion, exploration, and anxiety of rats exposed to SA for 60 days by analysing latency time (sec.), ambulation (peripheral and central squares), and the amount of time spent in each area (Table 4). The time that rats spent in the centre squares can be used to assess changes in anxiety-like behaviour; the more anxious rats spent remarkably less time in the central area and more time near the walls. The results show a significant increase ($p < 0.05$) in mean latency time (sec.) and the time spent in the peripheral square (sec.) of the rats treated with SA compared to the control group. The total mean ambulation number (square, peripheral, and central) and amount of time spent in the central square (sec.) of SA-treated rats showed a significant decrease with a significant increase in latency time compared to those of the control group, indicating anxiety-like behaviour.

Table 4: Exposure of rats to SA for 60 days led to locomotors reduction and anxiety behavior, measured by open-fled tests for 10 min. (n=10 per group).

Groups / parameters test	Control group mean±SD	SA 5mg group mean±SD	SA 30mg group mean±SD
latency time(sec.)	69.86 ± 18.54	185.62 ± 16.19*	281.83 ± 10.42*#
Ambulation	51.99 ±5.94	14.10 ± 3.90*	9.00 ± 1.41*#
Time spent in the central square(sec.)	243.37± 12.55	75.38 ± 8.08*	57.18± 8.79*#
Time spent in the Peripheral square(sec.)	379.22± 60.93	525.58 ± 8.11*	542.65± 6.98*

* The means are statistically significant compared with the control group at $P < 0.05$.
# The means are statistically significant compared with the SA group (5gm/kg) at $P < 0.05$.

3.6. Genetic polymorphisms of 5HTT

First, the genomic DNA (Fig. 2) was isolated from the tissue samples so that the target region of the third exon of the SLC6A4 gene in rats could be amplified.

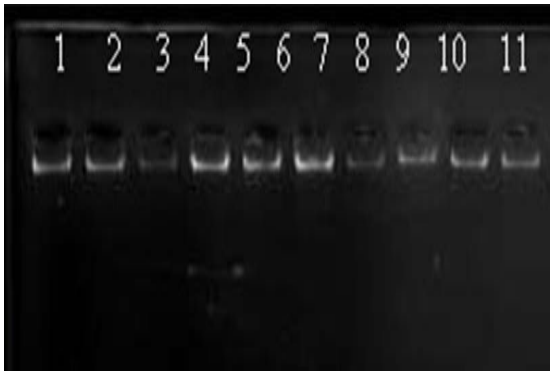

Figure 2. shows the electrophoretic pattern of genomic DNA derived from tissue samples. Lane 1 to lane 11 corresponds to the genomic DNA derived from tissue samples, which were subjected to electrophoresis under the following conditions: 1% agarose gel was used, and 75 volts with 20 milliamperes were applied for 1 hour. Each well contained 10 microlitres of the DNA sample, which was subsequently stained with ethidium bromide.

The process of SLC6A4 genotyping involved amplifying the genomic DNA using primers specifically designed for this purpose. This amplification was carried out using a thermocycler device, following the ideal conditions for this procedure. The findings indicate the detection of a solitary band (548 bp) representing the target sequence of the SLC6A4 gene on the agarose gel (Fig. 2).

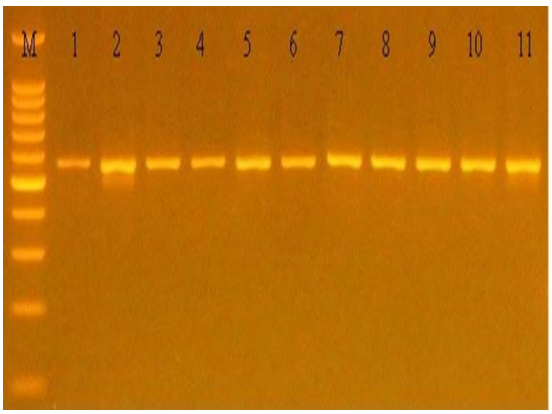

Figure 3. shows that agarose gel electrophoresis was conducted to analyse the patterns of the amplified products (SLC6A4). The DNA size marker, denoted as M, was used for reference. Lanes 1–11 exhibited amplified products that appeared as a single band with a size of 548 bp. The experimental parameters for electrophoresis were established as follows: The agarose gel used in this experiment had a concentration of 1%. Following electrophoresis, the gel was stained with ethidium bromide.

