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*Full Length Research Paper*

# Formulation and enhancement of dissolution rate of poorly aqueous soluble drug Aceclofenac by solid dispersion method: *In vitro* study

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Solid dispersion technique was successfully used to enhance the dissolution of poorly aqueous soluble drugs aceclofenac. Four carriers Hydroxyl propyl methylcellulose (HPMC), polyvinyl alcohol (PVA), Mannitol and Dextrose in two ratios (1:2, 1:3 w/w) were used to prepare solid dispersion. Eight different formulations were designed by the varying carrier and the drug: Carrier ratio and solvent wetting method was used for preparing solid dispersion. Acquired formulations were used for various micrometric and *in vitro* drug release studies. The solubility of aceclofenac in distilled water was  $0.0753 \pm 0.021$  mg/ml. The study shows that aceclofenac solubility is pH-dependent where the solubility observed was greater in phosphate buffer (pH 7.4) at  $5.76 \pm 1.23$  mg/ml compared to acid buffer (0.1 N HCl) at  $0.0214 \pm 0.012$  mg/ml. The percentage yield measured in F5 was higher at  $85.18 \pm 6.02\%$  and lower at  $70.43 \pm 5.028\%$  in F1. Micrometric study suggest that the Carr's index and Hausner's ratio value was smallest for F4 with  $5.018 \pm 0.0025$  and  $1.06 \pm 0.0025$  respectively, indicating an excellent flow property and greatest for F6 with  $15.35 \pm 0.0022$  and  $1.18 \pm 0.0022$ , indicating relatively poor flow. The angle of repose value was lower for F5 with  $20.77 \pm 2.9^\circ$  and higher for F2 with  $30.77 \pm 2.1^\circ$ . Both acid buffer (pH 1.2) and phosphate buffer (pH 7.4) were undertaken for *in vitro* drug release study of pure drug aceclofenac and eight separate formulations. The *in vitro* drug release after 180 min for aceclofenac in acidic buffer (pH 1.2) was  $3.89 \pm 0.41\%$  and was highest in F8 with  $54.73 \pm 4.60\%$  and lowest in F5 with  $31.75 \pm 3.10\%$ . Drug release was significant compared to pure drug aceclofenac ( $p < 0.05$ ) in acidic buffer. Similarly, *in vitro* drug release after 180 min for aceclofenac in phosphate buffer (pH 7.4) was  $23.79 \pm 2.20\%$  and was highest in F8 with  $76.65 \pm 6.50\%$  and lowest in F5 with  $64.09 \pm 5.70\%$ . The data was analyzed by Dunnett's multiple comparison tests while statistical significance was predefined at  $p < 0.05$ .

**Key words:** Aceclofenac, hydroxyl propyl methylcellulose (HPMC), polyvinyl alcohol (PVA)

## INTRODUCTION

Among all newly discovered chemical entities, about 40% of drugs are lipophilic and fail to reach the market due to

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their poor aqueous solubility (Ganesan et al., 2015). Drugs weak aqueous solubility and dissolution rate is one of the major problems in pharmaceutical growth and has become more prevalent among current drug candidates over the past two decades owing to the use of elevated-performance and combinatorial screening techniques during the drug discovery and selection phase (Mooter, 2011). A drug compound seems to be poorly soluble according to the Biopharmaceutical Classification System (BCS) if the utmost dose strength is insoluble in 250-ml aqueous media over the pH range at 37°C (FDA, 2017). These compounds are mostly categorized into Class II compounds that seem to be poorly soluble and extremely permeable depending on the pH of the gastrointestinal fluid and tend to deliver dissolution rate-limited absorption (Kawabata et al., 2011). Drugs assigned to BCS Class II are marked by elevated permeability of the membrane, slow dissolution rate (owing to low water solubility) and elevated oral dose. Consequently, a drug's solubility or dissolution rate is a crucial factor in determining its rate and magnitude of absorption. Improving the dissolution rate is essential to achieving an appropriate blood concentration for therapeutic effect since their dissolution rates are typically the rate-limiting step for bioavailability (Al-Hamidi et al., 2010). One of the most problematic aspects of drug development exists in the improvement of oral bioavailability of poorly aqueous soluble drugs. Though salt formation, co-solubilization, and decrease of particle size have been frequently used to boost the rate of dissolution and thus the oral absorption and bioavailability of such drugs, these methods have practical constraints (Bharti et al., 2015). The strategy of salt formation is not viable for neutral compounds and the synthesis of suitable salt forms of drugs that are weakly acidic or weakly basic may often not be functional (Choi et al., 2017). In many cases, although salts can be prepared, an enhanced rate of dissolution in the digestive tract might not be fulfilled due to the transformation of salts into aggregates of their corresponding acid or base forms (Frizon et al., 2013). The solubilization of drugs in organic solvents or aqueous media by the use of surfactants and co-solvents leads to liquid formulations that are generally unwanted from patient acceptance and marketing.

Although a decrease in particle size is frequently used to raise the dissolution rate, there is a practical limit to size reduction obtained through frequently used techniques such as controlled crystallization, grinding, pearl milling, etc. The use of very fine powders in a dosage form can sometimes be troublesome due to hardships in handling and weak wettability due to the development of charges (Mogal et al., 2012). In 1961, Sekiguchi and Obi created a practical technique by which most of the constraints with the improvement of achieved, which was named as "Solid Dispersion" (Leonardi et al., 2007). The dissolution rate in

conventional capsules and tablets is restricted by the size of the principal particles created after the dosage forms are disintegrated. In this situation, an average particle size of 5  $\mu\text{m}$  is generally the reduced limit, although greater particle sizes are favored for ease of handling, formulation, and production (Pande et al., 2014). On the other side, if a solid dispersion or a solid solution is used, a part of the drug will immediately dissolve to saturate the gastrointestinal fluid and the surplus drug will precipitate as fine colloidal particles or submicron-sized. Solid dispersion has, therefore, become one of the most influential exploration fields in the pharmaceutical sector owing to the promising rise in the bioavailability of poorly water-soluble drugs (Kurmi et al., 2016; Leuner et al., 2000). Solid dispersion technology is the science of diffusing one or even more principal components in a solid-state in an inert matrix to obtain enhanced dissolution or constant discharge of drugs, modified solid-state properties as well as increased stability (Leonardi et al., 2007). The word solid dispersion relates to a set of solid components composed of at least two distinct parts, a soluble matrix and a low aqueous-soluble drug in general. The matrix can be either crystalline or amorphous and the drug may be molecularly dissipated in amorphous particles (clusters) or crystalline particles (Mogal et al., 2012).

Aceclofenac is a derivative of phenyl acetic acid with powerful anti-inflammatory and analgesic properties. It is a novel NSAIDs that displays a multifactor mechanism of action. ACE's mode of action is based primarily on prostaglandin synthesis (PG) inhibition. ACE inhibits the enzyme of cyclooxygenase (Cox) involved in PG synthesis and also hinders synthesis inflammatory cytokines, interleukins, and tumor necrosis factors. The reduced nitrous oxide synthesis in human articular chondrocytes is also associated with its anti-inflammatory action. The aim of the study is focused on formulating the solid dispersion of poorly aqueous soluble drug aceclofenac by using different hydrophilic polymers to enhance the aqueous solubility and dissolution rate of the drug. The study also dwells on the effect of various polymers on drugs dissolution when formulated individually in different ratios.

## MATERIALS AND METHODS

Aceclofenac (API) was gifted from Quest Pharmaceuticals Pvt. Ltd. Pipara, Bara, Birgunj, Nepal. Hydroxyl propyl methylcellulose (HPMC) was purchased from Kemphasol. Mannitol and polyvinyl alcohol (PVA) were purchased from SD- Fine Chemicals. Dextrose was purchased from Qualigens and Isopropyl alcohol (IPA) was purchased from Rankem. All the chemicals used were of analytical grade.

### Formulation of solid dispersion

Solid dispersion of Aceclofenac was prepared by using four carriers

**Table 1.** Formulation design involving different Aceclofenac loaded solid dispersions.

Formulation	Drug	Carrier	Ratio (Drug: Carrier)
F1	Aceclofenac	Mannitol	1:2
F2	Aceclofenac	Dextrose	1:3
F3	Aceclofenac	HPMC	1:2
F4	Aceclofenac	PVA	1:3
F5	Aceclofenac	PVA	1:2
F6	Aceclofenac	HPMC	1:3
F7	Aceclofenac	Dextrose	1:2
F8	Aceclofenac	Mannitol	1:3

HPMC, PVA, Mannitol, Dextrose in two ratios (1:2, 1:3 w/w). Eight different formulations were designed by the varying carrier and the drug: carrier ratio as depicted in Table 1. The Solvent wetting method was used for preparing solid dispersion.

A weighed quantity of Aceclofenac was dissolved in an appropriate quantity of isopropyl alcohol. The amount of isopropyl alcohol used was 5 ml/gm polymer. The required amount of carrier was placed in the mortar, and then the drug solution was dropped into the carrier and was constantly stirred. Finally, the solvent was removed by evaporation at ambient temperature (25°C). The powder so obtained was ground in a mortar, dried and stored in a vacuum oven at 40°C for 24 h (Weerapol et al., 2017).

## Characterization

### Melting point determination

A small amount of the drug sample was taken and inserted in a thin-walled capillary tube; at one end, the tube was closed. The capillary comprising the sample was placed in the melting point apparatus and heated, and the melting point of the sample powder was observed when the drug sample was melted (Kala et al., 2016).

