PriMera Scientific
Medicine and Public Health
Volume 2 Issue 5 May 2023
DOI: 10.56831/PSMPH-02-059
ISSN: 2833-5627

Chronic Constipation and Its Curable Treatment with Senna Leaves in Wistar Rats

Type: Case Report
Received: April 02, 2023
Published: April 17, 2023

Citation:
BL Gupta., et al. "Chronic Constipation and Its Curable Treatment with Senna Leaves in Wistar Rats". PriMera Scientific Medicine and Public Health 2.5 (2023): 27-35.

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Summary

Chronic constipation developed by loperamide in Wistar rats were subjected to experimentation and their treatments with senna leaves extracts. It was observed that there was an increase of body weight, swollen stomach and belly size. The stools were hard and less in amount in constipated rats which all they got normalized with sanay treatments.

In chronic constipation the glutathione peroxidase activity was observed to be decreased by 18% approximately in case of male and 16% approximately in case of female. The glutathione reductase activity also increased by 22% in male and 25% in female rats red blood cells. The catalase activity has shown an increase of 14% in males and 32% approximately in female rats red blood cells. The GOT activity increased by 54% in males and 48% in females. The GPT activity in red blood cells was observed to be increased by 36% approximately in case of male and 44% in female. The catalase activity was found to be increased by 14% in male and 32% in female rats red blood cells. All these enzymes activities normalized after sanay leaves extract treatments to chronic constipated rats.

The metabolites levels of GSH also decreased by 25% in both male and female rats and the GSSG levels increased by 77% in male and 66% in female. The sanay treatments to both male and female rats normalized the metabolites levels.

In chronic constipation the creatinine levels were observed to be increased by 8% approximately in case of male and 13% in case of female. This metabolite level was observed to be normalized after sanay leaves extracts treatments to chronic constipated rats.

It may be concluded that the female rats suffer the most biochemical dysfunction in comparison to male in chronic constipation.

Introduction

Chronic constipation is defined as three or fewer bowel movements per week (Higgins and Johanson, 2004). It is a condition with a lower abdominal discomfort distension or bloating (Johanson et. al., 1989). Constipated patients typically report other symptoms, such as straining, passage of hard stool, inability to defecate at will, unproductive urges, and sensation of incomplete evacuation (Higgins and Johanson, 2004; Lembo and Camilleri, 2003).

Chronic constipation is prevalent among old age person (McCrea et.al.,2009). Most older persons perceive constipation as straining during defecation and difficulty in evacuation, rather than decreased frequency of bowel movements (De Lillo and Rose, 2000). In community-dwelling adults older than 65 years, about 20 percent have rectal outlet delay with need to self-evacuate (Talley et.al., 1996).

The holding of stool in the large intestine occurs for a longer time during chronic constipation. These stools carry bacteria and fungi which may be growing more and more in the large intestine disturbing various metabolic processes (Everhart, JE et. al., 2009). It has been reported that 8 to 10 pounds of bacteria are present in the gut's lining which generally comes through food and water (Guinane and Cotter, 2013). The number of the gut's bacteria increases and the person become at the risk of development of constipation (Cho and Blaser, 2012). It has been observed that constipated person feel weakness and laziness of the body. This weakness may relate to a decrease in defensive and immune system of the body (Maqbool, and Liacouras, 2020). It affects the work output in the offices.

The defensive enzymes like glutathione peroxidase, glutathione reductase and catalase play important roles in the defence of cells. The defensive metabolites like reduced glutathione and oxidized glutathione play the similar role, in keeping healthy defensive system (Mete R et. al., 2013). The data are scary to determine and support the defensive roles in red blood cells in animal model during constipation.

