Biological Forum – An International Journal 15(3): 839-850(2023)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of *in situ* Action of Serotonin in Transmembrane Ion Transport in Mice Exposed to Restraint Stress

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(Received: 20 January 2023; Revised: 16 February 2023; Accepted: 21 February 2023; Published: 15 March 2023)
(Published by Research Trend)

ABSTRACT: Serotonin (5-HT) is known to be a key modulator of stress effects. However, its role in ion transport during stress response is not yet understood. The dose-dependent effect of in situ serotonin was examined in Swiss albino mice. 10^{-9} M 5-HT perfusion dose seems to be effective in producing a pronounced effect on most of the tissues. Perfusion of serotonin at 10^{-9} M for 20 minutes produced a significant decrease in Na^+, K^+-ATPase activity in the kidney, liver, stomach and intestinal tissues. A dose-responsive decrease in cytosolic and mitochondrial H^+ ATPase activity was found in these tissues after serotonin perfusion. Likewise, the cytosolic and mitochondrial Ca^{2+} ATPase activities decreased in the kidney, liver, stomach and intestine. The mitochondrial Mg^{2+} ATPase activity decreased in the tested tissues in a dose-responsive manner. Subjecting mice to restraint stress for seven days increased the Na^+, K^+-ATPase, H^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase activities to significant levels in kidney, liver, stomach and intestinal tissues. On the contrary, in-situ perfusion of serotonin to stressed mice at 10^{-9} M caused decrease in the stress-induced hyperactivity of these transmembrane ion transporters. The above results show a role of serotonin in ion transporter activity and suggest the mitigation role of serotonin in ion transport during stress response in mice.

Keywords: ATPase, Serotonin, Mice, Stress.

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) the brain neurotransmitter has been well-known for its role in depression, migraine and other neuropsychiatric illnesses. It has been reported to be essential for maintaining physiological and psychological homeostasis in animals when they are exposed to stressors (Saito, 1976). This monoamine neurotransmitter, affects many systems in mediating the physiological processes that regulate anger, mood, sleep, appetite, and even learning are influenced by the influence of the serotonin the body (Fuller, 1990; Uphouse, 1997; Barnes and Sharp 1999). The serotonin system originates from a small number of neurons located in the midbrain raphe nuclei that project widely throughout the central nervous system which regulates a large array of inter-related biological functions (Paul and Chawki 2013). The neuroendocrine studies (Romero and Sapolsky 1996) have shown that neurochemical links between stress and mood disorders obey stressor-specific effects.

In mammals during stress response there will be an activation of serotonergic activation of the hypothalamic–pituitary–adrenal (HPA) axis which leads to an enhanced level of corticotrophin releasing hormone (CRH) from the hypothalamus and stimulates the secretion of adrenocorticotropic-releasing hormone (ACTH) from the pituitary, which in turn activates the secretion of glucocorticoids, such as cortisol, from the adrenal gland (Calogero *et al*., 1990).

The aim of the present study was to examine whether 5-HT perfusion influences the ion transport in mice during restraint stress. In addition, the effects of 5-HT on cytosolic as well as mitochondrial ion transporters during restraint stress may provide better understanding of transporter activity in mice during stress.

MATERIALS AND METHODS

Animal Holding Conditions

Healthy adult male Swiss albino mice (*Musmusculus*) born and reared in the laboratory were used in the study. Animals are housed in an isolated building located as far away from human habitations as possible and not exposed to dust, smoke, noise, wild rodents, insects and birds. Animals weighing 28–35 g were kept in polypropylene cages for mice (29 cm L x 22 cm W x 14 cm H) with mesh-wire top. All animals were maintained on a lighting schedule (lights on at 7 a.m., lights off at 7 p.m.), and acclimated to our climate controlled animal facility at room temperature of 26-30 ºC with relative

humidity of 70 ±10% with minimum noise levels and less handling, with minimum noise levels. Mice had *ad libitum* access to purified tap water and food (Sri SaiDurga Feeds and Products, Bangalore) on non-testing days. Cage bedding was changed once in every two days. The experimental procedures were carried out in a separate area away from the animal's house room, under the same environmental conditions as those in the where animals were housed. The experimental animals were handled and treated in accordance with Kerala University animal care protocol No. IAEC-KU-17/09-10-ZOO-MCSP (1) and follow the regulation of CPCSEA 2006.

EXPERIMENTAL DESIGN

Restraint Procedure

Animals were immobilized individually by using hollow tubes made of polyvinyl chloride having length and diameter 12 cm and 5 cm, respectively. One end of the tube was closed and the other end with few holes for ventilation. The tubes with animals were placed vertically. This restraint procedure minimized the space around the animal, prevented its normal horizontal posture and its movements thus providing a rather strong, stressful stimulus without being harmful to the animal. This procedure resulted in an almost complete immobilization of the animals.

The dose-dependent effect of (5-HT) in mice

In this experiment the dose-responsive *in situ* effects of serotonin in ion transporter activities were studied in the kidney, liver, stomach and intestine of mice to understand the short-term actions of this neurotransmitter. The effects of varied concentrations (10^{-9}, 10^{-8} and 10^{-7} M) of *in situ* serotonin (Sigma, India) perfusions were done in the test species.

The effect of serotonin in restraint-stressed mice

In the second set of experiments a selected dose of **5-HT** (10^{-9} M) was perfused to normal mice.

Likewise, **5-HT** was perfused to the mice which were exposed to the psychosocial stressor in the form of intermittent restraint stress for seven days.

Isolation of mitochondria

Mitochondria were isolated from kidney, liver, stomach and intestine tissues. Briefly, the tissues were kept in ice-cold 0.25M sucrose. A 10% tissue homogenate was prepared and subjected to differential centrifugation at 40C (Eppendorf R3435). First, it was centrifuged at 700 xg for 10 minutes to separate the cell debris and nuclei. The supernatant obtained was spun at 10,000 xg for 10 minutes and the pellets recovered were washed twice by repeating the centrifugation. This mitochondrial pellet was later suspended in fresh ice-cold 0.25 M SEI buffer containing 0.25 M sucrose, 10 mM Na2EDTA, 0.1 M imidazole. The supernatant collected first was taken as post-mitochondrial supernatant (PMS) and it served as the material for cytosolic transporter assays. The protein concentrations in mitochondria as well as in cytosol were determined using modified Biuret Assay.

Perfusion of varied doses of serotonin to normal mice

Animals were assigned to four groups (total n=16). The mice were anaesthetized with chloroform (Sisco Research Laboratories, Mumbai, India). Then they were swabbed with 70% alcohol to wet the fur and the abdominal skin was shaved. A midline abdominal incision was made and abdominal aorta and vena cava were exposed. These blood vessels were cleared of connective tissue and fat with cotton swabs. A 23 gauge needle was inserted in the aorta and arterial blood was removed with the help of a canula. Then, without any interruption of pressure/flow, desired concentration of the melatonin dissolved in the perfusion medium was perfused for 20 min. Krebs Ringer bicarbonate Buffer (KRB saline) (Sigma-Aldrich, USA) was used as perfusion medium. The control mice were perfused with KRB saline at the rate of 1 ml/min for 20 min. Similarly, the remaining mice were perfused with 10^{-9}, 10^{-8}, and 10^{-7} M serotonin for 20 min. After perfusion the mice were sacrificed and tissues such as stomach, intestine, kidney and liver were collected in SEI buffer and stored at -80 ^{o}C for further analysis.

Perfusion of melatonin to restrained mice

Animals (total n=16) were assigned to two sets. The first set comprised of two groups which were normal and served as non-stressed mice. The second set of mice was kept under restraint stress for 60 min daily between 11 am and 12 noon for one week. The non-stressed mice were maintained undisturbed for the same duration. At the end of the seventh day all the animals were anaesthetized and serotonin or KRB was perfused. The first group in non-stressed mice was perfused with KRB saline for 20 min. whereas the second group in non-stressed mice were perfused with 10^{-9} M serotonin for 20 min. On the other hand, the first group in the second set of stressed mice was perfused with KRB saline for 20 min. whereas the second group of stressed mice was perfused with 10^{-9} M serotonin. After perfusion the mice were sacrificed and tissues such as stomach, intestine, kidney and liver were collected in SEI buffer and stored at -80 ^{o}C for further analysis.