### Solubility study

An excess quantity of aceclofenac was placed in 100-ml conical flask containing 50 ml of different solutions (distilled water, 0.1 N HCl and phosphate buffer PH 7.4). The sample was stirred for 24 h. at 37°C in magnetic stirrer. The supernatant solution was then passed through a Whatmann filter paper 0.45 µm. The filtrate was appropriately diluted and the concentration of the Aceclofenac in the filtrate was determined by UV spectrophotometer at 273 nm (Samal et al., 2012).

### Percentage yield

The percent yield was helpful to observe the efficiency of the method of preparation and was evaluated as the ratio of the practical mass obtained (g) concerning the total theoretical mass of drug and carrier considered during formulation. The final yield was expressed in terms of percentage (Gaur et al., 2014; Patel et al., 2006).

$$\% \text{ Yield} = \frac{\text{Practical mass}}{\text{Theoretical mass}} \times 100$$

### Micrometric property

**Bulk density:** Appropriately weighted solid dispersions were transferred to a measuring cylinder (100 ml) and the true volume of the powder (bulk volume) was noted in the cylinder. The bulk density of the powder was measured as the ratio of the weight of the sample taken to the bulk volume of the loaded solid dispersion (Milling, 1991).

$$\text{Bulk density} = \frac{\text{Mass of solid dispersion}}{\text{Bulk volume of solid dispersion}}$$

**Tapped density:** Appropriately weighted solid dispersions were transferred to a measuring cylinder (100 ml). The measuring cylinder was subjected for 100 tapings or till constant volume was achieved. The achieved constant volume was considered as the tapped volume of solid dispersions. The tapped density of the powder was measured as the ratio of the weight of the solid dispersion taken to the tapped volume of the loaded solid dispersion (Singh et al., 2000).

$$\text{Tapped density} = \frac{\text{Mass of solid dispersion}}{\text{Tapped volume of solid dispersion}}$$

**Carr's (compressibility) index:** Carr's index value of solid dispersion was computed considering the tapped and the bulk density of the solid dispersion and was computed according to the following equation (Srivastava et al., 2005).

$$\% \text{ compressibility} = \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Tapped density}} \times 100$$

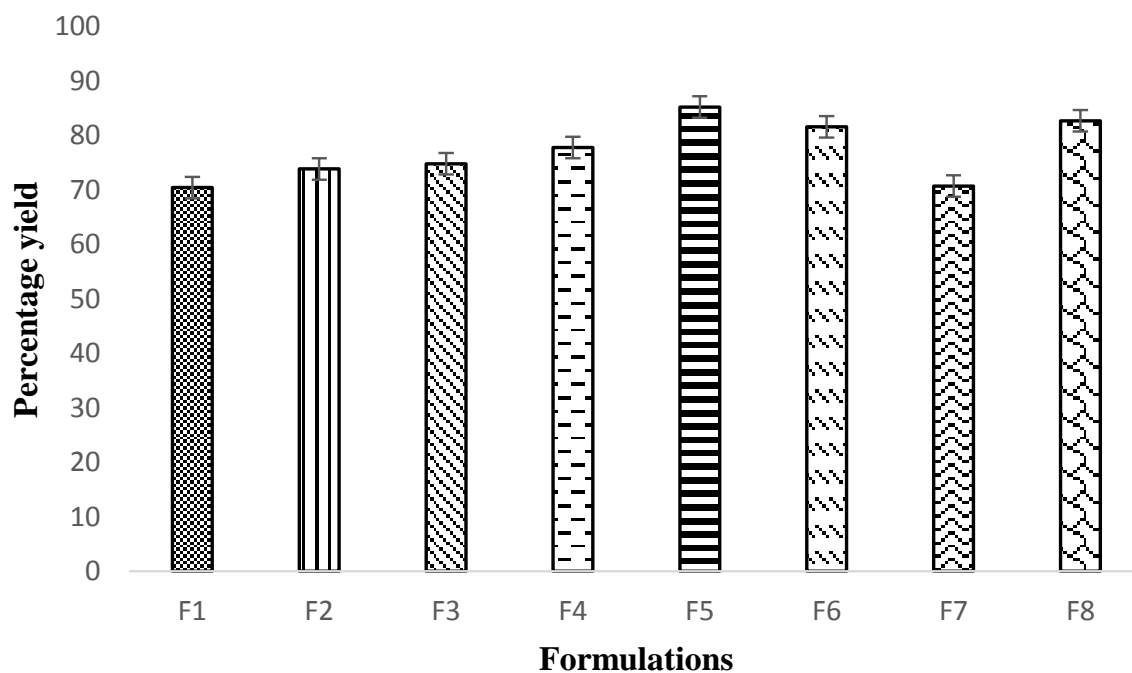
Powder with Carr's index value below 15% is usually considered to have good flow characteristics, while above 25% is considered to have poor flow ability.

**Hausner's ratio:** Hausner's ratio of solid dispersion was determined as the ratio of tapped density to bulk density using the following equation (Kannan et al., 2009).

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

A decent flow is shown by a Hausner ratio higher than 1.25, and a weak flow maybe 1.5.

**Angle of repose:** The angle of repose ( $\alpha$ ) of solid dispersion which measures the resistance of particle flow was determined by a fixed



**Figure 1.** Percentage yield of different Aceclofenac loaded solid dispersions.

funnel method. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of blends. Accurately weighed solid dispersion was allowed to pass through the funnel freely on the surface. The height and radius of the powder were measured and the angle of repose was calculated using the following equation (Bhardwaj et al., 2012).

$$\alpha = \tan^{-1} h/r$$

Where,

$\alpha$  = angle of repose

h = height

r = radius of the heap of powder or granule

#### ***In vitro* drug release study**

*In vitro* dissolution studies were carried out in a USP type II dissolution apparatus in both medium 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4) for both pure drug and prepared formulations. Sample equal to 50 mg of Aceclofenac was introduced to 900 ml dissolution medium at  $37 \pm 0.5^\circ\text{C}$  and stirring rate of 50 rpm. An aliquot sample (2 ml) was taken at 10, 20, 30, 40, 50, 60, 90, 120 and 180 min intervals with new medium substitute to retain sink condition. The sample was filtered and 1 ml of the sample was taken and diluted to 10 ml with the medium. Each sample was analyzed for Aceclofenac content by UV-Visible spectrophotometer at 273 nm.

#### **Statistical analysis**

All the experiments were run in triplicate and results were expressed as mean  $\pm$  SD. Statistical analysis was carried out using

Graph-Pad prism version 7 software (GraphPad software Inc., La Jolla, CA). The data was analyzed by Dunnett's multiple comparison tests. Statistical significance was predefined at  $p < 0.05$ .

## **RESULTS AND DISCUSSION**

### **Melting point and solubility**

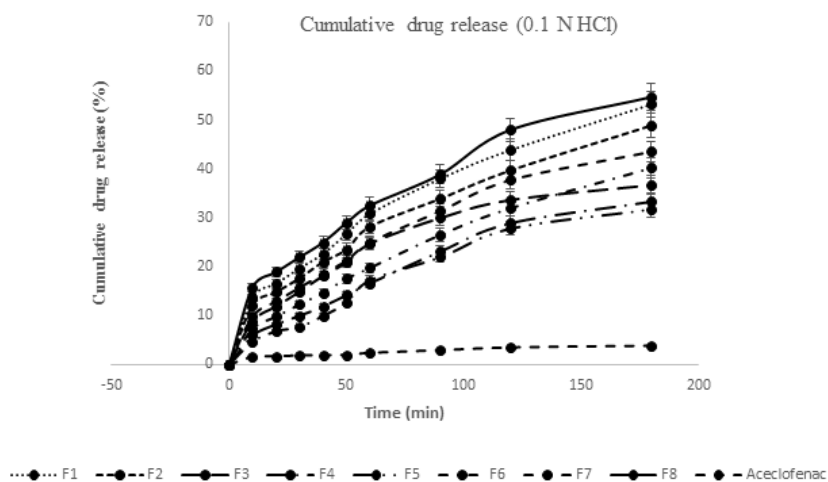
The melting point of the drug was observed at  $149.66^\circ\text{C}$ . The melting point of the drug was within the range of literature specification,  $149-150^\circ\text{C}$  indicating the identity and purity of the drug sample as Aceclofenac (Kala et al., 2016). The solubility of aceclofenac was  $0.0753 \pm 0.021$  mg/ml in distilled water. The study suggests that the solubility of aceclofenac was pH-dependent where the observed solubility was higher in Phosphate buffer (pH 7.4) with  $5.76 \pm 1.23$  mg/ml compared to acidic buffer (0.1 N HCl) with  $0.0214 \pm 0.012$  mg/ml. This might be because aceclofenac being weakly acidic remains unionized in lower pH while it remains ionized in higher pH value rendering the remarkable increase of drug solubility in aqueous media.