The remedial method to get relief from constipation, the people used toxicants like cigarette smoking, alcoholic consumption, hot milk intake in the night, lots of drinking water in the morning time etc. are generally practiced emptying the stomach. In such a situation the people become addict to intoxicants and many tissues in the body get damaged and also invite many diseases. There are different neuro-modulators which have been used in the gut function to get relieve from constipation such as serotonin, lubiprostone, neurotrophinin-3 etc. (Crowell, 2001; Amitiza, 2006; Parkman et.al, 2003). To get relieve from constipation the people use different kinds of laxatives which may be a pharmaceutical drug or herbal drug. The use of excess laxatives may cause toxicity in the body particularly the liver (Hallann. F, 2000). Screening many herbal drugs through the literature it has been decided to choose sanay leaves (*Cassia angustifolia*) which contain anti-microbial and anti-inflammatory properties (Nascimento, MNG et. al.,2020) which may holistically treat the constipated animals which may kill bacteria and fungi in the gut and also show anti-inflammatory action of the gut to get relief permanently from constipation.

Methodology

Development of chronic constipation

Wistar rats with a mean weight 190±10gm. were obtained from the experimental animal house of Jamia Millia Islamia (JMI) in the laboratory of Prof. S A Husain, Department of Bioscience. Rats were grouped into three categories: (a) Control (b) Chronic Constipation (C) Chronic constipation rats treated with sanay extract.

Constipation was developed in experimental rats by giving oral administration of 1ml of loperamide with a dose of 3mg/kg body weight for three days (Hughes S et al., 1984). The stools were tested and found to be hard with less quantity and mostly devoid of water. The fourth day after loperamide administration, the day first was counted for constipation duration.

The experimental animals were kept for three to six month for chronic constipation, out of twenty experimental rats ten rats were kept untreated and ten rats treated with sanay leaves and 5 rats for control.

The constipation rats were daily monitored like observing the stool quality and its amount, abdomen enlargement and activeness of the animal. The constipation rats were not given any type of fibrous food. The feeds were made of rice pellets and leafy vegetables. The treated rats were given standard food pellets and other vegetables.

Sennea leaves (Cassia angustifolia) extract preparation

3-4gm. of senna leaves were added to 100 ml of boiling for one minute. It was then cooled down and filtered. This filtered water (supernatant) is ready for treatment to constipated rat to drink from water pipe.

Treatment to constipated rats

Sanay leaves extract was given to experimental constipated rats in a clean bottle especially during night time because rats are nocturnal animals. During day time, the normal water was given to rats.

Blood collection from rats

The blood was collected from Wistar rat's caudal tail commonly used because through sampling minimum pain or invasiveness. The tail is first of all washed with warm water so that the vein is visible. The needle of the syringe is punched in the vein and then piston of the syringe was slowly lifted so that the blood is sucked from the vein. Only 2ml of blood was collected from control, constipated and sanay treated rats during the period of 3, 4 and 6 months of experimentation in a syringe washed with anticoagulant i.e. Na_2 EDTA. The blood is then transferred to a centrifuged tube having 2ml of normal saline.

Isolation of Red blood cells from blood

The blood sample was centrifuged in Sorvall centrifuged machine at 3000 rpm 5 minute for the separation of RBCs. These cells were washed three times with cold phosphate buffer saline (Gupta and Baquer, 1998).

Preparation of RBC hemolysate

The hemolysate was prepared by forceful lysis with phosphate buffer (5 mM, pH 8.0). The hemolysate was used for the assay of enzymes and metabolites. The forceful lysis was done by taking 1 ml of RBC in 50ml of test tube with 39ml of phosphate buffer was taken in a syringe. It becomes 1:40 hemolysate.

Blood Hemoglobin Measurement

Blood is diluted in a solution containing potassium ferricyanide and potassium cyanide. Potassium ferricyanide oxidizes the iron in heme to the ferric state to form methemoglobin, which is converted to hemiglobincyanide (HiCN) by potassium cyanide.

Measurement of enzymes activities
Glutathione peroxidase (E.C 1.11.1.9)

The activity of glutathione peroxidase was assayed by the method of Beutler (1976). All the ingredients in the final concentration were namely 0.1M Pot. Phosphate buffer pH 7.0,1mM GSH, 4mM EDTA, 3U glutathione reductase, 4mM sodium azide, 0.2mM NADPH and 50µl of 1:40 erythrocytes hemolysate were taken in 1ml cuvette. The blank without GSH and the assay cuvette were incubated at 37°C for 10 minute in a water bath. The reaction was started by the addition of 0.1mM tert-butyl hydroperoxide (tBHP) and the decrease in absorbance was followed at 340nm in a Shimadzu 260 spectrophotometer (Gupta et.al., 2004).