The sequencing results (Fig. 3) show the presence of three SNPs determined in the third exon of the SLC6A4 gene depending on Primer 3 plus reference sequences: XM_017597042.2 (https://www.ncbi.nlm.nih.gov/nuccore/XM_017597042.2). These SNPs were T\C 270, G\T 272, and G\T 525, located at exon 3 according to the reference sequence (ID: XM_017597042.2). Three sequences from 30 sequence isolates were deposited in GenBank at the DNA Data Bank Japan (DDBJ) database (LC777458, LC777459, LC777460).

The results demonstrate a significant association ($p < 0.05$) between DNA polymorphisms according to the three SNPs determined in the third exon of the SLC6A4 gene with treatment compared with the control groups in the table below.

Figure 4. Sequences alignment ID: XM_017597042.2 results for a rat in the third exon of SLC6A4 gene depending on Primer3 [plus] reference sequences: XM_017597042.2 by Bio Edit program version 7.2.5.

Table 5. Mutations distribution in the third exon of SLC6A4 gene

	SNP1(T\C)	SNP 2(G\T)	SNP 3 (T\G)	The chi-square	P- Value
Group 1	8	0	2		
Group2	5	5	0	10.53	0.03*
Group3	6	1	3		
Colum total	10	10	10		

*P ≤ 0.05

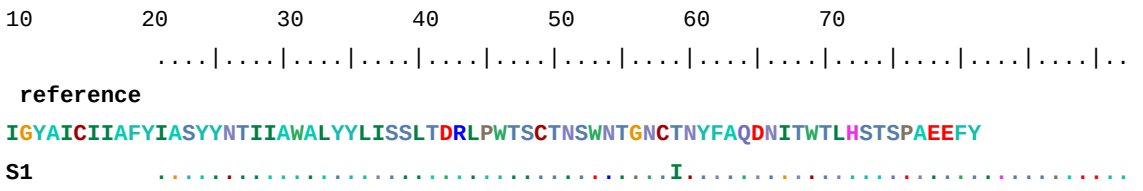

Figure 5. Protein sequence alignment of the genotyped sample with their corresponding reference sequences of the 72 amino acids SLC6A4 gene. The symbol "ref." refers to the NCBI referring sequence. The "S" refers to the genotyped sample. This SNP caused an amino acid substitution of serine to Isoleucine in position 39.

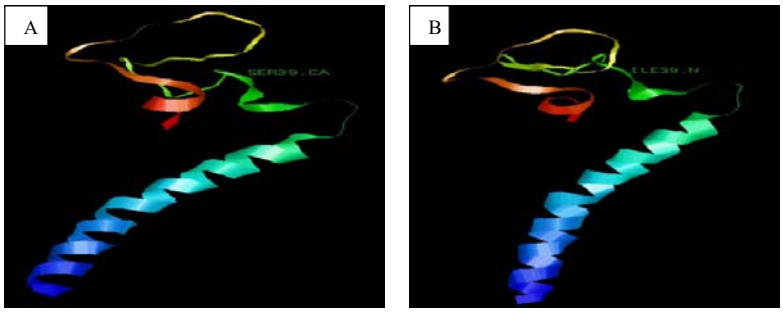

Figure 6. Substitution of serine to Isoleucine in position 39 of SLC6A4 gene (A normal 3D of secondary structure ribbon model, B abnormal 3D of secondary structure ribbon model

4. Discussion

Ingesting contaminated food and water or contacting consumer-infected goods can put people in considerable danger of SA poisoning (Lu *et al*., 2023). In the present study, chronic administration of SA showed a significant rise in the levels of serotonin and pro-inflammatory cytokines (such as IL-12, IL-8, IL-6, and TNFα), probably due to damage in the intestinal epithelium tissues induced by SA, which usually causes a mucosal inflammation accompanied with changes in the number of enterochromaffin cells and the levels of secreted serotonin in the gastrointestinal tract (Acharyya *et al*., 2015). Elevated serotonin levels trigger the immune system by generating different kinds of pro-inflammatory cytokines. These cytokines can cross the blood-brain barrier and send signals to the brain, which responds by altering various behavioural acts. Intestinal serotonin acts as a signal that regulates immune responses and behaviour (Waclawiková and Aidy, 2018). AS causes a block of serotonin receptors resulting in raised severity of colitis; in opposition, serotonin receptor agonists result in an anti-inflammatory impact (Vijayakaran *et al*., 2014). Recently, reports showed that activation of serotonin receptors causes blockade of oxidative stress and pro-inflammatory markers in rats. This indicates that activation of serotonin receptors may appear an efficient and new approach to developing potent therapeutics for inflammatory diseases like inflammatory bowel diseases (Quintero-Villegas and Valdés-Ferrer, 2020). Oxidative stress is one of the main mechanisms underlying SA-induced cell damage. Therefore, one of the possible therapeutic strategies, which may follow to eliminate or reduce the SA toxicity in vivo, is by employing varıous antioxidants. Based on recent studies, nanoselenium treatment during SA intoxication can alleviate the harmful ımpacts of both toxic elements on rodent tissues and cells. Co-administration of nano selenium with SA prohibited apoptosis induction, DNA damage, lipid peroxidation, decline of SA accumulation, serotonın system defect and anxiety-like behaviours (Samad *et al*., 2022).