### **Percentage yield**

The percentage yield was conducted in eight different formulations. The results for the percentage yield of eight different formulations are depicted in Figure 1. The

**Table 2.** Summary result of micrometric properties of different Aceclofenac loaded solid dispersions.

Formulation	Bulk density	Tapped density	Carr's index	Hausener's ratio	Angle of repose
F1	0.6250±0.0024	0.7259±0.0022	13.89±0.0025	1.16±0.0024	29.03±2.7
F2	0.6122±0.0027	0.7031±0.0024	12.92±0.0019	1.14±0.0025	30.77±2.1
F3	0.6271±0.0023	0.7305±0.0023	14.15±0.0018	1.16±0.0022	27.88±1.9
F4	0.6165±0.0024	0.6544±0.0026	5.79±0.0025	1.06±0.0025	25.81±2.2
F5	0.6250±0.0022	0.7258±0.0029	13.88±0.0022	1.16±0.0022	20.77±2.9
F6	0.6338±0.0019	0.7438±0.0024	14.78±0.0022	1.17±0.0022	21.56±1.5
F7	0.6315±0.0024	0.7275±0.0022	13.19±0.0019	1.15±0.0029	30.61±2.9
F8	0.6428±0.0020	0.7408±0.0027	13.22±0.0022	1.15±0.0022	29.43±3.1



**Figure 2.** Cumulative percentage release of pure aceclofenac and aceclofenac from solid dispersion in 0.1 N HCl (pH 1.2).

observed percentage yield was highest in F5 with 85.18±6.02% while the percentage yield of F1 was the lowest of all the formulations accounting value of 70.43±5.028%.

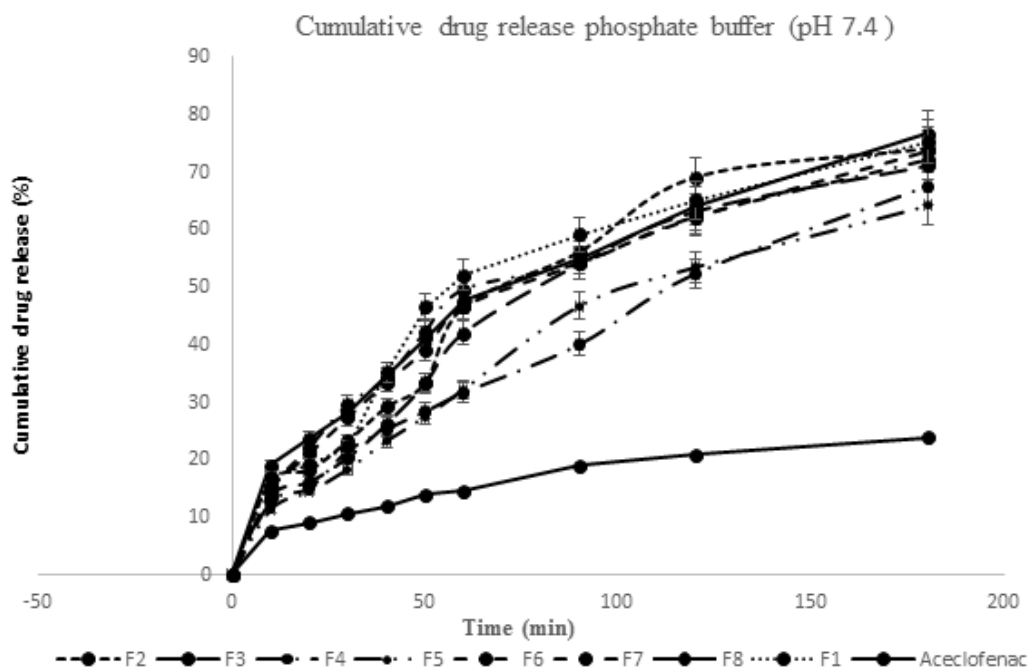
**Micrometric study**

The different micrometric study was conducted for eight different formulations. The summary result of the micrometric properties of eight different formulations is depicted in Table 2. The observed response indicated that the bulk density of F8 was greatest with 0.6428±0.002 g/ml while bulk density was the smallest for F2 with 0.6122±0.0027. The observed tapped density was highest for F6 with 0.7438±0.0024 g/ml while tapped density was lowest for F4 with 0.6544±0.0026. The Carr's index and Hausner's ratio value was smallest for F4 with 5.018±0.0025 and 1.06±0.0025 respectively indicating an excellent flow property, while Carr's index and Hausner's

ratio value was greatest for F6 with 15.35±0.0022 and 1.18±0.0022 indicating relatively poor flow. The angle of repose value was lower for F5 with 20.77±2.9° while the angle of repose value was higher for F2 with 30.77±2.1°. The solvent wetting method was found to be an efficient method to produce solid dispersions with good flow properties.

**In vitro drug release study**

The test on *in vitro* drug release was undertaken in both acid buffer (pH 1.2) and phosphate buffer (pH 7.4) for pure drug aceclofenac and eight separate formulations. The cumulative percentage drug release of pure aceclofenac in 0.1 N HCl and phosphate buffer is depicted in Figures 2 and 3 respectively. The cumulative *in vitro* drug release after 180 min for aceclofenac in acidic buffer (pH 1.2) was 3.89±0.41%, while according to observed study of cumulative percentage drug release



**Figure 3.** Cumulative percentage release of pure aceclofenac and aceclofenac from solid dispersion in Phosphate buffer (pH 7.4).

from eight different formulations as depicted in Figure 2, cumulative percentage drug release was highest in F8 with  $54.73 \pm 4.60\%$  and lowest in F5 with  $31.75 \pm 3.10\%$  as found under the same dissolution medium and interval. The *in vitro* drug release of different formulations in acidic buffer (pH 1.2) were significant compared to pure drug aceclofenac ( $p < 0.05$ ). Similarly, the cumulative *in vitro* drug release after 180 min for aceclofenac in phosphate buffer (pH 7.4) was  $23.79 \pm 2.20\%$  while, as per the observed result depicted in Figure 3, cumulative percent drug release was highest in F8 with  $76.65 \pm 6.50\%$  and lowest in F5 with  $64.09 \pm 5.70\%$  as in the same dissolution medium and interval. The *in vitro* drug release of different formulations in phosphate buffer (pH 7.4) were not significant compared to pure drug aceclofenac ( $p > 0.05$ ). The study also suggests that the intrinsic solubility as well as the rate of aceclofenac dissolution is poor, which may be attributed due to poor wettability and agglomeration of particles, so there is a strong need to enhance its solubility and dissolution. Meanwhile, when formulated as a solid dispersion relative with pure aceclofenac as a whole, there was a significant increase in the dissolution rate of aceclofenac. The probable mechanism might be due to the reduction of particle size of aceclofenac and increase in effective surface area of drug, amorphization and improved wettability of aceclofenac by hydrophilic carrier at diffusion layer (Yadav et al., 2013). In the order of Mannitol > dextrose >

HPMC > PVA, the rise in dissolution rate was noted. The release profile was higher with the formulation containing mannitol in the ratio 1:3 (F8) in both of the medium which might be due to generation of fine crystals of the drug when it comes in contact with dissolution medium resulting increase in wettability of the crystals, while release profile was lower with the formulation containing HPMC and PVA which might be due to the result of formation of viscous layer at the interface of drug and dissolution medium that hinders the diffusion of drug from the diffused layer to bulk layer (Rane et al., 2007; Zaini et al., 2017; Madgulkar et al., 2016). Similarly, the lower dissolution rate of PVA may be attributed to the weaker drug-polymer interaction in the PVA system that leads to the lower degree of amorphization and also due to its higher viscosity in solution, might hinder the transformation of the drug domain during dissolution (Chan et al., 2015).

## Conclusion

Aceclofenac solid dispersion was prepared by the solvent wetting method. The aqueous solubility of the drug aceclofenac was considerably lower resulting in its poor dissolution rate. Moreover, the solubility of the drug aceclofenac was found to be pH-dependent as a result of which the *in vitro* drug release of aceclofenac was higher

in phosphate buffer (pH 7.4) and lower in acidic buffer (pH 1.2) with the value of  $23.79 \pm 0.0022$  and  $3.89 \pm 0.0024\%$  respectively. The formulation of aceclofenac in a solid dispersion resulted in significant increase in *in vitro* drug release in both acidic and phosphate buffer. A comparative study of *in vitro* drug release of eight different formulations was carried. The observed result suggested that the percent cumulative drug release in acidic buffer was highest in F8 and lowest in F5 with  $54.73 \pm 0.0020$  and  $31.75 \pm 0.0022\%$  respectively. Similarly, the percent cumulative drug release in phosphate buffer was highest in F8 and lowest in F5 with  $76.65 \pm 0.0018$  and  $64.09 \pm 0.0019\%$ , respectively.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# **Histopathological and genetic study on the protective role of $\beta$ -Carotene on testicular tissue of adult male albino rats treated with titanium dioxide nanoparticles**

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Titanium dioxide nanoparticles are one of the famous widely produced nanoparticles in the world that are widely used in paints, cosmetics, plastics, pharmaceutical preparations, water treatment and purification and filtration of air. Beta carotene is provitamin A carotenoid with antioxidant and anti-apoptotic activities. The aim of this study is to evaluate the histopathological changes that occur in the testicular tissue of adult albino rats after intraperitoneal injection of titanium dioxide nanoparticles and to clarify possible protective role of beta carotene against this toxicity. 48 adult male albino rats weighing 180 to 200 g was divided into 4 groups: (I) *Control group* which consisted of 24 rats divided into 3 equal subgroups (Subgroup Ia (negative control): Received balanced diet for 14 days; Subgroup Ib (positive control): received intraperitoneal injection of distilled water for 14 days; Subgroup Ic: received corn oil by gastric gavage for 21 days (positive control)). *Beta carotene group* (positive control) which consisted of 8 rats received oral beta carotene 10 mg/kg once daily by gastric gavage for 21 days. *The treated group* which consisted of 8 rats received intraperitoneal (IP) injection of TiO<sub>2</sub> nanoparticles (300 mg/kg) daily for 14 days. *The protective group* which consisted of 8 rats received 10 mg/kg beta carotene for 7 days by gavage then intraperitoneal (IP) injection of TiO<sub>2</sub> nanoparticles (300 mg/kg) for 14 days together with 10 mg/kg beta carotene by gavage. Testicular sections were stained with H&E, Masson's trichrome, vimentin immunohistochemical staining. Serum testosterone level along with quantitative real-time PCR for TNF $\alpha$  gene was measured and statistical analysis was done. Results revealed that marked histopathological changes in testicular tissue was observed in TDN treated group which was improved by co-administration of beta carotene. In addition, statistically significant difference in TNF $\alpha$  expression in testicular tissue, testosterone level, body weights, testes weights and sperm count, motility, tubular dimensions, thickness of capsule in TDN treated group compared to the control group and protective group which showed significant improvement. Thus, titanium dioxide nanoparticles have hazardous effect on testis and that can be improved by beta carotene co-administration.