Additional blanks were also taken in which all ingredients were present except GSH and blanks without tBHP were also used. Additional blanks were carried to substract this value from the assay for interference of other non specific peroxidases.

Glutathione Reductase (EC 1.6.4.2)

The activity of glutathione reductase was assayed at 25°C by the modified method of Erden and Bor (1984). 3μ1 of packed erythrocytes was added to the assay mixture containing the following in the final concentration of : 4.1 mM Tris-HCl buffer pH 7.5, 15mM $MgCl_2$, 5.7mM EDTA, 60mM KCl, 0.017% Saponin, 2.6mM GSSG and 0.2mM NADPH in 1.0 ml final volume. The decrease in absorbance was measured at 340nm after 10 minute of incubation against blanks containing all ingredients except GSSG (Gupta et.al., 2004).

Catalase (EC 1.11.1.6)

The catalase activity was measured by the method of Beutler (1986), within half an hour of 1:2000 hemolysate preparation. 2ml of assay mixture contained the following in the final concentration of 0.1ml Tris-HCL/EDTA, 0.1ml, pH 6.5; 1ml 10mM H_2O_2 and 0.8ml distilled water. The mixture was incubated at 37°C for 10 minute. 0.1ml of 1:2000 hemolysate was added to start the reaction and the change in absorbance was read at 230nm.

Glutamate Oxaloacetate Transaminase (GOT) (EC 2.6.1.1)

Glutamate Oxaloacetate Transaminase (GOT) were measured by the method of Bergmeyer and Bernt (1974) for AsAT assay the reaction mixture in 1.5 ml in a final concentration contained potassium phosphate buffer 80 mM, pH 7.4, L-aspartate 66 mM, pH 7.4, malate dehydrogenase (dialysed) one unit; 2mM NADH and 10 ml of 1:40 diluted hemolysate. The reaction was started by adding 2-oxoglutarate 12 mM, pH 7.4 and the change in absorbance was measured at 340 nm.

Glutamate Pyruvate Transminase (EC 2.6.1.2)

AlaAT was measured by taking the following in the final concentration of assay mixture: potassium phosphate buffer 80 mM, pH 7.4; L-alanine 200 mM, pH 7.4 lactate dehydrogenase (LDH) dialysed 2 unit; NADH 2mM and 50 ml of 1:40 diluted hemolysate. The reaction was started by adding 2- oxoglutarate 0.18 mM, pH 7.0 and the change in absorbance was measured at 340 nm.

Unit of the Enzyme Activity

One unit of enzyme activity was defined as one µ mole of NADPH oxidized per unit time per gm of hemoglobin.

Measurment of metabolites by spectrophotometer
Reduced glutathione (GSH)

Reduced glutathione (GSH) was measured by the method of Griffith (Griffith 1980). The reaction mixture contained the following in a final concentration of: 0.20 mm NADPH, 0.6 mm 5,5¢ - dithiobis- (2-nitrobenzoic acid, 0.5U glutathione reductase in 125 mm sodium phosphate buffer (pH 7.5), 6.3mm EDTA and appropriate sample volume. The rate of reduction of 5,5-dithiobis- (2-nitrobenzoic acid) was measured at 412nm.

Oxidised Glutathione (GSSG)

GSSG level was assayed by the method of Beutler (Beutler 1986). 0.01ml of packed red cells was mixed with 20 µ1 of 0.25mM N-ethyl maleimide. This was precipitated with 30% TCA and the supernatant was extracted three times with ice-cold diethyl ether. The remaining diethyl ether was removed by passing a stream of air. The assay was performed in one ml of cuvettes containing DTNB buffer ($Na2HPO_4$, 0.1M, pH 7.2; EDTA,10mM;BSA, 0.04% and DTNB, 0.2mM); HCl, 1mM; NADPH 2mM and glutathione reductase, 3 U/ml. the calculation was performed by making a standard curve of GSSG (10nM - 100nM).