Quantification of Na^+, K^+-ATPase specific activity

A portion of the tissue homogenized in SEI buffer (pH 7.4) and centrifuged at 700 xg for 10 minutes was used for analyzing the Na^+, K^+-ATPase activity. The ouabain-sensitive Na^+, K^+-ATPase-specific activity in the intestinal and liver tissue homogenates was quantified. Saponin (0.2 mg protein^{-1}) was routinely added to optimize substrate accessibility. The samples in duplicate were added to a 96-well microplate containing 100 mM L^{-1} NaCl, 30 mML^{-1} immidazole (pH 7.4), 0.1 mM L^{-1} EDTA and 5 mM L^{-1} $MgCl_2$ with or without souabain and incubated at 37 ^{o}C. The reaction was initiated by the addition of ATP and was terminated with addition of 8.6% TCA. The liberated inorganic phosphate was determined in Autoreader 4011 (Spam Diagnostics Ltd., Surat, India) at 700 nm and expressed in μM Pi h^{-1} mg protein^{-1}.

Quantification of H^+-ATPase specific activities

Bafilomycin-sensitive H^+-ATPase activity was assayed in tissue homogenates. Briefly, about 100 mg of anterior portion of intestine, part of pyloric stomach, part of posterior kidney and lower lobe of liver tissue was homogenized in of 0.25 M SEI buffer (pH 7.4) and centrifuged at 1800 for 10 min. The supernatant obtained was centrifuged at 10,000 g for 10 min. and the supernatant and was collected and store as Post Mitochondrial Fraction (PMS). This aliquot is used to

measure the specific activity of cytosolic H^+-ATPase. The pellet was washed again with 1 ml SEI buffer and centrifuged at 10,000 g for 10 minutes. The pellet was resuspend in fresh SEI buffer and this aliquot was used to measure the specific activity of mitochondrial H^+-ATPase. Homogenate samples were quantified in microplate containing Bafilomycin A. The reaction was initiated by the addition of ATP for 15 min at 37 °C and terminated by adding 8.6% TCA. The inorganic phosphate content was measured with a Systronics Double Beam Spectrophotometer 2202 at 700 nm and expressed in µmol P*i* h^{-1} mg protein. The protein contents in the tissues were measured using modified Biuret assay with bovine serum albumin as standard.

Quantification of Ca^{2+}-dependent ATPase specific activity

The Ca^{2+}-dependent ATPase activity in the isolated mitochondria was estimated as described previously with some modifications. Mitochondrial samples of various tissues were incubated in 96-well microplate containing the assay medium with Vanadate. ATP was added for 15 min. at 37 ºC and the reaction was terminated by adding 8.6% TCA. Inorganic phosphate content released was measured with a Span Autoreader 4011 at 700 nm and expressed in µmol Pi h^{-1} mg $protein^{-1}$. The protein contents in the tissues were measured using modified Biuret assay with bovine serum albumin as standard.

Quantification of Mg^{2+} dependent ATPase specific activity

The specific activity of oligomycin-sensitive Mg^{2+}-dependent ATPase of isolated mitochondria was estimated as described earlier with some modifications. Mitochondrial samples of tissues were added to a 96-well microplate containing medium oligomycin. The reaction was started by the addition of ATP for 15 min. and incubated at 37 ºC. The reaction was terminated by adding 8.6% TCA and the inorganic phosphate content released was measured with a Span Autoreader 4011 at 700 nm and expressed in µmol P*i* h^{-1} mg $protein^{-1}$. The protein contents in the tissues were measured using modified Biuret assay (Alexander and Ingram, 1980) with bovine serum albumin as standard.

Statistics. Data were collected from four animals in each group. Statistical difference among groups were tested by means of one-way analysis of variance (ANOVA) followed by SNK comparison test. Significance between the groups were analyzed with the help of Graphpad Software (Graphpad Instat-3, San Digeo) and the level of significance was accepted if $P<0.05$.

RESULTS AND DISCUSSION

Effect of varied doses of in situ 5-HT on ion transporters in mice

Na^+/K^+-ATPase activity

The perfusion of 5-HT at varied concentration decreased ($P<0.05$) the Na^+/K^+-ATPase activity in the gastric tissues at 100 nM concentration when compared to normal mice (Fig. 1). In hepatic tissues perfusion at 5-HT decreased the Na^+/K^+-ATPase activity at 1 nM ($P<0.01$) and 10 nM ($P<0.05$) concentrations when compared to the control (Fig. 1). Na^+/K^+-ATPase activity decreased ($P<0.05$) in intestinal tissues after the perfusion of 5-HT at 1 nM concentration. In hepatic tissues 5-HT perfusion reduced the Na^+/K^+-ATPase activity at 1 Nm ($P<0.01$) and 10 nM ($P<0.05$) concentrations (Fig. 1 and Fig. 2). The perfusion of 5-HT at varied concentrations reduced the renal Na^+/K^+-ATPase activity ($P<0.001$) 1 nM and 10 nM concentrations.

Cytosolic Ca^{2+} ATPase activity

5-HT perfusion reduced ($P<0.001$) the Ca^{2+} ATPase activity in gastric tissues at 1 nM concentration (Fig. 3) and 10 nM concentration ($P<0.01$) when compared to normal mice. In hepatic tissue 5-HT perfusion at 1nM concentration decreased the ($P<0.05$) Ca^{2+} ATPase activity when compared to normal mice (Fig. 4). In intestine, 5-HT perfusion decreased ($P< 0.001$) the Ca^{2+} ATPase activity at 1 nM concentration when compared to normal mice (Fig. 5). 5-HT perfusion at the rate of 1 nM concentration significantly reduced ($P<0.01$) the Ca^{2+} ATPase activity in renal tissues when compared to normal mice (Fig. 4).

Cytosolic H^+- ATPase activity

The renal tissues showed a reduced ($P<0.001$) H^+-ATPase activity at 1 nM 5-HTwhen compared to normal mice. In hepatic tissue, at 1 nM 5-HT perfusion the H^+-ATPase activity decreased ($P<0.001$) from that of control (Fig. 4). In gastric tissue at 1 nM and10 nM concentrations of 5-HT, the H^+-ATPase activity went down ($P< 0.01$) from that of control. In intestine H^+-ATPase activity showed reduced activity ($P<0.05$) at 1 nM concentration when compared to normal mice (Fig. 5).

Mitochondrial Ca^{2+} ATPase activity

In renal tissue a 5-HT perfusion at 1 nM concentration reduced the Ca^{2+} ATPase activity significantly ($P<0.01$) compared to normal mice. In this tissue 5-HT perfusion rate at 100 nM concentration also have reduction ($P<0.05$) in the Ca^{2+} ATPase activity when compared to normal mice. The Ca^{2+} ATPase activity in hepatic tissues decreased ($P<0.05$) with perfused 5-HT at 1 nM concentration when compared to normal mice (Fig. 6). 5-HT perfusion reduced ($P< 0.05$) the Ca^{2+} ATPase activity in intestinal tissue at a 1 nM concentration when compared to normal mice. In gastric tissues both at 1 nM ($P<0.001$) and 10 nM ($P<0.01$) concentrations of 5-HT perfusion, Ca^{2+} ATPase activity went down ($P<0.001$) when compared to normal mice (Fig. 7).

Mitochondrial Mg^{2+} ATPase activity

5-HT perfusion at 100 nM concentration significantly increased ($P<0.001$) the Mg^{2+} ATPase activity of intestinal tissues when compared to normal mice. 5-HT perfusion at 100 nM concentration increased ($P<0.001$) the Mg^{2+} ATPase activity of gastric tissues when compared to normal mice (Fig. 7). In renal tissues of 5-HT at 1 nM perfused dose down regulated the Mg^{2+} ATPase activity ($P<0.05$) when compared to normal mice. 5-HT perfusion at 100 nM concentration significantly reduced ($P<0.01$) the Mg^{2+} ATPase activity of hepatic tissues when compared to normal mice (Fig. 6).