**Key words:** Adult albino rats, testes, titanium dioxide nanoparticles,  $\beta$ -carotene and gene expression.

## **INTRODUCTION**

Three decades ago, nanotechnology had been significantly flourished and has transitioned from bench

top science to applied technology. Whereas, nanomaterials (NMs) have been widely developed and applied in industry, medicine, cosmetics and personal care products (Shah et al., 2017; Wu and Tang, 2018), nanomaterials are predicted to soon become the cornerstone of the microelectronics, materials, textiles, energy, healthcare, and cosmetics industries (McIntyre, 2012).

The extensive use of nanotechnology raised concern about their adverse effects. There is increasing need to evaluate risk associated with their use, as humans potentially exposed to nanoparticles (NPs) due to their wide use in diagnosis or therapy (Shi et al., 2013; Faddah et al., 2013).

Owing to their minute size, NPs can get an entrée to many biological structures, interacting with molecules such as nucleic acids, proteins and lipids, which may, in turn, meddle with their normal function, damage the subcellular organelles and cause cellular death (Tay et al., 2014).

Nano-sized Titanium dioxide particles are one of the most commonly synthetic nanoparticles (Liang et al., 2009). Titanium dioxide particle is an odorless, low-solubility crystal which has thermal stability and combustibility, excellent physical properties, corrosion resistance, biocompatibility and excellent electrical and optical performance (Morgan et al., 2006).

Titanium dioxide NPs are found in toothpaste, food colorants, candies, sweets and chewing gums (Weir et al., 2012) as well as in sunblock creams for protecting the skin from ultraviolet light (Wiesenthal et al., 2011). They are also used for implanted medical devices as cardiovascular stents, dental implants, joint replacements and spinal fixation devices. However, under mechanical stress or altered physiological conditions such as low pH, titanium-based implants can release large amounts of NPs debris (Cunningham et al., 2002).

Contact to nanoparticle can be either by accident due to occupational exposure, or purposely through different routes such as nose by inhalation, mouth by intake, skin contact or intravenous injection (Zhu et al., 2012).

Carotenoids are yellow or orange, containing organic pigments found in the chloroplasts of plants, some bacteria, and fungi (Altincicek et al., 2011). Beta carotene is the most efficient pro-vitamin A carotenoid which acts as an antioxidant that protect the cells against free radicals, due to its unique structure and cleavage efficacy (Orazizadeh et al., 2014). The present study is aimed at evaluating the possible toxic effect of Titanium dioxide NPs on the structure of the testis of adult albino rats and to determine the protective role of beta carotene to

minimize its toxicity.

## MATERIALS AND METHODS

### Chemicals

Titanium dioxide NPs (TiO<sub>2</sub>NPs) nano powder comes with the following characters: <100 nm particle size, surface area of 35-65 m<sup>2</sup>/g and purity ≥99.5% trace metals basis and CAS No 634662. It is a white odorless fine powder mixture of rutile and anatase manufactured by Sigma-Aldrich Chemical Company, Germany and purchased from Sigma-Egypt. B-carotene is presented in red orange powder form, obtained from Sigma-Aldrich Chemical Company, Germany and was purchased from Sigma-Egypt with code c 9750. Corn oil was obtained from SEKEM Company, Cairo, Egypt and primers were obtained from Delta Company Egypt.

### Animals

The present study was conducted on forty-eight adult male albino rats weighing about 180 g. They were housed in a temperature-controlled and light-controlled room (12-h light/dark cycle), with free access to food and water. All experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Faculty of Medicine; Zagazig University, Egypt. The animals were divided equally into 4 groups:

**Control group (I):** this contains 24 rats and is divided into 3 equal subgroups:

*Subgroup Ia (negative control):* 8 rats received balanced diet for 14 days.

*Subgroup Ib (positive control):* 8 rats received intraperitoneal injection of distilled water for 14 days.

*Subgroup Ic (positive control):* 8 rats received corn oil by gastric lavage for 21 days.

**Beta carotene group (II):** 8 rats received oral beta carotene 10 mg/kg once daily by gastric lavage for 21 days.

**The treated group (III) (TDN group):** this contains 8 rats that received intraperitoneal (IP) injection of TiO<sub>2</sub> NPs (300 mg/kg) daily for 14 days (Xu et al., 2013).

**The protective group (IV) (TDN+BC group):** This contains 8 rats that received 10 mg/kg beta carotene for 7 days by gavage then intraperitoneal (IP) injection of TiO<sub>2</sub> NPs (300 mg/kg) for 14 days together with 10 mg/kg β-carotene by gavage (Lyama et al., 1996). By the end of the experiment, all animals were anesthetized by thiopental inhalation, venous blood samples were collected by means of micro-capillary glass tubes from vein of rat tail for assessment of testosterone level and then animals were sacrificed. Laparotomy was performed and the testis was carefully dissected out and weighed then subjected to histopathological, immunohistochemistry and morphometrical examination.

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**Table 1.** primers used for Semi-quantitative PCR analysis

Target gene	Primer sequence: 5' - 3'
TNF- $\alpha$	Forward: 5'- CCACCACGCTCTTCTGTCTAC-3'
	Reverse: 5'-ACCACCAGTTGGTTGTCTTTG-3'
GAPDH	Forward: 5'-AGATCCACAACGGATACATT-3
	Reverse: 5-TCCCTCAAGATTGTCAGCAA-3'

## Approach

### Testosterone level

Venous blood samples for each animal, about 2 mL were collected into a glass tube for measuring testosterone hormone levels. Blood samples were left for serum separation, and then centrifuged at 3000 r.p.m. for 15 min. Serum samples were collected and kept frozen at -20°C for performing hormonal analysis by using Testosterone Rat/Mouse ELISA (Enzyme-linked immunosorbent assay) (Zirkin and Chen, 2000).

### Epididymal spermatozoa examination

Spermatozoa collection was done from the content of the Epididymis of each rat. It was obtained immediately by cutting the tail of the epididymis and squeezing it gently to obtain the fresh undiluted semen in a clean Petri dish to proceed with the following examinations:

**Sperm motility:** A small droplet of undiluted semen was added to one drop of sodium citrate solution in 2.9-3% on warm slide, several fields were examined under light microscope and incidence of motile sperms were estimated and recorded (Bearden and Flyquary, 1980).

**Sperm count:** The hemocytometer pipette was used to withdraw the undiluted semen up to the 0.1 mark and was then filled up to the mark 101 by normal saline stained with eosin; thereafter, the pipette content was vigorously shaken by holding the ends of the pipette between the thumb and index finger. A cover slide was placed over the hemocytometer counting chamber and a drop of diluted semen was spread between the hemocytometer chambers and its cover. Thereafter, the sperm were counted in 5 large squares at 400X magnification. Sperm concentration was estimated by multiplying the counted number of sperm by 100 (depth) and 1000 (dilution) (Blazak et al., 1993).

### Real time PCR detection of TNF $\alpha$ gene

**RNA extraction and cDNA synthesis:** For preparation of total RNA, testes (approximately 100 mg per sample) were collected from rats, frozen in liquid nitrogen and subsequently stored at -70°C

in 1 mL Qiazol (QIAGEN Inc., Valencia, CA). Frozen samples were homogenized using a Polytron 300 D homogenizer (Brinkman Instruments, Westbury, NY). Thereafter, 0.3 mL chloroform was added to the homogenate. The mixtures were shaken for 30 s followed by centrifugation at 4°C and 12,500 rpm for 20 min. The supernatant layer was transferred to a new set of tubes and an equal volume of isopropanol was added to the samples, shaken for 15 s and centrifuged at 4°C and 12,500 rpm for 15 min. The RNA pellets were washed with 70% ethanol, briefly dried up, then dissolved in Diethylpyrocarbonate (DEPC) water. The prepared RNA integrity was checked by means of electrophoresis. RNA concentration and purity were determined spectrophotometrically at 260 nm. The ratio of the 260/280 optical density of all RNA samples was 1.7-1.9. For synthesis of cDNA, mixture of 2  $\mu$ g total RNA and 0.5 ng oligo dT primer in a total volume of 11  $\mu$ L sterilized DEPC water was incubated in the PeX 0.5 thermal Cycler (Thermo Electronic Corporation, Milford, Ma) at 65°C for 10 min for denaturation. Thereafter, 4  $\mu$ L of 5X RT-buffer, 2  $\mu$ L of 10 mM dNTPs and 100 U Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase (SibEnzyme Ltd. Ak, Novosibirsk, Russia) were added and the total volume was completed up to 20  $\mu$ L by DEPC water. The mixture was then re-incubated in the thermal Cycler at 37°C for 1 h, then at 90°C for 10 min to inactivate the enzyme.