Measurement of creatinine by Alkaline Kits

Measurement of serum creatinine by kits purchased from Ank Cares Pvt.Ltd., Ahmedabad, Gujrat, India.

Results

General Parameters changes after sanay leaves extracts treatments.

The constipated rats have a higher body weight in comparison to controls than sanay treated conditions in 3, 4 and 6 months of constipation. The stomach and belly size were found to be increased in shape in constipated rats which normalized after sanay treated condition.

The stools were hard and less in amount in constipated conditions which normalized in sanay treated condition. All these general parameters changes in 3, 4 and 6 months treatments of sanay leaves to constipated rats have been presented in Table 1.

		Chronic Constipation (CC)	*Treated with Sanay leaves extract*
Body Weight		196 ± 18	190 ± 15
Stomach size (in inch)		Enlarged	Normal
Abdomen Swelling		Belly - bulged	Normal
Stool Quality	Amount	Less and Hard	Normal
	Shape and size	Hard and Oval size	Normal

Table 1: Physical parameters of chronic constipations rats and treated with sanay leaves extract.

Changes of Enzymes Activities in Red Blood Cells and Sanay leaves treatments

Glutathione Peroxidase

The glutathione peroxidase (GPx) activity in red blood cells of control was less in female in comparison to male rats. In chronic constipation, the glutathione peroxidase activity was decreased by 18% in the case of male rats and 16% in female rats red blood cells. The sanay leaves extracts treatments to chronic constipated rats normalized the enzyme activity in both male and female rats. All these changes have been presented in Table 2.

Glutathione Reductase

The glutathione reductase increased by 22% in male and 25% in female rats red blood cells. The sanay treated constipated rats showed a reversal of enzyme activity to normal values.

Glutamate Oxaloacetate Transaminase (GOT)

The activity of GOT was measured in red blood cells of chronic constipation condition of rats for 3, 4 and 6 months duration. It was found that the enzyme activity was observed to be increased by 54% in male and 48% in female chronic constipation rats red blood cells which reversed to normal values after sanay leaves extract treatments.

Glutamate Pyruvate Transaminase (GPT)

Glutamate pyruvate transaminase (GPT) activity was measured in red blood cells of chronic constipated rats, was increased by 36% in the case of female rats and 44% in male rats respectively. The sanay leaves extracts treatments to chronic constipated rats reversed the enzyme activity to normal in both male and female rats.

Catalase

The catalase (CAT) activity was found to show an increase by 14% in male and 32% in female rats red blood cells which normalized after sanay treatments.

All the above enzymes activities have been presented in Table 2.

Enzymes	Male			Female		
	Control	Cronic Const.	CC + Sanay	Control	Chronic Const.	CC + Sanay
GPX	109.2±12.8	89.8±7.9[a]	106.6±13.9	103.2±14.3	86.3±8.7[a]	104.2±15.6
GR	1.32±0.23	1.74±0.06[a]	1.36±0.08	1.30±0.31	1.69±0.11[a]	1.33±0.09
GOT	110.4±12.3	170.6±13.8[a]	115.5±11.5	109.6±17.6	162.3±16.1[a]	116.8±13.9
GPT	57.6±3.2	78.8±8.9[a]	68.8±5.3	55.4±4.4	80.2±9.6[a]	62.1±4.6
Catalase	6.3±0.4	7.2±0.3[a]	6.4±0.3	5.6±0.6	7.4±0.5[a]	5.8±0.8

The enzyme activity is expressed as μ ml/min/g Hb. Values are Mean ± SEM of 14 experimental rats. Fisher's 'p' values are shown as a. $p < 0.001$, b. $p < 0.005$.

Table 2: The levels of defensive enzymes (GPx, GR & CAT), GOT & GPT in RBCs of chronic constipated rats and its treatment with sanay leaves extracts.