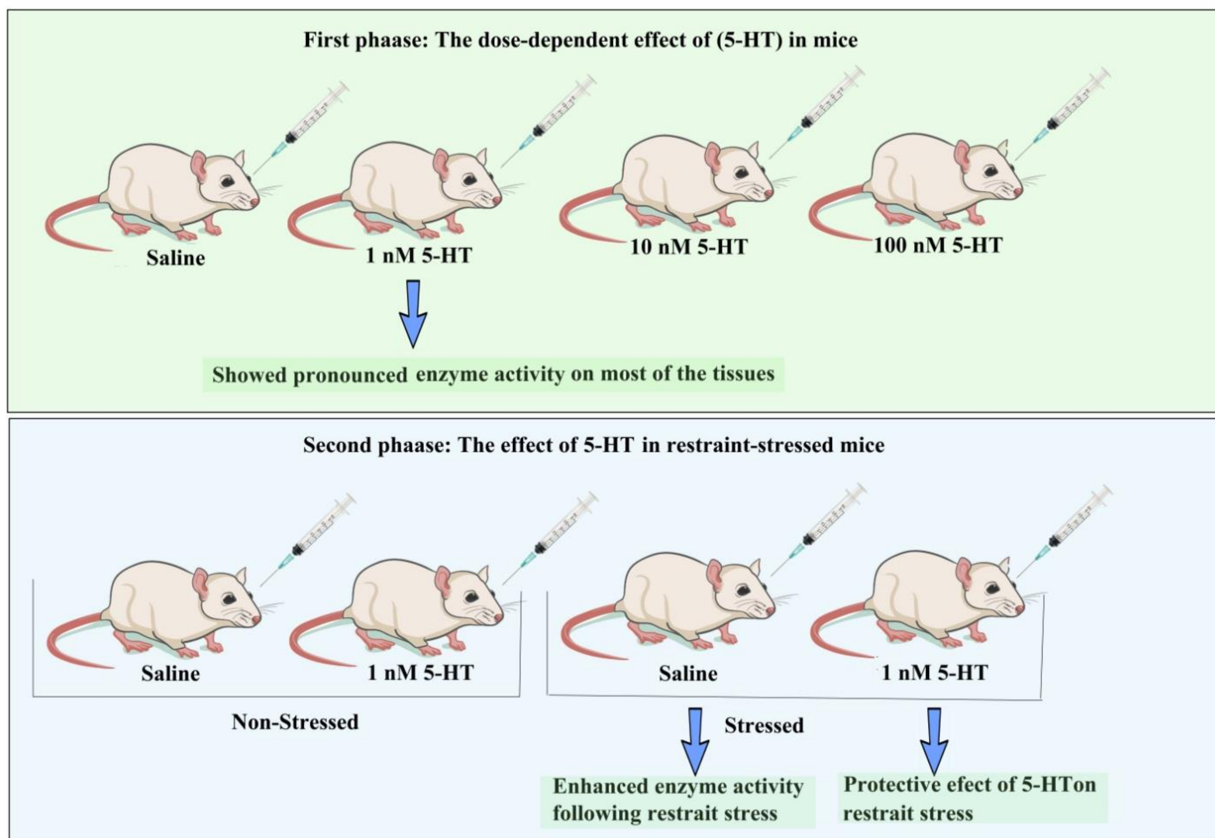

Fig. 1 and 2. Showing phase wise dose dependent treatment of serotonin (5-HT) and its effect on transmembrane ion transporters in mice. In phase I dose 1 nM 5-HT showed pronounced enzyme activity in mice compared to other doses. In phase II dose 1 nM 5-HT showed a protective effect on enzyme activity in stressed mice compared to non-stressed mice.

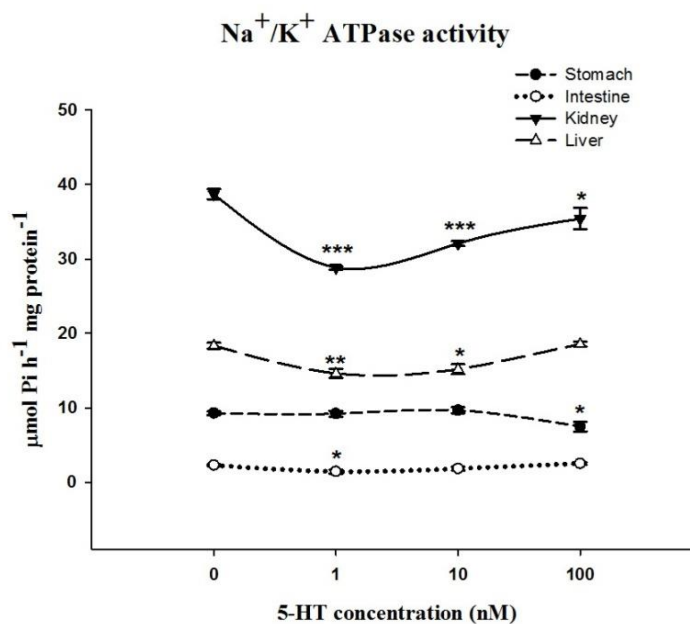

Fig. 3. Na^+, K^+-ATPase activities in the gastric, intestinal, renal and hepatic tissues of 5-HT perfused mice. Each point represents mean ±SEM for five mice. *P<0.05 and ***P< 0.001 denote significant difference from that of control.

Mitochondrial H^+-ATPase activity

At 1 nM and 100 nM perfused doses of 5-HT, H^+-ATPase activity went down significantly (P<0.001) in renal tissues, compared to control. 5-HT perfusion at 1nM concentration and 10 nM concentrations decreased (P<0.001) the H^+-ATPase activity in hepatic tissues when compared to normal mice (Fig. 6). Intestinal tissues showed decreased (P<0.001) H^+-ATPase activity at perfused 5-HT doses of 1 nM and 10 nM concentrations when compared to normal mice (Fig. 7). Gastric tissues showed reduced (P<0.001) H^+-ATPase

activity in perfused 5-HT dose at 1 nM concentration when compared to normal mice (Fig. 5).

mice

Na^+/K^+-ATPase activity

In the gastric tissues of the stressed mice where saline was perfused, the Na^+/K^+-ATPase activity increased (P<0.001) when compared to saline perfused normal mice. In gastric tissues of the stressed mice where 5-HT was perfused, the Na^+/K^+-ATPase activity increased (P<0.001) from that of 5-HT perfused normal mice. In gastric tissues of the normal mice where 5-HT was perfused, the Na^+/K^+-ATPase activity declined (P<0.01) from that of saline perfused normal mice. In gastric tissues of the stressed mice where 5-HT was perfused, the Na^+/K^+-ATPase activity declined significantly (P<0.01) from that of saline perfused normal mice.

Effect of in situ 5-HT on ion transporters in stressed

When compared to saline perfused restraint group, the Na^+/K^+-ATPase showed a decline (P<0.01) in the intestine of 5-HT perfused stressed mice. Saline perfused stressed mice showed an increase (P<0.01) in intestinal Na^+/K^+-ATPase activity from when compared to saline perfused normal mice (Fig. 8).

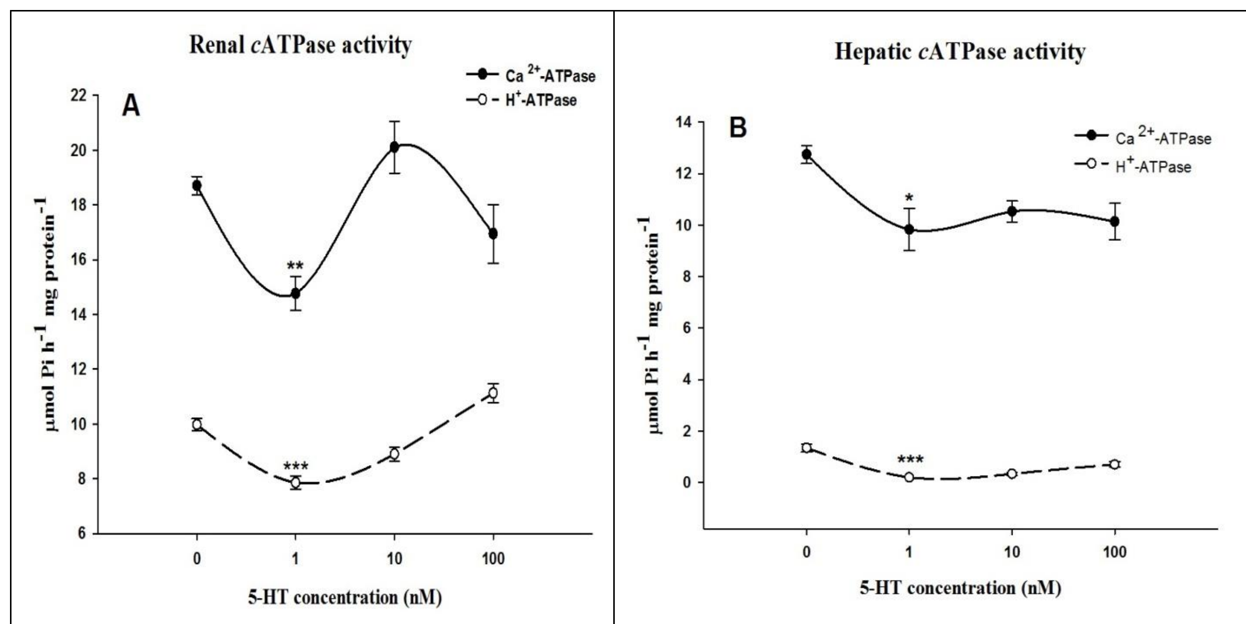

Fig. 4. Ca^{2+} ATPase activity and H^+ ATPase activity in the cytosol of renal and hepatic tissues of 5-HT perfused mice. Each point represents mean ± SEM for five mice. *P<0.05, ***P<0.001 and **P<0.01 denote significant difference from that of control.