Recently, there is evidence that the gut microbiota effects are not restricted to the intestine but may include all physiological functions of the organism, such as brain functions, inflammation response and regulation of behaviour. Specifically, when the structures of the gut microbiota are changed by exposure to chemical compounds such as heavy metals or drugs, gut cells respond to these alterations, and thus send signals to the central nervous system by releasing neurotransmitters, hormones and neuropeptides (Baldissarelli *et al*., 2021).

Attention to the gut-brain axis (GBA) has grown with our developed understanding of this axis importance in the pathophysiology of several common clinical disorders including neurodegenerative diseases and behaviour defects. The gastrointestinal tract and brain are important sensory organs accountable for detecting, transporting, and responding to external and internal environmental signals (González Delgado et al., 2022). This can happen through a two-way system called the gut-brain axis (GBA), which involves the production of neurotransmitters such as serotonin. Immune cells in both the brain and intestines consistently respond to environmental agents, leading to changes in immune activity that signal the body's current physiological state (Banskota *et al*., 2019). Recent research reveals that the gut-brain axis regulates inflammatory cytokines, inflammatory nociception and immune homeostasis (Rutsch *et al*., 2020).

According to Keil *et al*. (2011), it has been demonstrated that 95% of orally administered SA which absorbed in the intestine enters the bloodstream, resulting in systemic toxicity and increasing oxidative stress by elevating the levels of the created ROS. Hence, this result indicates that SA administration through drinking water may cause pathological damage to colon cells, as concluded by Chiocchett *et al.* (2018), who demonstrated that exposure to inorganic arsenic induces changes in the

immune system and serotonin levels in the gut, with the severity of these effects contingent upon dosage and duration of exposure. Therefore, it can be postulated that pro-inflammatory cytokines have the capacity to exhibit either an inflammatory or protective function, contingent upon the specific immunological condition of the individual. The acute exposure to arsenic has been found to contribute in the injury of the gut epithelium tissue, as well as increased serotonin levels, pro-inflammatory cytokines, and oxidative stress. Continuous exposure to inorganic substances can lead to an immune-toxic impact (Acharyya *et al*., 2015).

The intestinal epithelium tissue forms a selective permeability barrier that effectively inhibits any harmful substances from passing through it and causing infections. Therefore, any damage would decrease the efficiency of this barrier, leading to a non-specific and unregulated increase of the intestinal permeability, which mostly facilitates the entry of hazardous materials or microbes that probably interact with the immune system components located in the lamina propria (Chiocchetti *et al*., 2018). The commencement of an inflammatory process is associated with a disruption in the integrity of the intestinal barriers, which will elevate the serotonin levels and develop persistent inflammatory conditions (Ferrario *et al*., 2016). These interactions normally stimulate an immunological response; for instance, the expression of tight junction proteins, namely occludin, claudin-5, and zonula occludens-1, is reduced by pro-inflammatory cytokines within the tight junctions. Reduction in protein expression will decrease the integrity and structure of the epithelium cells and ultimately increase permeability (Zhao *et al*., 2023). Inflammation serves as a key mechanism to remove the damaged tissues or start the healing process. Interleukin-1β (IL-1β) and interleukin-18 (IL-18) are cytokines that play a crucial role in promoting inflammation (Waclawiková and Aidy, 2018). These cytokines, as multi-protein complexes found within cells, are processed by inflammasomes; particularly, nucleotide-binding domain leucine-rich repeat (NLR) proteins NLRP3 and NLRC4 in macrophages induce and activate caspase-1. The activated NLRP3 will lead to the proteolytic cleavage of IL-1β and IL-18 precursors, which induce apoptosis of the inflammatory cells (Bauernfeind *et al*., 2011; Ahn *et al*., 2016).