Changes of Metabolites Level in Red Blood Cells and Sanay leaves treatments.

Reduced Glutathione (GSH)

In chronic constipation, the GSH level decreased by 25% approximately in the case of both male and female rats red blood cells. The sanay leaves extracts treatments to chronic constipated rats reversed the GSH levels to normal in both male and female rats.

Oxidised Glutathione (GSSG)

In chronic constipation, the GSSG levels were increased by 77% in the case of male and 66% in female rats red blood cells. The sanay leaves extracts treatments to chronic constipated rats normalized the GSSG levels in both male and female rats.

Creatinine

In chronic constipation, the creatinine levels were shown to be increased by 8% approximately in case of male and 13% in female rats. The sanay leaves extracts treatments to chronic constipated rats normalized the creatinine levels.

All the metabolites level have been presented in Table 3.

Enzymes	Male			Female		
	Control	Chronic Const.	CC + Sanay	Control	Chronic Const.	CC + Sanay
GSH	7.8±0.6	5.8±0.8[a]	7.6±0.2	7.2±0.7	5.3±0.4[a]	7.1±0.8
GSSG	0.18±0.3	0.32±0.06[a]	0.19±0.02	0.21±0.4	0.35±0.07[a]	0.22±0.04
Creatinine	1.31±0.04	1.42±0.08[b]	1.34±0.06	1.30±0.07	1.48±0.07[b]	1.32±0.03

The values of GSH expressed as um/gm Hb for GSSG and GSH; Creatinine as ml/minute. Values are p of Mean ± SEM of 14 experimental rats (7 males & 7 females). Fisher's P values are shown as a. $p < 0.001$, b. $p < 0.005$.

Table 3: Levels of GSH, GSSG and Creatinine in chronic constipation of male and female rats and its treatment with sanay leaves extract.

Discussion

Constipation has been a serious problem all over the world due to change in life style, food quality and unhygienic condition around the place where they are living (Wald A et. al., 2007). Constipation leads to economic loss of the country in which the people efforts to do the daily work, is decreased. It may due to discomfort and laziness of the constipated person who feel incomplete evacuation of stools always. The holding of stools in the large intestine occurs for a longer time. What these stools do in the large intestine is not known completely. But the stools carry bacteria and fungi which may be growing more and more in the large intestine disturbing various metabolic processes. There may be occurrence of gases formation like sulphur dioxide, nitrogen oxide, ammonia etc (Feldman M

et. al., 2016). The roles of these gases present in the gut are not known completely. But it may be presumed that these gases may cause changes in physiology and biochemistry of the cells affecting the digestion process and liver dysfunction. As we know that males and females have different sexual hormones which also affect our defensive and immune system. (Taneja V, 2018).

The six month constipated rats showed decreases in GPx and increases of GR. This may be due to enhanced production of free radicals products during course of constipation. The red blood cells tried to revert the defence caused by free radicals and their products by enhancing in the GR activity. A balance of GR and GPx activities correlate the production of glutathione in red blood cells (Dalvi SM et.al., 2012). If the glutathione reductase is more than it may be inferred that production of hydrogen peroxide is more. At one hand glutathione peroxidase is compatible to detoxify hydrogen peroxide levels but its level is decreased and on the other hand glutathione reductase activity is to produce hydrogen peroxide for which the activity was found to be increased. In such a situation constipation leads to formation of more GSSG. In our present investigation it has been observed that sanay leaves treatment to constipated rats relaxes the intestine to release the stored stools through the anus.

Both GOT & GPT activities increased in chronic constipation. In chronic constipation both the enzymes activities are enhanced more in red blood cells for the duration of 6 months. Both GOT & GPT were found to be increased about 50%. This shows that liver function during constipation was highly altered in chronic constipation. The protein break down is more in this condition and it may be presumed that these progressive products like urea and uric acid may be more in this condition.

In chronic condition there is an increase of catalase activity by 14% in male condition whereas in female there is about 32%. It shows that the female is more sensitive than males when constipation is taken into account. It also shows that the females are severely affected than male in constipation condition. Furthermore, it also shows that females has produced more hydrogen peroxide in RBCs in comparison to males. It may be due to less protective female hormones than in male condition. The other possible causes of this enhancement of the enzyme are not clear.