### Semi-quantitative PCR analysis

Specific primers for tested genes (Table 1) were designed using Oligo-4 computer program and synthesized by Macrogen (Delta Company, Egypt). PCR was conducted in a final volume of 25  $\mu$ L consisting of 1  $\mu$ L cDNA, 1  $\mu$ L of 10 picomolar (pM) of each primer (forward and reverse) and 12.5  $\mu$ L PCR master mix (Promega Corporation, Madison, WI); the volume was brought up to 25  $\mu$ L using sterilized, deionized water. PCR was carried out using a PeX 0.5 thermal Cycler with the cycle sequence at 94°C for 5 min one cycle, followed by different cycles each of which consisted of denaturation at 94°C for one minute, minute with additional final extension at 72°C for 7 min. As a reference, expression of Glyceraldehyde-3-Phosphate Dehydrogenase (G3PDH) mRNA was detected by using specific primers (Table 1). PCR products were electrophorized on 1% agarose gel (Bio Basic INC. Konrad Cres, Markham Ontario), and stained with ethidium bromide in TBE (Tris-Borate-EDTA) buffer. PCR products were visualized under UV light and photographed using gel documentation system. The intensities of the bands were quantified densitometrical using ImageJ software (El-Kirdasy et al., 2014).

**Table 2.** Comparison between mean values of testes weights and rat weights in different studied groups.

Parameter	Control	$\beta$ -Carotene	Titanium	Titanium + $\beta$ -Carotene	ANOVA
	Mean $\pm$ SD				
Rat weight (g)	215.63 $\pm$ 11.78 <sup>a</sup>	216.25 $\pm$ 10.26 <sup>a</sup>	165.63 $\pm$ 17.20 <sup>b***</sup>	200.63 $\pm$ 17.82 <sup>A</sup>	< 0.0001***
Testis weight (g)	1.30 $\pm$ 0.06 <sup>a</sup>	1.30 $\pm$ 0.13 <sup>a</sup>	1.03 $\pm$ 0.03 <sup>b**</sup>	1.21 $\pm$ 0.20 <sup>A</sup>	0.0009***

Groups with different letters are statistically significant P value <0.05. Different stages of significance were considered. High significance (\*\*\*) when P value < 0.001, Moderate significant (\*\*\*) at 0.01 >P value >0.001 and low significance (\*) when 0.05 >P value >0.01.

### Histopathological examination

The testes of male albino rats were collected at the end of experiment from tested groups. The samples were fixed in Bouin's solution, then dehydrated in ascending grades of alcohols, cleared in xylene and embedded in paraffin. The samples were casted, then sliced into 5  $\mu$ m in thickness and placed onto glass slides. The slides were stained by hematoxylin and Eosin (H&E) stains and Masson's Trichrome (Bancroft and Gamble, 2008).

### Immunohistochemical study

Immunohistochemistry was performed following the method of Ramos-Vara et al. (2008). The paraffin sections were processed by Streptavidin-biotin complex (Strep ABC) paraffin deparaffinized in xylene, hydrated and then placed in phosphate buffered saline (PBS; pH 7.6). Antigen retrieval was performed by boiling for 15 min in citrate buffer (0.01 M). Sections were treated with 3% hydrogen peroxide for 5 min to quench endogenous peroxidase activity, rinsed with deionized water and then washed with PBS. Sections were incubated first with 1% pre-immune rabbit serum to decrease non-specific staining and then with a monoclonal antibody against Vimentin (Dako, Carpinteria CA, USA) at 23°C in a moist chamber for 1 h. Detection of the antibody was performed using a biotin-streptavidin detection system (Bio Genex, San Ramon CA, USA) with 3-amino 9-ethyl carbazole (AEC) as chromogen (Dako, Carpinteria CA, USA). Sections were counterstained with Mayer's hematoxylin and sections were evaluated using a light microscope.

### Morphometric study

The morphometric study was done using Image analyzer software (Leica Imaging System Ltd., Cambridge, England). Ten readings were obtained in each specimen and the mean values were obtained. According to this method, we used an optical magnification of 100 for calculating the mean tubular diameter, epithelial height and interstitial space thickness, and tunica albuginea thickness were performed on routine H&E sections while thickness of capsule was performed on sections stained with Masson's trichrome.

### Statistical analysis

Results are expressed as means  $\pm$  S.D of 8 different rats per each group. Statistical analysis was done using ANOVA with p<0.05 being considered as statistically significant.

## RESULTS

### Body weight

Regarding weight of rats, titanium treated group showed significant decrease than all other groups. Moreover, there is no significant difference between control and both  $\beta$ -carotene and  $\beta$ -carotene in combination with titanium (Table 2).

### Testis weight

Considering testicular weight, titanium group showed significant decrease than all other groups. Although,  $\beta$ -Carotene and  $\beta$ -Carotene in combination with titanium showed non-significant difference with control group (Table 2).

### Laboratory results

There was non-significant difference in sperm count between both control and  $\beta$ -Carotene groups; sperm count of Titanium treated group was significant less than control and  $\beta$ -Carotene groups. Moreover, on administration of beta carotene in combination with titanium, sperm count is significantly less than control but significantly more than treated (Table 3).

As regards both sperm motility and testosterone level, there is significant decrease in Titanium treated group in comparison to all other groups. Moreover, in the group protected by  $\beta$ -Carotene, sperm count was significantly less than control but significantly more than treated (Table 3).

Considering TNF  $\alpha$ , there was non-significant difference between control,  $\beta$ -Carotene and Titanium +  $\beta$ -Carotene groups, but titanium treated group was significantly increased in comparison with all other groups (Table 3).

### Histopathological examination

#### Hematoxylin and eosin (H&E)

Examination of H&E sections obtained from testis in

**Table 3.** Comparison between mean values of sperm count, motility, testosterone and TNF  $\alpha$  in different studied groups.

Parameter	Control	$\beta$ -Carotene	Titanium	Titanium + $\beta$ -Carotene	ANOVA
	Mean $\pm$ SD				
Sperm count (million/mm <sup>3</sup> )	24.75 $\pm$ 4.53 <sup>a</sup>	24.50 $\pm$ 3.74 <sup>a</sup>	10.58 $\pm$ 2.02 <sup>b***</sup>	16.19 $\pm$ 3.25 <sup>c***</sup>	< 0.0001***
Sperm motility (%)	85.63 $\pm$ 5.73 <sup>a</sup>	84.63 $\pm$ 7.03 <sup>a</sup>	37.50 $\pm$ 9.64 <sup>b***</sup>	63.75 $\pm$ 8.35 <sup>c***</sup>	< 0.0001***
Testosterone ( $\mu$ g/ml)	4.25 $\pm$ 0.59 <sup>a</sup>	4.28 $\pm$ 0.42 <sup>a</sup>	1.72 $\pm$ 0.48 <sup>b***</sup>	3.22 $\pm$ 0.66 <sup>c**</sup>	< 0.0001***
TNF $\alpha$	1.025 $\pm$ 0.35 <sup>a</sup>	1.03 $\pm$ 0.18 <sup>a</sup>	1.60 $\pm$ 0.21 <sup>b**</sup>	1.21 $\pm$ 0.36 <sup>A</sup>	0.0006

Groups with different letters are statistically significant P value <0.05. Different stages of significance were considered. High significance (\*\*\*) when P value < 0.001, Moderate significant (\*\*) at 0.01 >P value >0.001 and low significance (\*) when 0.05 >P value >0.01.

**Table 4.** Comparison between mean values of seminiferous tubule diameter, germinal epithelium thickness, interstitial space thickness, basement membrane thickness, *Tunica albuginea* thickness.

Parameter	Control	$\beta$ -Carotene	Titanium	Titanium + $\beta$ -Carotene	ANOVA
	Mean $\pm$ SD				
Seminiferous tubule diameter ( $\mu$ m)	233.03 $\pm$ 12.43 <sup>A</sup>	229.37 $\pm$ 18.01 <sup>a</sup>	123.59 $\pm$ 9.97 <sup>b***</sup>	165.83 $\pm$ 14.68 <sup>c***</sup>	<0.0001***
Germinal epithelium thickness ( $\mu$ m)	58.62 $\pm$ 6.89 <sup>A</sup>	59.77 $\pm$ 5.11 <sup>a</sup>	36.30 $\pm$ 6.30 <sup>b***</sup>	47.41 $\pm$ 6.35 <sup>c**</sup>	<0.0001***
Interstitial space thickness ( $\mu$ m)	9.81 $\pm$ 1.62 <sup>A</sup>	9.60 $\pm$ 1.19 <sup>a</sup>	18.91 $\pm$ 3.40 <sup>b***</sup>	10.15 $\pm$ 1.42 <sup>A</sup>	<0.0001***
Tunica albuginea thickness ( $\mu$ m)	12.79 $\pm$ 1.87 <sup>a</sup>	12.15 $\pm$ 1.32 <sup>a</sup>	62.34 $\pm$ 12.79 <sup>b***</sup>	15.21 $\pm$ 4.05 <sup>a</sup>	<0.0001***
Vimentin OD	0.671 $\pm$ 0.177 <sup>a</sup>	0.670 $\pm$ 0.145 <sup>a</sup>	0.273 $\pm$ 0.058 <sup>b***</sup>	0.554 $\pm$ 0.094 <sup>a</sup>	<0.0001***

Groups with different letters are statistically significant P value <0.05. Different stages of significance were considered. High significance (\*\*\*) when P value < 0.001, Moderate significant (\*\*) at 0.01 >P value >0.001 and low significance (\*) when 0.05 >P value >0.01.

control group revealed normal seminiferous tubules lined by stratified germinal epithelium. Spermatozoa were observed in the lumina of the tubules (Figure 1a).