The open field tests were employed to assess the anxiety levels in animals and examine the potential neurobehavioral deficit resulting from exposure to SA. The test outlined in the study has been widely used to detect anxiety-like behaviours in rats (Knight *et al*., 2021). The results revealed that rats exposed to SA exhibited reduced locomotor activity in an open field environment, mirroring the findings of Firdaus *et al*. (2018) study, which suggested that arsenite exposure may have a suppressive effect on both balance and motor activity in rats. Furthermore, cytokine receptors in the brain for IL-1β and TNF-α will be activated by cerebral NF-κB signalling pathways. This activation will lead to the production of secondary cytokines, which have been associated with anxiety and mood disorders. Taken together, these pathways reveal multiple brain signalling pathways that may be activated by chemicals produced during gastrointestinal inflammation, which in turn may trigger glaring alterations in brain functioning that underlie the development of anxiety and a decline in locomotor activity (Waclawiková and Aidy, 2018).

The SLC6A4 gene encodes a protein known as the serotonin transporter, which facilitates the reuptake of serotonin molecules from the synaptic cleft into the presynaptic neurons. The impact of polymorphisms in the SLC6A4 gene on the rate of serotonin reuptake and its function in different disorders has been revealed in studies conducted by Taylor (2013), and Rao et al. (2017). Another study conducted by O'Mahony *et al*. (2015) demonstrated that serotonin (5-HT) functions as a neurotransmitter that has an impact on both immune responses and the communication between the intestines and the brain. A decrease in the copy number variation of the intestinal SLC6A4 transporter can lead to an accumulation of serotonin (5-HT) in the intestines (Dell'Osso *et al*., 2013; Nguyen *et al*., 2014; Catena-Duman and Canli, 2015), which might increase stimulation of the intestinal mucosa and ultimately result in the occurrence of diarrhoea (Sjaarda *et al*., 2017). Depressive symptoms and higher pro-inflammatory cytokine levels are associated with a specific genetic susceptibility that the present study has identified. This vulnerability involves the serotonin transporter gene. This study found that serotonin plays a significant role in the pathophysiology of depression and modulates neural-immune interactions; furthermore, the findings support the concept that shared pathophysiological pathways connect inflammation and depression.

Numerous investigations have demonstrated that the manifestation of a genetic variant in the serotonin transport gene (SLC6A4) in rodents leads to deficits in behaviour (Lam et al., 2018; Veenstra-Vander Weele *et al*., 2012). The investigation of genetic abnormalities associated with 5-HT in individuals with deviant attitudes suggested a potential association with Slc6a4, the gene responsible for encoding SERT. Carneiro *et al.* (2008) conducted a comprehensive investigation of individuals afflicted with diverse disorders, revealing significant associations with SERT across the entire genome. Apart from the present study, an investigation was conducted on uncommon mutations in the Slc6a4 gene. The observed alterations led to the occurrence of a gain-of-function mutation and an increase in SERT activity, as reported by Muller. et al. (2015). The SERT Ala56 mutation, along with the other polymorphisms documented in this study, induces a hyper-functional SERT protein that exhibits enhanced efficiency in the reuptake of 5-HT (Sutcliffe *et al*., 2005).

Arsenic toxicity impacts various systems and pathways involved in learning, memory, locomotion, decision-making, and emotion, according to research in mouse models. Future studies should concentrate on developing therapies to alleviate the neurological effects of arsenic poisoning by delving further into the underlying mechanisms of action, such as epigenetics. To determine the actual effect of arsenic on the central nervous system, more data on health and exposure during the intrauterine period would be useful.

5. Conclusion

This study indicates that variants within the serotonin gene exon correlated with elevated colonic serotonin

levels, which subsequently led to: first, increased pro-inflammatory cytokine (IL-1β, TNF-α, and IL-12) levels; second, elevated oxidative stress (ROS, H2O2, and NO) levels in the colon tissues of rats exposed to sodium arsenite; and finally, increased activity of the caspase-1 enzyme and anxiety behaviour with decreased levels of the anti-inflammatory cytokine IL-10.

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Authors' Contribution Statement

This manuscript was carried out in cooperation between all authors. H.AH.CH. designed the experiment, collected tissue and blood samples from rats, and conducted behavioural and physiological tests. Z.I.J. designed the genetic study. W.M. and H.AH.CH. were responsible for the writing, editing, and data analysis of the manuscript. All authors read and approved the final manuscript.

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