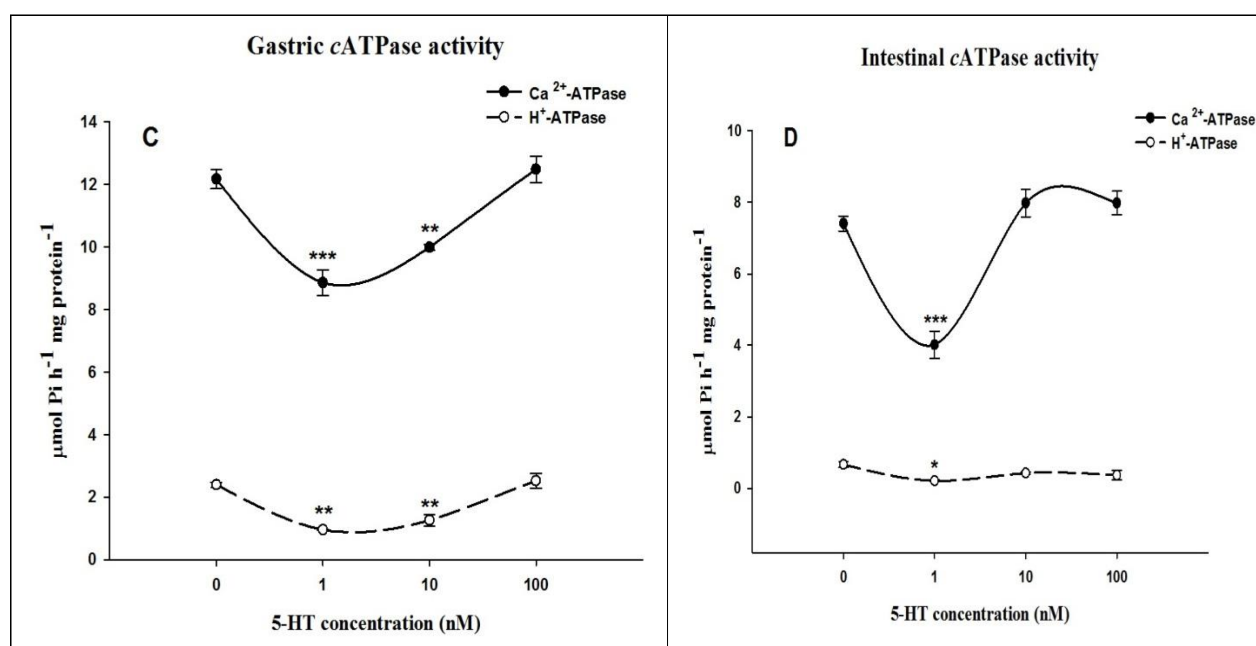

Fig. 5. Ca^{2+} ATPase activity and H^+ ATPase activity in the cytosol of gastric and intestinal tissues of 5-HT perfused mice. Each point represents mean ± SEM for five mice. *P<0.05, ***P<0.001 and **P<0.01 denote significant difference from that of control.

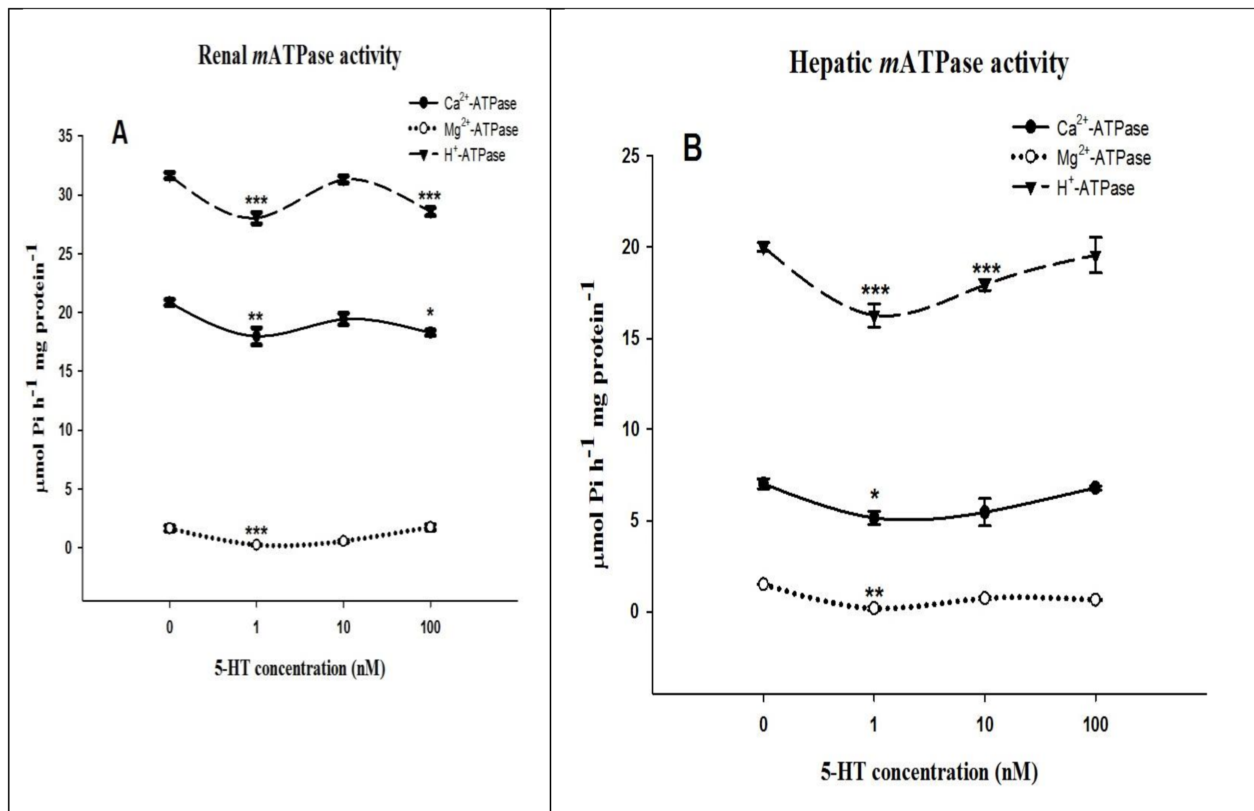

Fig. 6. Ca^{2+} ATPase activity, Mg^{2+} ATPase activity and H^+ ATPase activity in the mitochondria of renal and hepatic tissues of 5-HT perfused mice. Each point represents mean ± SEM for five mice. *P<0.05, ***P<0.001 and **P<0.01 denote significant difference from that of control.