The germinal epithelium revealed two types of cells; spermatogenic and Sertoli. Sertoli cells were detected in-between spermatogenic cells as pyramidal cells. The spermatogenic cells were seen in regularly arranged rows at different stages of spermatogenesis. They were arranged from the basal compartment to the lumina of the tubules starting from spermatogonia, primary spermatocytes, and spermatids till mature spermatozoa in the lumen (Figure 1b).

On the other hand, examination of H&E sections obtained from testes in treated group demonstrated highly congested sub-capsular blood vessel (Figure 2a). Other sections showed loss of normal hexagonal uniform of seminiferous tubules. They appeared with irregular contour with detached and separated basement membrane (Figure 2c).

Others tubules showed detached germinal epithelium from basement membrane (Figure 2a) and loss of layers of germinal epithelium (Figure 2b). And others exhibited disorganized germinal epithelium with darkly stained pyknotic nuclei and vacuolation within seminiferous tubule (Figure 2d).

Lumina of some tubules appeared either empty or with many sloughed germinal cells and deposition of hyaline

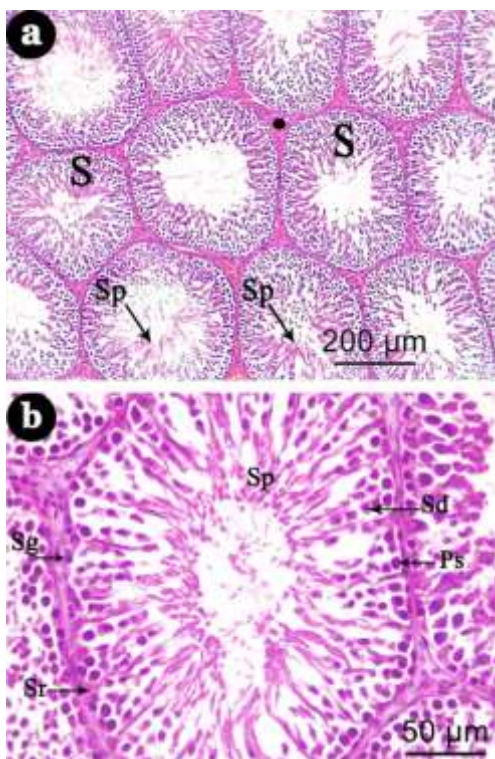
acidophilic material in interstitium and some acidophilic material also appeared in the lumina of some seminiferous tubules (Figure 2b).

Examination of H&E sections obtained from testes in the protective group demonstrated wide areas of testicular tissue similar to the examined control sections. Most seminiferous tubules had nearly normal architecture lined by stratified germinal epithelium formed of several layers. Their lumina of some of them showed aggregated spermatozoa and few seminiferous tubules lined by disorganized detached germinal epithelium from basement membrane (Figure 3a, b and d). Other section revealed congested blood vessels between seminiferous tubules (Figure 3c).

### Masson's trichrome stain

Masson's trichrome stained sections of control group revealed normal distribution of collagen fibers in the testicular capsule and around the blood vessels (Figure 4a), while stained sections obtained from treated group revealed marked increase of collagen fibers in the capsule and around blood vessels (Figure 4b).

On the other hand, Masson's trichrome stained sections obtained from protective group revealed mild increase of collagen fibers in testicular capsule and



**Figure 1.** (a) A photomicrograph of a section of control adult albino rat testis showing oval to round closely packed seminiferous tubules (S) with little interstitial tissues in between (black dot) and cavity full of sperms (Sp). (H&E  $\times 100$ ). (b): A photomicrograph showing seminiferous tubules lined by Sertoli cells appeared pyramidal cells with pale nucleus (Sr) and different germ cells namely, spermatogonia were seen close to the basement membrane with their dark nuclei (Sg), primary spermatocyte were the largest cells (Ps) spermatids appeared smaller than primary spermatocytes and lying near the lumen (Sd) and spermatozoa (Sp) from outward inward. (H&E  $\times 400$ ).

around blood vessels (Figure 4c).

### Vimentin immunostaining

Vimentin immunostaining testicular section of the control group showed Vimentin prominently expressed in the Sertoli cells with clear continuity between both Sertoli cells' apical and basal segments (Figure 5a). Vimentin immunostaining of testicular section of the treated group showed Sertoli cells destruction exhibited clearly. There was nearly complete destruction of the apical segment of Sertoli cells with scarce appearance of basal segment in most of tubules (Figure 5b).

However, Vimentin immunostaining of testicular section of protective group showed moderate improvement with

conservation of the vimentin expression pattern of the control in some Sertoli cells with apical and basal segments. However, few Sertoli cells showed some destruction (Figure 5c).

### Morphometrical results

There is non-significant difference in tubular diameter and germinal epithelium thickness between control and  $\beta$ -Carotene groups. Tubules of titanium treated group were significant less than control and  $\beta$ -Carotene groups. Moreover, on administration of beta carotene in combination with titanium, tubular diameter is significantly less than control but significantly more than treated. (Table 4).

Considering interstitial space thickness and tunica albuginea, there is non-significant difference in between control and  $\beta$ -Carotene and Titanium +  $\beta$ -Carotene groups. Interstitial space thickness of Titanium treated group was significant more than all other groups. (Table 4).

There is non-significant difference in Vimentin OD (Optical Density) between control, beta carotene and Titanium +  $\beta$ -Carotene groups. Vimentin OD in titanium treated group was significant less than all other groups. (Table 4).

### DISCUSSION

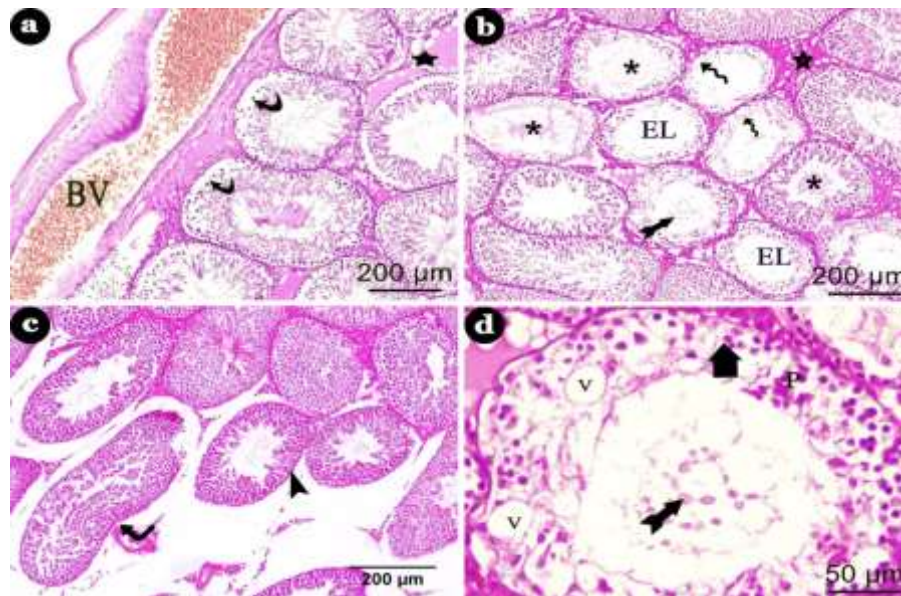
The testes are the most vital organs of the male reproductive system and are highly sensitive to genetic, hormonal and environmental insults (e.g., X-ray exposure, infectious diseases, and toxicants) (Lewis and Aitken, 2005), as it possess highly sensitive cellular composition of the spermatogenic epithelium and the high rate of mitotic activity (EL Shafai et al., 2011).

Rats have well-defined reproductive systems and the compounds which can cause infertility in human males were also noticed to be active in rats (Cho et al., 2014).

In this study, Titanium dioxide was injected by i.p route. The drug absorption by i.p injection is good and rapid because of the intensive blood and lymph vessels in the peritoneum beside its large surface area, thus the drug easily reached the circulation. Moreover, injection can avoid the common gastrointestinal side effects related to oral route of administration (Nebendahl, 2000). Moreover, Shi et al. (2013) used intraperitoneal method to evaluate the possible toxic effects of  $\text{TiO}_2$ NPS used in nanomedicine.

$\beta$ -carotene is a precursor of vitamin A which acts as potent antioxidant agent that blocks the free radicle formation or scavenges them (Yüncü et al., 2015). The present study was designed to investigate protective effect of  $\beta$ -carotene on TNP-induced testicular damage.