In our present investigation it has been observed that GSH decreases in red blood cells in constipation. The reason may be a more oxidative stress in comparison to less defence capsule present in RBC as arisen by production of lipid peroxides, lipid hydroperoxides, etc. What leads to these more productions is not known completely but some other principles of causes of these production may be pronounced.

An increase in oxidised glutathione levels in red blood cells may either leads to extracellular transported or converted into reduced glutathione (Deponte et.al.,2013). Though, it has been observed in our research that excess amount of oxidized glutathione as observed in chronic constipated rats may show injurious to cell structure, integration and function.

The creatinine levels are important in diagnosing kidney function tests. As the dysfunction in muscles leads to more formation of creatinine which comes in blood circulation and reaches kidney. If the kidney is not functioning well then creatinine is not being excreted therefore, increasing the blood creatinine.

As constipation prolongs it has been observed a decrease of 5% body weight till the treatment. It may be concluded that sanay leaves may be possessing the compounds inside it which are showing the effects of decreasing that inflammation. As also observed an increase in stomach size before treating constipated rats along with swelling in stomach. The treatment of constipated rats with sanay leaves led to normalizing the stomach size and abdominal swelling through out the experiments. It may be interpreted that the holding of stool in the intestine is never exist after relieve from constipation. The stool quality which was less in quantity and hard in character was normalized throughout the experiments.

Long-term use of any laxative particularly irritant laxatives such as senna, often results in laxative dependency syndrome, characterized by poor gastric motility in the absence of repeated laxative administration. Other reports of laxative abuse include laxative-induced diarrhea (Cummings JH et.al.,1974) and osteomalacia and arthropathy associated with prolonged use of the product (Frier and Scott, 1977). Senna may cause hepatotoxicity. This may be attributed to the exposure of the liver to high amounts of toxic metabolites of anthraquinone glycosides (Vanderperren B et.al.,2005). But in our present investigation, senna leaves have been boiled and then

cooled, killing all the possible existence of bacteria and fungi. Furthermore, sanay leaves extract is used and other solid compounds remain in the pellets. This may be highly useful in treating constipation with a least occurrence of side effects.

Conclusion

Observing all the results it may be concluded that the constipated rats show a decreased defense, liver dysfunction more in females than males and the kidney dysfunction more in females in comparison of males. Therefore, the females are more affected in comparison to males during chronic constipation. The sanay leaves treatments to constipated rats normalized the physical and biochemical parameters.