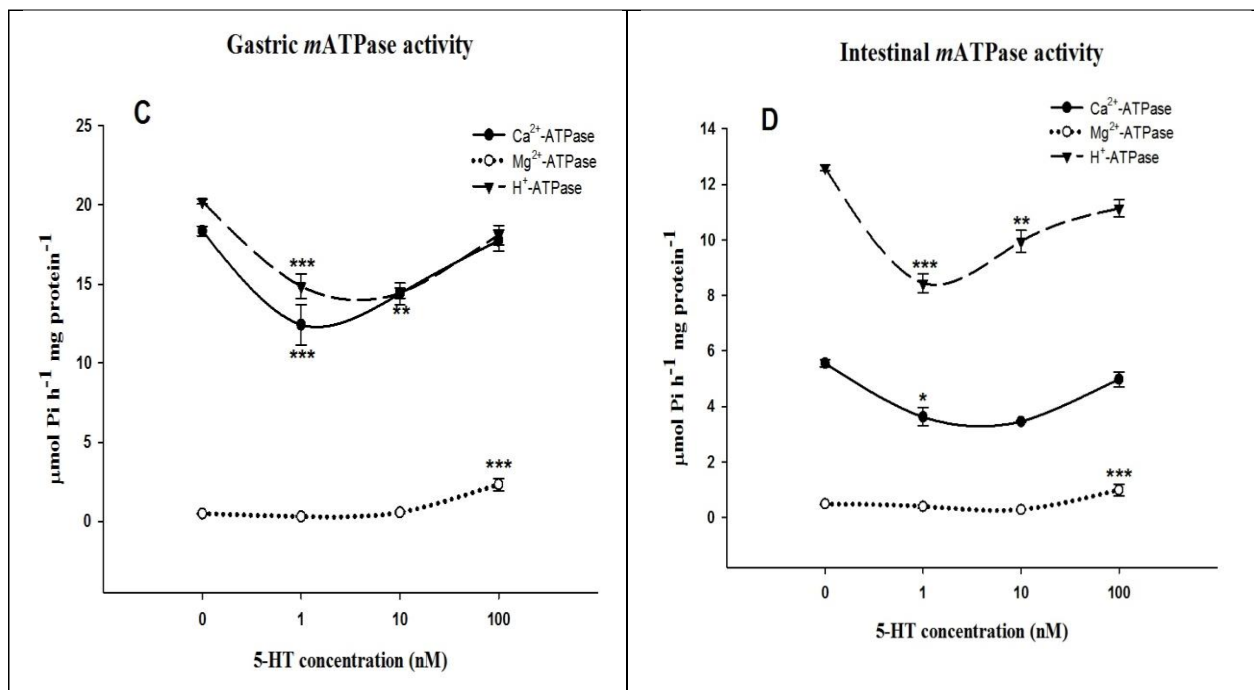

Fig. 7. Ca^{2+} ATPase activity, Mg^{2+} ATPase activity and H^+ ATPase activity in the mitochondria of gastric and intestinal tissues of 5-HT perfused mice. Each point represents mean ± SEM for five mice. *P<0.05, ***P<0.001 and **P<0.01 denote significant difference from that of control.

In renal tissues, when compared to saline perfused stressed mice, the Na^+/K^+-ATPase showed a decline ($P<0.001$) in 5-HT perfused stressed mice. Saline perfused stressed mice showed an increase ($P<0.05$) in renal Na^+/K^+-ATPase activity from that of saline perfused normal mice. 5-HT perfused stressed mice showed an increase ($P<0.05$) in renal Na^+/K^+-ATPase activity from that of 5-HT perfused normal mice. 5-HT perfused normal mice showed a decline ($P<0.001$) in renal Na^+/K^+-ATPase activity from that of saline perfused normal mice.

In hepatic tissues the activity of Na^+/K^+-ATPase was found increased ($P<0.001$) in saline perfused stressed mice from its normal mice. In 5–HT perfused stressed mice hepatic Na^+/K^+-ATPase activity was increased ($P<0.05$) when compared to 5–HT perfused normal mice. 5-HT perfused stressed mice showed a decline ($P<0.001$) in hepatic Na^+/K^+-ATPase activity from that of saline perfused stressed mice. Decline ($P<0. 01$) in hepatic Na^+/K^+-ATPase activity is observed in 5-HT perfused normal mice from that of saline perfused normal mice (Fig. 9).

Cytosolic Ca^{2+} ATPase activity

Renal Ca^{2+} ATPase of saline perfused stressed mice showed a significant rise ($P<0.001$) in its level from that of saline perfused normal mice (Fig. 9). In this tissue 5-HT perfusion showed a decline ($P<0.001$) Ca^{2+} ATPase activity in normal mice when compared to saline perfused normal mice. 5-HT perfused stressed mice showed a significant increase ($P<0.01$) in renal Ca^{2+} ATPase from that of 5-HT perfused normal mice. Reduced ($P<0.001$) renal Ca^{2+} ATPase activity is observed in 5-HT perfused stressed mice, compared to saline perfused stressed mice.

Hepatic Ca^{2+} ATPase of saline perfused stressed mice showed a significant rise ($P<0.001$) in its level from that of saline perfused normal mice. 5-HT perfused control group showed a significant decline ($P<0.05$) in hepatic Ca^{2+} ATPase from that of saline perfused normal mice. Reduced ($P<0.001$) hepatic Ca^{2+} ATPase activity was observed in 5-HT perfused stressed mice, compared to the saline perfused stressed mice (Fig. 10).

Gastric Ca^{2+} ATPase of saline perfused stressed mice showed a significant rise ($P<0.001$) in its level from that of saline perfused normal mice. 5-HT perfused normal mice showed a significant decline ($P<0.01$) in gastric Ca^{2+} ATPase from that of saline perfused normal mice. 5-HT perfused stressed mice showed an increased ($P<0.01$) in gastric Ca^{2+} ATPase activity when compared to 5-HT perfused normal mice. Reduced ($P<0.001$) gastric Ca^{2+} ATPase activity is observed in 5-HT perfused stressed mice, compared to the saline perfused stressed mice (Fig. 11).

Cytosolic H^+- ATPase activity

Gastric H^+-ATPase activity in 5-HT perfused normal mice has showed a declining ($P<0.01$) effect when compared to saline perfused normal mice (Fig. 10). In 5-HT perfused stressed mice the activity of gastric H^+-ATPase reduced ($P<0.001$) from that of saline perfused stressed mice. Restraint stress increased ($P<0.001$) the gastric H^+-ATPase activity in saline perfused group from that of its normal mice (Fig. 11).

Hepatic H^+-ATPase activity in 5-HT perfused normal mice has showed a declining ($P<0.05$) effect from that of saline perfused normal mice. In 5-HT perfused stressed mice the activity of hepatic H^+-ATPase is reduced ($P<0.01$) from that of saline perfused stressed mice.

Renal H^+-ATPase activity in 5-HT perfused normal mice has showed a declining ($P<0.01$) effect from that of saline perfused normal mice. In 5-HT perfused stressed mice the activity of renal H^+-ATPase is reduced ($P<0.001$) from that of saline perfused stressed mice (Fig. 10).

Mitochondrial Ca^{2+} ATPase activity

In saline perfused stressed mice, renal Ca^{2+} ATPase activity levels increased ($P<0.001$) from that of saline perfused normal mice. In 5-HT perfused normal mice, decrease ($P<0.001$) in renal Ca^{2+} ATPase activity was observed when compared to saline perfused normal mice. In 5-HT perfused stressed mice, renal Ca^{2+} ATPase activity levels decreased ($P<0.001$) from that of saline perfused stressed mice.

In saline perfused stressed mice, hepatic Ca^{2+} ATPase activity levels increased ($P<0.001$) from that of saline perfused normal mice. In 5-HT perfused normal mice, decline ($P<0.001$) in hepatic Ca^{2+} ATPase activity from that of saline perfused normal mice was observed. In 5-HT perfused stressed mice, hepatic Ca^{2+} ATPase activity levels decreased ($P<0.001$) from that of saline perfused stressed mice (Fig. 6). In 5-HT perfused stressed mice, hepatic Ca^{2+} ATPase activity levels increased ($P<0.001$) from that of 5-HT perfused control normal mice (Fig. 12).

In saline perfused stressed mice, gastric Ca^{2+} ATPase activity levels increased significantly ($P<0.01$) from that of saline perfused normal mice. In 5-HT perfused normal mice, decline ($P<0.001$) in gastric Ca^{2+} ATPase activity from that of saline perfused normal mice was observed. In 5-HT perfused stressed mice, gastric Ca^{2+} ATPase activity levels decreased ($P<0.001$) from that of saline perfused stressed mice. In 5-HT perfused stressed mice, gastric Ca^{2+} ATPase activity levels increased ($P<0.01$) from that of 5-HT perfused normal mice.

In saline perfused stressed mice, intestinal Ca^{2+} ATPase activity levels increased significantly ($P<0.001$) from that of saline perfused normal mice. In 5-HT perfused normal mice, decline ($P<0.01$) in intestinal Ca^{2+} ATPase activity from that of saline perfused normal mice was observed. In 5-HT perfused stressed mice, intestinal Ca^{2+} ATPase activity levels decreased ($P<0.001$) from that of saline perfused stressed mice. In 5-HT perfused stressed mice, intestinal Ca^{2+} ATPase activity levels increased ($P<0.01$) from that of 5-HT perfused normal mice (Fig. 13).

Mitochondrial Mg^{2+} ATPase activity

In stressed mice where saline was perfused, we observed a significant rise ($P<0.001$) in renal Mg^{2+} ATPase activity from saline perfused normal mice. Decline ($P<0.01$) in renal Mg^{2+} ATPase activity was observed in 5-HT perfused normal mice from saline perfused normal mice. In 5-HT perfused stressed mice, renal Mg^{2+} ATPase activity is reduced ($P<0.001$) when compared to saline perfused stressed mice.