Regarding the effect of  $\text{TiO}_2$  on body weights, our



**Figure 2.** (a) A photomicrograph of a section of adult male albino rat testis of the  $\text{TiO}_2$  treated group showing highly congested sub-capsular blood vessel (BV) some seminiferous tubules showing detached germinal epithelium from basement membrane (curved arrow) and vacuolated acidophilic material in interstitium (star). (H&E  $\times 100$ ) (b) A photomicrograph showing some shrunken tubules with different shape and irregular outlines (head of arrow) also with irregular corrugated outline (bent arrow) (H&E  $\times 100$ ). (c) A photomicrograph showing distorted lining of some seminiferous tubules and loss of some layer of germinal epithelium (wavy arrow), sloughing of some cells in the lumen (thick tailed arrow) other tubule show empty lumen (EL) acidophilic hyaline material appears in the lumen of some seminiferous tubules (astrike) and interstitium (star) (H&E  $\times 100$ ). (d) A photomicrograph showing disorganized germinal epithelium (thick short arrow) with marked vacuolation (V), dark pyknotic nuclei (P), and sloughing of some cells in the lumen (thick tailed arrow) (H&E  $\times 400$ ).

results revealed that rats fed on  $\text{TiO}_2$  had lowered mean value of body weights compared with that of the control group; this result is in agreement with Khayal et al. (2019) who observed that Titanium dioxide NPS treated rats revealed a significant reduction in body weight in comparison with other groups. El-Sharkawy et al. (2010) explained that the weight loss is paralleled with anorexia, depression, and white feces among the different dose levels of  $\text{TiO}_2$  treated male rats along the experimental period.

Protective group showed a significant increase in body weight in comparison to TDN treated group; this result is in disagreement with Orazizadeh et al. (2014) who reported that the body weight in all groups (control group, TDN treated group and TDN + BC group) was the same and no difference was detected between them.

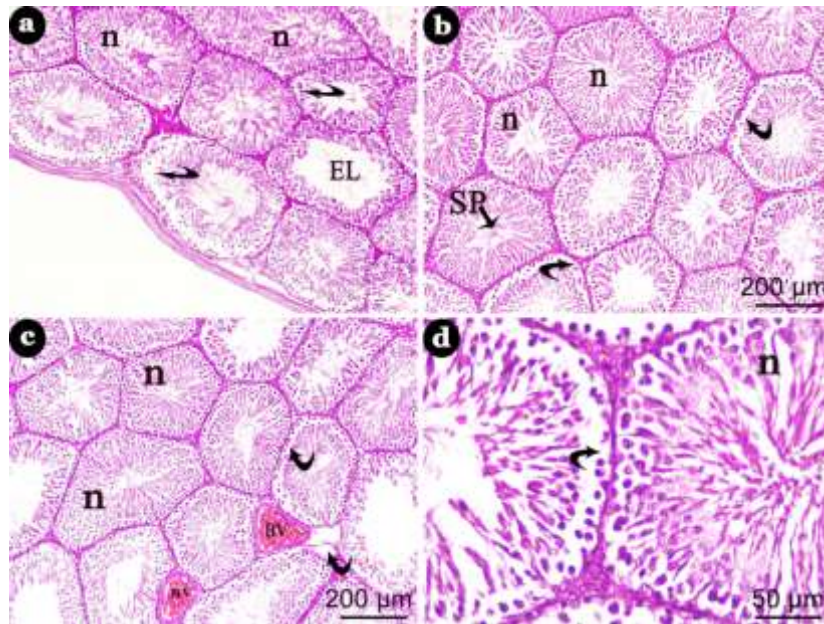
This study revealed that rats fed on  $\text{TiO}_2$  had lowered mean value of testicular weights; this result is in agreement with Morgan et al. (2015) who observed significant decrease in relative testis weights after 8 weeks of  $\text{TiO}_2$  exposure. Contrary to our results, Jia et al. (2013) found no significant difference in absolute or

relative weights of testes and epididymides between TDN-exposed mice and control group after oral exposure to 10, 50 and 250 mg/kg/day TDN (25 nm) for 42 days, but in protective group there was significant increase in testis weight in comparison to TND intoxicated group; this result is in agreement with Orazizadeh et al. (2014) who also observed significant increase in testis weight in BC + TDN group in comparison with TND treated group.

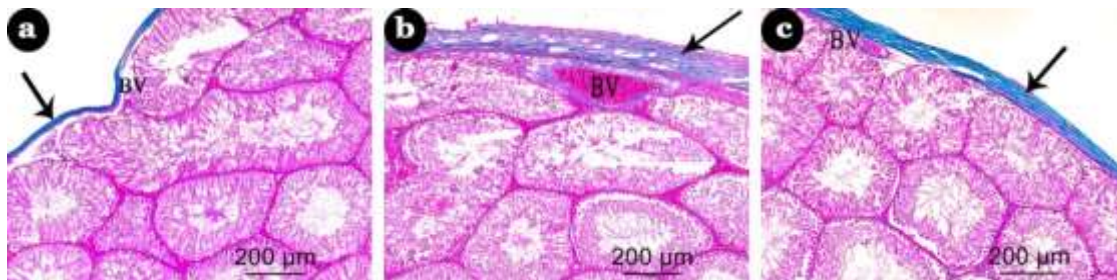
This discrepancy between our results and the previous literatures concerning the suppressive effect of TDN on body and sex organs weights could be related to particle size, species, strain, route, dose and duration of exposure variation.

This study revealed that intraperitoneal administration of TDN resulted in a significant decrease in serum testosterone level and reduction in sperm count and sperm motility compared to control. This is consistent with Karimi et al. (2019) who reported that there was significant reduction in testosterone concentration in the  $\text{TiNO}_2$  treated animals. However, in the protective group there was significant increase in testosterone level and sperm count; this result is in agreement with Orazizadeh





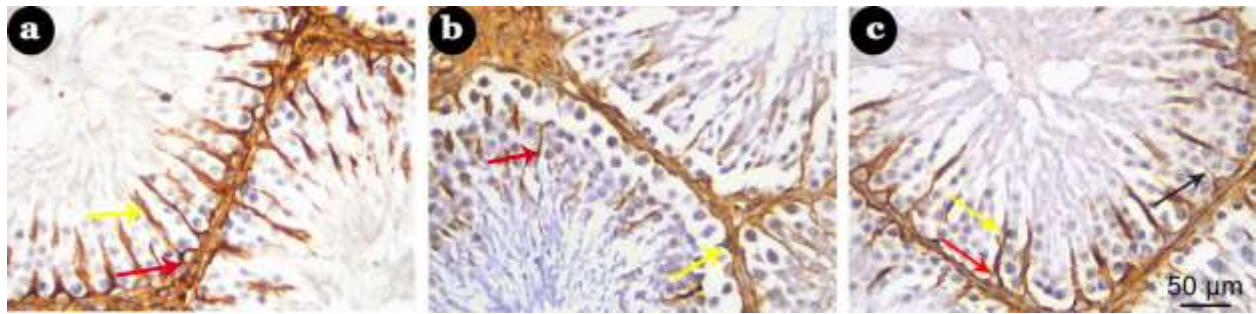
**Figure 3.** (a) A photomicrograph of a section of adult male albino rat testis of the TiO<sub>2</sub>&beta carotene treated group showing some tubules appear normal (n) while others appear affected with detached germinal epithelium (curved arrow) and some tubule appear with empty lumen (EL) (H&E ×100). (b) A photomicrograph showing tubules with disorganized detached germinal epithelium from basement membrane (curved arrow) between others appear normal (n) with aggregation of sperms in the lumen (SP) (H&E ×100). (c) A photomicrograph showing few disorganized seminiferous tubules with detachment of spermatogenic cell (curved arrow) between many normal seminiferous tubules (n) and congested blood vessels between seminiferous tubules (BV) (H&E ×100). (d) A photomicrograph showing seminiferous tubules lined by detached germinal epithelium from basement membrane (curved arrow) and another tubule showing nearly normal organization of seminiferous tubule (n) (H&E ×400).



**Figure 4.** (a) A photomicrograph of a section of the testis of an adult male albino rat of control group showing normal distribution of collagen fibers in the capsule (arrow) and around blood vessels (BV) (Masson's trichrome ×100). (b) Photomicrograph of a section of the testis of an adult male albino treated group showing marked increase of collagen fibers in the capsule (arrow) and around the blood vessels (BV). (Masson's trichrome ×100) (c) Photomicrograph of a section of the testis of an adult male albino rat protective group showing mild increase of collagen fibers in the capsule ( arrow) and around blood vessels (BV) (Masson's trichrome ×100).

et al. (2014) who reported that BC elevated the testosterone concentration in TDN-intoxicated mice, Sofikitis et al. (2008) assumed that the protection in

gametogenic activity in BC pre-treated mice may be the result of restoration of testicular androgenesis, as androgen is a prime regulator of gametogenesis.



**Figure 5.** (a) A photomicrograph of testicular section of the control group showing Vimentin prominently expressed in the Sertoli cells with clear continuity between both Sertoli cells apical (yellow arrow) and basal segments (red arrow) (Vimentin X400). (b) A photomicrograph of testicular section of the treated group showing Sertoli cells destruction exhibited clearly, there was nearly complete destruction of the apical segment of Sertoli cells (red arrow) with scarce appearance of basal segment in most of tubules (yellow arrow) (Vimentin x400). (c) A photomicrograph of testicular section of protective group showing conservation of the vimentin expression pattern of the control in some Sertoli cells with apical (yellow arrow) and basal segments (red arrow). However, few Sertoli cells still showing some destruction (arrow) (Vimentin x400).