References

1. Amitiza R. Soft gelatin capsules [prescribing information] Bethesda, Md: Sucampo Pharmaceuticals, Inc. (2006).
2. Bergmeyer HU and Bernt E. Glutamate-oxaloacetate transaminase in Method of enzymatic analysis (ed) H U Bergmeyer (New York Academic Press) (1974): 727-733.
3. Beutler E, West C and Blume KGJ. 'The removal of leucocytes and platelets from whole blood". Lab Cin Med 88 (1976): 328-333.
4. Beutler E. Method in Hematology (Beautler, E.Ed, Longman, UK 16 (1986): 52-72.
5. Crowell MD. "The role of serotonin in the pathophysiology of irritable bowel syndrome". Am. J. Manag. Care 78 Supp l (2001): S252-S260.
6. Cho I and Blaser M. "The human microbiome: at the interface of health and disease". Nat Rev Genet 13 (2012): 260-270.
7. Cummings JH. "Laxative abuse". Gut 15.9 (1974): 758-766.
8. Dalvi SM, Patil VW and Ramraje NN. "The roles of glutathione, glutathione peroxidase, glutathione reductase and the carbonyl protein in pulmonary and extra pulmonary tuberculosis". J Clin Diagn Res 9 (2012): 1462-5.
9. De Lillo AR and Rose S. "Functional bowel disorders in the geriatric patient: constipation, fecal impaction, and fecal incontinence". Am J Gastroenterol 95.4 (2000): 901-905.
10. Deponte M. "Glutathione catalysis and the reaction mechanisms of glutathione dependent enzymes". Biochemica et Biophysica Acta 1830 (2013): 3217-3266.
11. Erden M and Bor NM. Change of reduced glutathione, glutathione reductase, and glutathione preoxidase after radiation in guinea pigs Biochm Med. 312 (1984): 217-27.
12. Everhart JE and Ruhl CE. "Burden of digestive diseases in the United States part II: lower gastrointestinal diseases". Gastroenterology 136 (2009): 741-54.
13. Feldman M., et al. Intestinal gas. In: Sleisenger and Fordtran's Gastrointestinal and Liver Disease: Pathophysiology, Diagnosis, Management. 10th Ed. Philadelphia, Pa.: Saunders Elsevier (2016).
14. Frier BM and Scott RD. "Osteomalacia and arthropathy associated with prolonged abuse of purgatives". Br J Clin Pract 31.1-3 (1977): 17-19.
15. Griffith OW. "Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine". Anal Biochem 107 (1980): 207-212.
16. Guinane CM and Cotter PD. "Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ". Therap Adv Gastroenterol 4 (2013): 295-308.
17. Gupta BL and Baquer NZ. "Hexokinase, G-6PDH and antioxidant enzyme in diabetic reticulocytes: Role of insulin and vanadate". Biochem. Mol.Biol. Int 48 (1998): 1145-1152.
18. Gupta BL, Preet A and Baquer NZ. "Protective effects of sodium orthovanadate in diabetic reticulocytes and ageing red blood cells of Wistar rats". J Biosci 29 (2004): 73-79.
19. Hallmann F. "Toxicity of commonly used laxatives". Med Sci Monit 3 (2000): 618-28.
20. Higgins PD and Johanson JF. "Epidemiology of constipation in North America: a systematic review". Am J Gastroenterol 99(4) (2004): 750-759.
21. Hughes S, Higgs NB and Turnberg LA. "Loperamide has antisecretory activity in the human jejunum in vivo". Gut 25 (1984): 931-

935.

22. Johanson JF, Sonnenberg, A, and Koch TR. "Clinical epidemiology of chronic constipation". J Clin Gastroenterol 11.5 (1989): 525-536.
23. Lembo A and Camilleri M. "Chronic constipation". N Engl J Med 349.14 (2003): 1360-1368.
24. Maqbool A and Liacouras CA. "Major symptoms and sign of digestive tract disorders". In Kliegman RM, St. Geme JW, Blum NJ, Shah SS, Tasker RC, Wilson KM, eds. Nelson Textbook of Pediatrics. 21 st ed. Philadelphia, PA: Elsevier (2020).
25. McCrea GL., et al. "A review of the literature on gender and age differences in the prevalence and characteristics of constipation in North America". J Pain Symptom Manage 37.4 (2009): 737-745.
26. Mete R., et al. "The role of oxidants and reactive nitrogen species in irritable bowel syndrome: a potential etiological explanation". Med Sci Monit 19 (2013): 762-6.
27. Nascimento MNG., et al. "Antimicrobial and cytotoxic activities of Senna and Cassia species (Fabaceae) extracts". Industrial Crop and Products 148 (2020): 1120-81.
28. Parkman HP., et al. "Neurotrophin-3 improves functional constipation". Am J Gastroenterol 98 (2003): 1338-1347.
29. Talley NJ., et. al. "Constipation in an elderly community: a study of prevalence and potential risk factors". Am J Gastroenterol 91.1 (1996): 19-25.
30. Taneja V. "Sex Hormones Determine Immune Response". Front Immunol 9 (2018): 1931.
31. Vanderperren B., et al. "Acute liver failure with renal impairment related to the abuse of senna anthraquinone glycosides". Annals of Pharmacotherapy 39.7-8 (2005): 1353-1357.
32. Wald A., et al. "The burden of constipation on quality of life: results of a multinational survey". Aliment Pharmacol Ther 26 (2007): 227-36.