In stressed mice where saline was perfused, we observed a significant rise (P<0.001) in hepatic Mg^{2+} ATPase activity from saline perfused normal mice. Decline (P<0.001) in hepatic Mg^{2+} ATPase activity was observed in 5-HT perfused normal mice when compared to saline perfused normal mice. In 5-HT perfused stressed mice, hepatic Mg^{2+} ATPase activity is decreased (P<0.001) from that of saline perfused stressed mice (Fig. 12).

In stressed mice where saline was perfused, we observed a significant rise (P<0.001) in gastric Mg^{2+} ATPase activity from saline perfused normal mice. Decline (P<0.01) in gastric Mg^{2+} ATPase activity was observed in 5-HT perfused normal mice when compared to saline perfused control group. In 5-HT perfused stressed mice, gastric Mg^{2+} ATPase activity is decreased (P< 0.001) from that of saline perfused stressed mice.

In stressed mice where saline was perfused, we observed a significant rise (P<0.01) in intestinal Mg^{2+} ATPase activity from saline perfused normal mice Decline (P<0.001) in intestinal Mg^{2+} ATPase activity was observed in 5-HT perfused normal mice when compared to saline perfused normal mice In 5-HT perfused mice restraint stress elevates (P<0.01) Mg^{2+} ATPase activity in intestinal tissues when compared to 5-HT perfused normal mice. In 5-HT perfused stressed mice, intestinal Mg^{2+} ATPase activity is decreased (P<0.001) when compared to saline perfused stressed mice (Fig. 13).

Mitochondrial H^+- ATPase activity

5-HT perfused normal mice showed decreased (P<0.05) renal H^+-ATPase activity, compared to saline perfused normal mice. In 5-HT perfused stressed mice decreased (P<0.01) renal H^+-ATPase activity, when compared to saline perfused normal mice was observed. A decline (P<0.01) in hepatic H^+-ATPase activity in 5-HT perfused stressed mice, when compared to saline perfused stressed mice was observed (Fig. 12).

5-HT perfused normal mice showed decreased (P<0.05) gastric H^+-ATPase activity, compared to saline perfused normal mice. In 5-HT perfused stressed mice decreased (P<0.05) gastric H^+-ATPase activity, when compared to 5-HT perfused normal mice was observed. Increased (P<0.001) gastric H^+-ATPase activity was observed in saline perfused stressed mice when compared to saline perfused normal mice. A decline (P<0.001) in gastric H^+-ATPase activity in 5-HT perfused stressed mice, when compared to saline perfused stressed mice was observed.

5-HT perfused normal mice showed decreased (P<0.05) intestinal H^+-ATPase activity, compared to saline perfused normal mice. Increased (P<0.05) intestinal H^+-ATPase activity in saline perfused stressed mice was observed in saline perfused stressed mice when compared to saline perfused normal mice. A decline (P<0.01) in intestinal H^+-ATPase activity in 5-HT perfused stressed mice, when compared to saline perfused stressed mice is observed (Fig. 13).

Serotonin as a neurotransmitter is vital for communications between brain and body. Not only are neurotransmitters important for our physical health but also play a significant role in our mental health, affecting our mood, sleep, memory and concentration. When they are out of balance (or when receptors on cells responsible for receiving neurotransmitter signals are impaired) they have significant effects on our mood and behaviour.

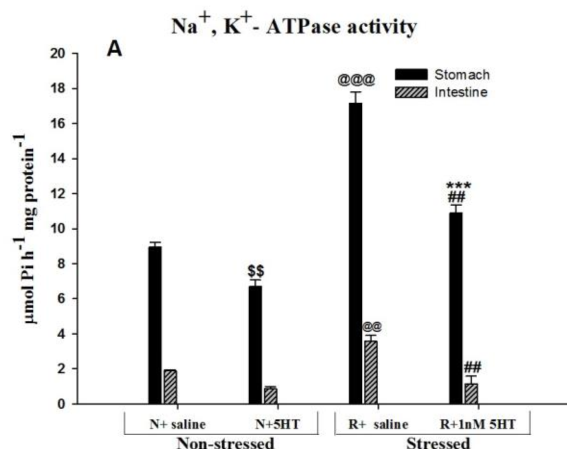

Fig. 8. Na^+, K^+-ATPase activities in the gastric and intestinal tissues of stressed mice. Each column represents mean ± SEM for five mice. ****P<0.001* denotes significant difference of 5HT perfused stressed mice from that of 5-HT perfused normal mice. \$\$*P<0.01* denotes significant difference of 5HT perfused normal mice from that of saline perfused normal mice. $^{\#\#}$*P<0.01* denotes significant difference of 5-HT perfused stressed mice from that of saline perfused stressed mice. $^{@@}$*P<0.01* and $^{@@@}$*P<0.001* denotes significant difference of saline perfused stressed mice from that of saline perfused normal mice.

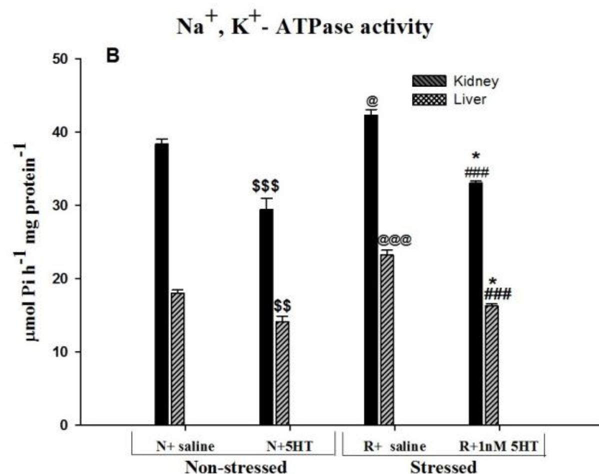

Fig. 9. Na^+, K^+-ATPase activities in the kidney and liver tissues of stressed mice. Each column represents mean ± SEM for five mice. **P<0.05* denotes significant difference of 5-HT perfused stressed mice from that of 5HT perfused normal mice. \$\$*P<0.01*, \$\$\$*P<0.001* denotes significant difference of 5HT perfused normal mice from that of saline perfused normal mice. $^{\#}$*P<0.05* denotes significant difference of 5HT perfused stressed mice from that of saline perfused stressed mice. $^{@}$*P<0.05* and $^{@@@}$*P<0.001* denotes significant difference of saline perfused stressed mice from that of saline perfused normal mice.

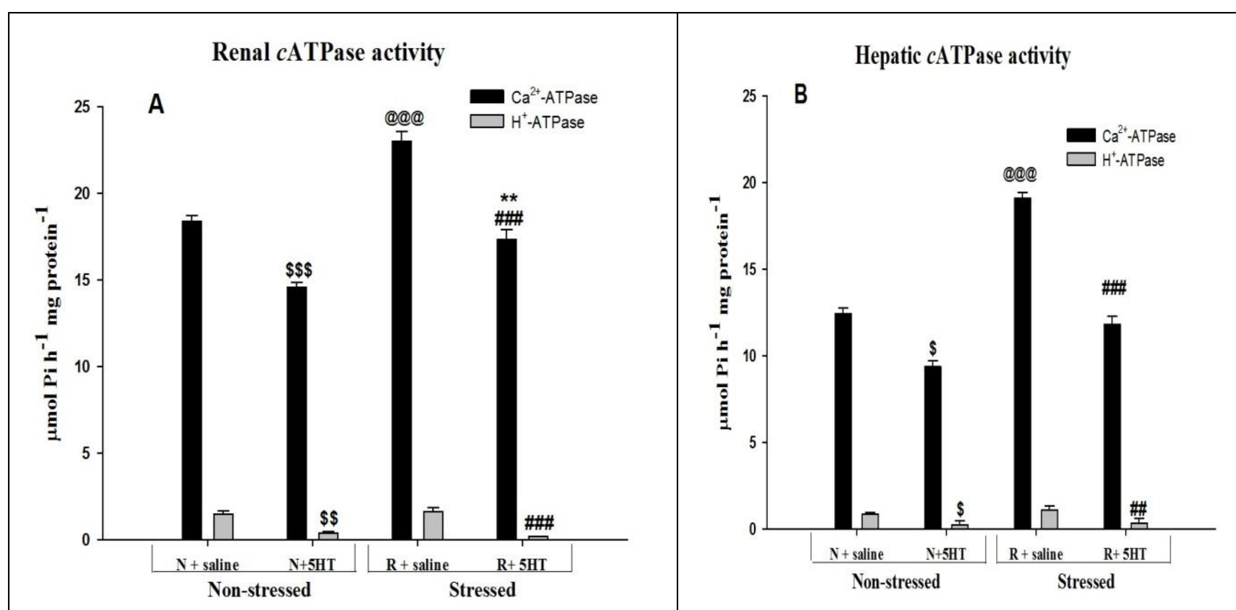

Fig. 10. Ca^{2+} ATPase and H^+-ATPase activities in the cytosol of renal and hepatic tissues of stressed mice . Each column represents mean ± SEM for five mice. **P<0.01 denotes significant difference of 5HT perfused stressed mice from that of 5-HT perfused normal mice. $^{\$}P<0.05$, $^{\$\$}P<0.01$, $^{\$\$\$}P<0.001$ denotes significant difference of 5-HT perfused normal mice from that of saline perfused normal mice. $^{\#\#}P<0.05$ and $^{\#\#\#}P<0.001$ denotes significant difference of 5HT perfused stressed mice from that of saline perfused stressed mice. $^{@@@}P<0.001$ denotes significant difference of saline perfused stressed mice from that of saline perfused normal mice.

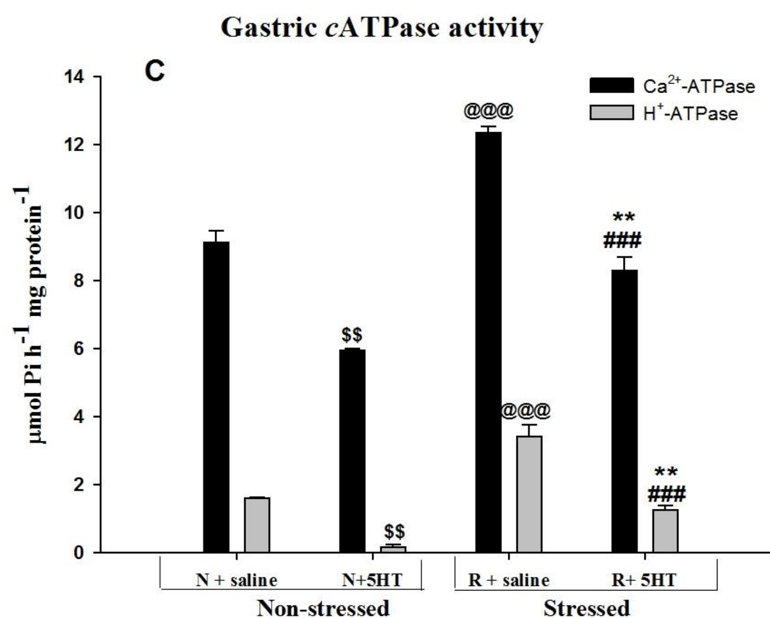

Fig. 11. Ca^{2+} ATPase and H^+- ATPase activities in the cytosol of gastric tissues of stressed mice. Each column represents mean ± SEM for five mice. $^{**}P<0.01$denotes significant difference of 5HT perfused stressed mice from that of 5-HT perfused normal mice. $^{\$\$}P<0.01$ denotes significant difference of 5-HT perfused normal mice from that of saline perfused normal mice. $^{\#\#\#}P<0.001$ denotes significant difference of 5-HT perfused stressed mice from that of saline perfused stressed mice. $^{@@@}P<0.001$ denotes significant difference of saline perfused stressed mice from that of saline perfused normal mice.

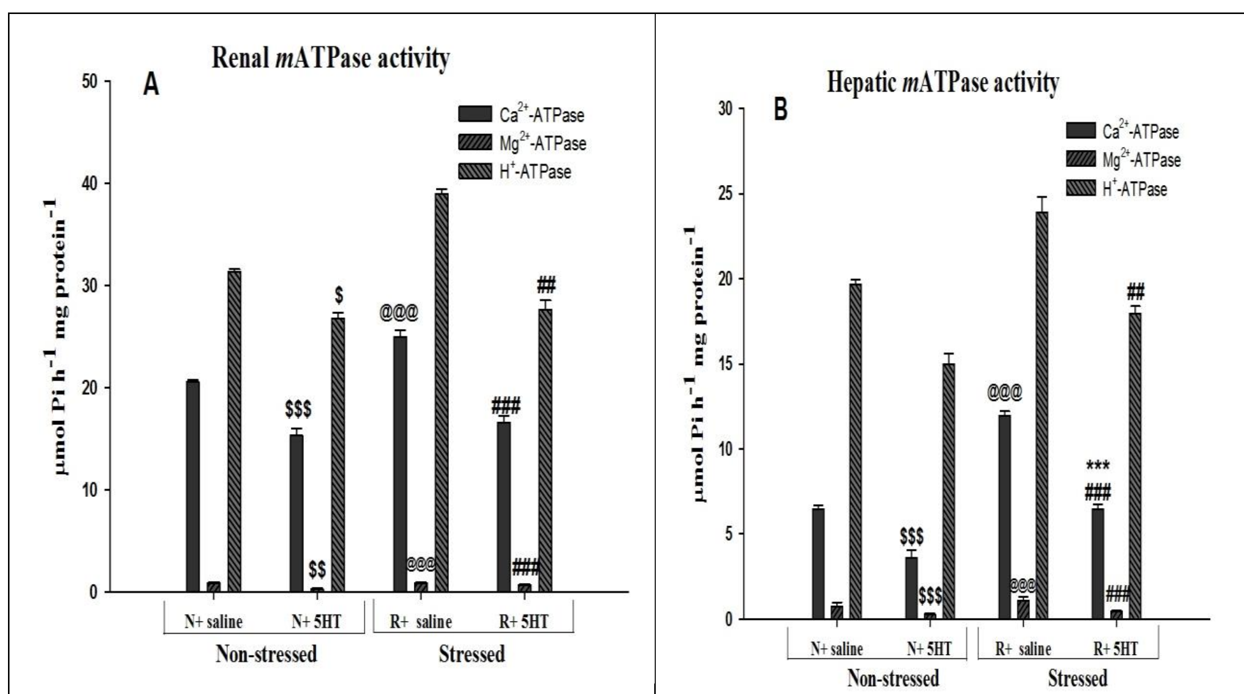

Fig. 12. Ca^{2+} ATPase, Mg^{2+} ATPase and H^{+}-ATPase activities in the mitochondria of renal and hepatic tissues of stressed mice. Each column represents mean ± SEM for five mice. *$P<0.05$*, **P<0.01, ***P<0.001 denotes significant difference of 5HT perfused stressed mice from that of 5-HT perfused normal mice. $^{\$}P<0.05$, $^{\$\$}P<0.01$, $^{\$\$\$}P<0.001$ denotes significant difference of 5-HT perfused normal mice from that of saline perfused normal mice. $^{\#}P<0.05$ and $^{\#\#\#}P<0.001$ denotes significant difference of 5-HT perfused stressed mice from that of saline perfused stressed mice. $^{@@@}P<0.001$ denotes significant difference of saline perfused stressed mice from that of saline perfused normal mice.

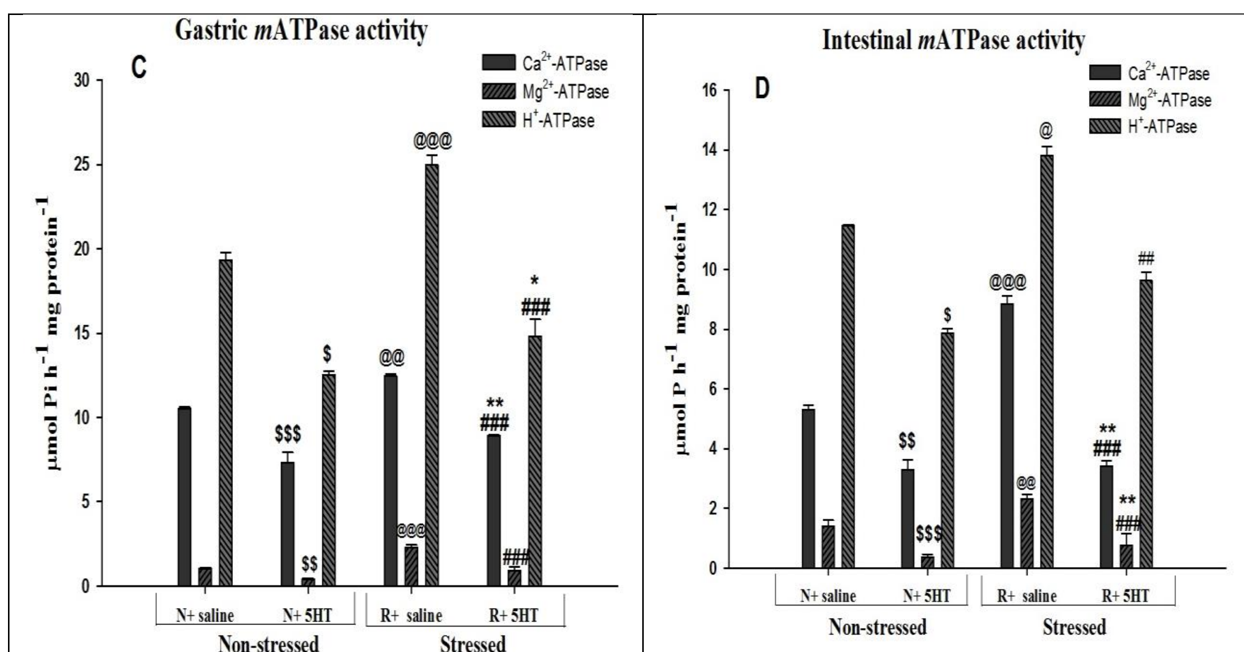

Fig. 13. Ca^{2+} ATPase, Mg^{2+} ATPase and H^{+}-ATPase activities in the mitochondria of gastric and intestinal tissues of stressed mice mice. Each column represents mean ± SEM for five mice. *$P<0.05$*, **$P<0.01$ denotes significant difference of 5-HT perfused stressed mice from that of 5-HT perfused normal mice. $^{\$}P<0.05$, $^{\$\$}P<0.01$, $^{\$\$\$}P<0.001$ denotes significant difference of 5-HT perfused normal mice from that of saline perfused normal mice. $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ denotes significant difference of 5-HT perfused stressed mice from that of saline perfused stressed mice. $^{@}P<0.005$, $^{@@}P<0.01$ and $^{@@@}P<0.001$ denotes significant difference of saline perfused stressed mice from that of saline perfused normal mice.

The serotonin plays a multifunctional part in mammalian homeostasis serving as a neurotransmitter within the central nervous system a gut-derived element of peristalsis, and a circulating hormone that directs appetite cardiovascular work, and hemostasis (Kawai and Rosen 2010). It acts as it is a gut-derived paracrine factor, a modulator of gastrointestinal motility, a neurotransmitter, an appetite mediator, a controller of circadian rhythmicity, a determinant of platelet contraction and hemostasis, an integrator of vitality modulation, and a particle that when changed in central nervous system (CNS) synapses is related to depression. High concentrations of serotonin is found in certain parts of the CNS acting as a neurotransmitter to control metabolic rate and appetite in hypothalamic nuclei, in this way making it another potential modulator of energy status. Serotonin also influences cognitive as well as psychological function in higher centers of the brain. Serotonin's peripheral effects occur during its biosynthesis in enterochromaffin cells of the gut where its principal function is to regulate intestinal peristalsis, and its secretion upon triggered by food in the gut.

In the first part of our work, we tested the response of enzyme's activity to 5-HT perfusion (10^{-7} M, 10^{-8} M and 10^{-9} M) of 5-HT. In our study it was found that tissue specific variations in ATPase enzyme's activity occur in both cytosolic as well as mitochondrial fractions of liver, kidney, intestine and stomach. It was observed a decline in tissue's enzyme activity from its basal levels when these were perfused with 5-HT. 10^{-9} M 5-HT perfusion dose seems to be effective in producing a pronounced effect on most of the tissues. In the second part of our experiment, we tested whether this 10^{-9} M 5-HT perfusion dose had any response to ion transporters during restraint stress.

As seen in our previous works, restraint induced stress resulted in an enhanced activity of ion transporters in specific tissues. It appears that the rise in ATPase function directs the Na and K transport in these cells. The enhancement was seen both in non-stressed as well as stressed mice. In contrast perfusion of 5-HT in stressed mice showed a reduced transporter activity. In cytosolic as well as mitochondrial tissues, 5-HT perfusion showed a protective role of 5-HT in ATPase activity.

Restraint stress caused, increased Na^+/K^+-ATPase activity. The restraint stress might lead to increased intracellular Na^+ concentration which increases the Na^+/K^+-ATPase activity. The action of 5-HT perfusion reduced the Na^+/K^+-ATPase activity in compensation to the increased intracellular Na^+ concentration arising from the restraining procedures. This suggests that pump activity can be altered in response to extracellular 5-HT. Repeated restraint stress application could be associated with a respective decrease in Na^+/K^+-ATPase activity resulting from high glucocorticoid levels, especially in the cerebral cortex and hippocampus (Rodrigo *et al.,* 2011). Antidepressants exert their clinical impacts through direct regulation of the glucocorticoid receptor (GR) is one of the foremost striking and inventive models of the mechanism of activity of this class of drugs (Barden, 1999; Holsboer, 2000; McQuade and Young 2000; Pariante and Miller 2001). In support of the above evidences, it is suggested that 5-HT administration might have enhanced the glucocorticoid levels and thereby reduced the Na^+/K^+-ATPase activity.

The cytosol employs a key arrangement and energetic properties of Ca^{2+} transporters and channels to create spatially and temporally complex Ca^{2+} change, which balance a number of cellular functions (Lee *et al.,* 1997; Wilson *et al.,* 1998; Kasai, 1999). As reported in the previous studies restraint stress increased calcium mobilization in cytosol. The mitochondrial Ca^{2+} uniportersarises as a result of this increased calcium mobilization. The membrane potential regulates the magnitude Ca^{2+} entry by controlling the driving force for Ca^{2+}, the Ca^{2+}ATPase. Increased Ca^{2+} mobilization increased the Ca^{2+}ATPase levels. In 5-HT perfused stressed mice, extracellular 5-HT could lower the raised Ca^{2+}ATPase activity, due to restraint stress, to baseline levels in cytosol as well as mitochondria. It is then suggested that repleting Ca^{2+} stores 5-HT undoubtedly plays a physiological role in the stress response of mice. At the sub cellular level, magnesium directs contractile proteins, modulates transmembrane transport of Ca^{2+}, Na^+ and K^+, acts as an fundamental cofactor within the activation of ATPase, controls metabolic regulation of energy-dependent cytoplasmic and mitochondrial pathways and impacts DNA and protein blend (Hoenderop *et al.,* 2005; Laurant *et al*., 2000). The Mg^{2+} level in the cell varies during changing physiological conditions. Dissipation the membrane potential release mitochondrial Mg^{2+} into the cytoplasm (Kubota *et al.,* 2005). Restraint stress raises the mitochondrial Mg^{2+}-ATPase levels. The free Mg^{2+} level increases slightly as ATP levels decrease. Extrusion of Mg^{2+} from mitochondria to cytosol may be the reason for increased ATPase activity and thereby reduced ATP level. Extracellular 5-HT acts to reduce the Mg^{2+}-ATPase levels in stressed mice. The total cellular Mg^{2+} level changes upon the addition of several hormones to cells (Romani and Maguire 2002). The improved glucocorticoid levels through 5-HT activity might work within the cells which may control the whole Mg^{2+} levels. Small changes in extracellular Mg^{2+} levels and intracellular free Mg^{2+} concentration have critical impacts on cardiac excitability and on vascular tone, contractility, reactivity, and development (Tammaro *et al.,* 2005; Touyz and Yao 2003).

Diminished serotonin movement is to a great extent related with depression and for the most part antidepressants which are effective have been appeared to improve the functioning of serotonin (Ninan and Philip 1999). Amid stress the increased tryptophan availability, tryptophan hydroxylase hyperactivity happens due to increased 5-HT synthesis/turnover Exposure to a metabolic or a psychological stressor leads to pronounced increases in brain tryptophan availability by means of stressor-specific mechanisms (Chaouloff, 1993). The level of cation stimulation of ATP hydrolytic activity in 5-HT administered control and stressed mice showed a marked reduction which was in contrast to the elevation noted after the saline treatment in restraint and control group. The data indicates that extracellular 5-HT

play a protective role in stress-induced imbalance of ion transport activity in mice.

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How to cite this article: Manish, K., Sajeeb Khan, A., Praveen Kumar P.P.,Nandini N.J. and Ayana Gayathri R.V. (2023). Effect of in situ Action of Serotonin in Transmembrane Ion Transport in Mice Exposed to Restraint Stress. *Biological Forum – An International Journal, 15*(3): 839-850.