In our study, administration of  $TiO_2$  to rats increased  $TNF-\alpha$  expression in testes and their expression was normalized in rats administered  $\beta$  carotene together with  $TiO_2$ . This result is in agreement with El-Kirdasy et al.

(2014) who mentioned that  $TiO_2$  induced oxidative stress and increased IL-6 and  $TNF-\alpha$  expression in testes and their expression was normalized in protective group. It is also in agreement with Khayal et al. (2019) who reported that the rats treated with  $TiO_2$ NPs showed significant elevations of IL6 and  $TNF-\alpha$  levels with significant reduction in IL10 in the ileal tissue compared to other groups.

In this study, normal architecture of the seminiferous tubules and intact germinal epithelium were observed in BC ( $\beta$ - carotene) group. This result in agreement with Orazizadeh et al. (2014); also, there was no difference in the histopathology criteria between BC and control groups.

In the present study, H&E stained sections of the testis of treated group revealed highly congested sub capsular blood vessel, distortion and loss of normal organization of the seminiferous tubules. Moreover, some tubules appeared shrunken and had variable shapes with irregular outlines and wide spacing from each other. Detachment of germinal epithelium from the underlying basement membrane and disorganized epithelial lining with marked depletion in germ cell number were noticed. Some tubules showed wide empty lumens while others showed exfoliation of germ cells within their lumens. This result in agreement with Karimi et al. (2019) who observed that in the  $TiO_2$  NPs treated group there were disorganization, detachment, sloughing and atrophy of germ cell layer.

In this study, the testis in  $\beta$ -Carotene and  $TiO_2$  administered rats showed partial improvement in the testicular tissue, some of seminiferous tubules nearly

retained their normal architecture, they revealed regular rounded contour and were lined by healthy germinal epithelium showing several types of spermatogenic cells and lumens contained aggregations of sperms and other seminiferous tubules lined by disorganized germinal epithelium, acidophilic hyaline material appears in interstitium and seminiferous tubules and mild congestion of the testicular blood vessels still persist; these results are in agreement with Orazizadeh et al. (2014).

In this study, Vimentin immunostaining in control group showed distinct expression of Vimentin in Sertoli cell cytoplasm. Vimentin positive reaction was found around the nucleus with apical extensions projecting towards the developing spermatids, a result that is in agreement with Alam and Kurohmaru (2014); however, in treated group, Sertoli cells showed loss of vimentin apical extensions towards the lumen, with the perinuclear condensation preserved and is in agreement with those of Dalgaard et al. (2000, 2001) who suggested that this alteration may be related to fragmentation of vimentin filaments into small subunits which become collected in the perinuclear region. As vimentin filaments of sertoli cells play an important role in the maintenance of spermatogenesis, therefore any alteration in the distribution of vimentin filaments correlate with sloughing of spermatogenic cells from the seminiferous epithelium (Zhu et al., 2010). The protective group showed conservation of the expression pattern of the control in most of sertoli cells. However, some sertoli cells showed loss of vimentin apical extensions towards the lumen, results that were consistent with Erkekoglu et al. (2011).

As regards Masson's trichrome, staining sections from control group revealed normally distributed collagen fibers in the capsule and around blood vessels, results that were in agreement with those described by Abd Ellatief et al. (2015); but in treated group revealed marked

increase of collagen fibers especially in the capsule and around blood vessels; this result is in accordance with Salem et al. (2017) and Altindag et al. (2007) who proposed that the increased collagen fibers are due to the decrease in collagen metabolism that may be related with oxidative stress. In protective group, there was normal distribution of collagen fibers especially in testicular capsule and around blood vessels, a result that is in agreement with Gopal et al. (2015).

Regarding this study, BC has an ameliorating effect against TDN induced testicular germ cell damage. BC may be a valuable protective agent to ameliorate spermatogenesis dysfunction and cell loss; also, BC treatment increased the weight of testis, serum testosterone and sperm count.

## Conclusion

Taken together, the results of this study revealed that TiO<sub>2</sub>NPs induced histological, genetic and immuno-histochemical abnormalities in testis of adult albino rats. Beta carotene improved the changes associated with TiO<sub>2</sub>NPs administration. This study supports the usage of beta carotene as a protective agent against the toxic effects of TiO<sub>2</sub>NPs.

## Recommendation

Much more attention should be paid for limiting the occupational and environmental exposure to TiO<sub>2</sub>NPs by continuous monitoring of TiO<sub>2</sub>NPs level in work environment and keeping it within the recommended exposure limits and increased awareness about the health hazards caused by TiO<sub>2</sub>NPs. Moreover, highly exposed individuals are advised to take β-Carotene supplementation to limit the toxic effects of TiO<sub>2</sub>NPs on the testes. In addition, toxicity studies should be done to clarify the mechanisms of TiO<sub>2</sub>NPs's toxicity.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Evaluation of pharmaceuticals in household waste in Senador Canedo, State of Goiás, Brazil

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The disposal of pharmaceuticals has become a problem for society and public health and causes risks to the environment. Therefore, the objective of this work was to evaluate the number of pharmaceuticals and the classes of the most discarded pharmaceuticals in household waste in Senador Canedo, Goiás, Brazil. The study was conducted in Senador Canedo, GO, Brazil (16° 42' 28" S, 49° 5' 34" W, and altitude of 801 m), which presents maximum air temperature of 35 to 37°C and a minimum of 11 to 13°C, along with mean annual rainfall depth of approximately 1,350 mm. Exploratory and descriptive research was conducted to characterize and quantify the disposal of pharmaceuticals in household wastes in the municipality of Senador Canedo, GO. The sample size required to estimate the production of household pharmaceuticals waste in a population (considered infinite) was defined through the following statistical criteria: 95% confidence level and 5% sampling error. The research consisted of a collection of 10 random samples (garbage bags) in each neighbourhood of Senador Canedo, GO. The most disposed pharmaceuticals in household wastes are from the analgesic, anti-inflammatory, antihypertensive, and antibiotic pharmaceutical classes, with percentages above 10%. Antiulcer, diuretics, and antidepressants are the other pharmaceutical classes that present the highest quantity in household wastes.

**Key words:** Analgesic, anti-inflammatory, antibiotic, toxic medicines, solid urban.

## INTRODUCTION

The metropolitan region of Goiânia is the most populous urban centre of the state of Goiás (GO), Brazil. This region produces most of the solid urban waste of the state, with the municipalities of Aparecida de Goiânia, Goiânia, and Senador Canedo presenting solid waste production per capita ranging from 0.83 to 1.00 kg day<sup>-1</sup> (Goiás, 2014; Ribeiro, 2017). Part of these wastes

consists of medicine leftovers that are disposed of by the population; moreover, the use of medicines has been increased and the continuous introduction of new, highly bioactive and potent medicines has increased the diversity of toxic medicines disposed of to the environment (Daughton, 2003; Medeiros et al., 2014).

The disposal of pharmaceuticals has become a

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problem for society and public health and causes risks to the environment; they are a toxic waste that can contaminate the environment and cannot have the same final destination of common wastes (Vaz et al., 2011; Feitosa, 2016).

Even though medical waste is classified as chemical waste (health care waste), it has commonly been disposed of together with urban solid waste when it is from people that consume them at home. The medicine disposal made by these people is a great concern because of the lack of control and management of this waste (Boer and Fernandes, 2011; Vargas, 2014).

Concerns about medicine disposal are not new but have been little addressed and left to the constituted authorities and agents directly involved, such as pharmacies, laboratories, and users of medicines; consequently, scientific information can be important to further this issue in all contexts, including environmental (Rosa, 2017).

Therefore, the objective of this work was to evaluate the number of pharmaceuticals and the classes of the most discarded pharmaceuticals in household waste in Senador Canedo, GO, Brazil.

## MATERIALS AND METHODS

The study was conducted in Senador Canedo, GO, Brazil (16° 42' 28" S, 49° 5' 34" W, and altitude of 801 m), which presents maximum air temperature of 35 to 37°C and a minimum of 11 to 13°C, and mean annual rainfall depth of approximately 1,350 mm.

Exploratory and descriptive research was conducted to characterize and quantify the disposal of pharmaceuticals in household wastes in the municipality of Senador Canedo, GO. In general, exploratory researches obtain qualitative and quantitative descriptions of the study object that conceptualize the interrelationships between the properties of the phenomenon, fact, or environment observed (Lakatos and Marconi, 2010).

The descriptive process is used to identify factors or variables that are related to the phenomenon or process under study; after data collection, the analysis of the relationships between variables is performed for subsequent determination of the effects (Perovano, 2014; Calimerio and Miyasato, 2016). Exploratory and descriptive researches are, in general, a preliminary step to obtain scientific explanations (Gil, 1991).

The sample size required to estimate the production of household pharmaceuticals waste in a population (considered infinite) was defined through the following statistical criteria: 95% confidence level and 5% sampling error.

Quantity of pharmaceuticals in household waste (QFW) and quantity of discarded blister packs containing tablets (DBPCT) were evaluated.

The research consisted of the collection of 10 random samples (garbage bags) in each neighbourhood of Senador Canedo, GO. The collection of garbage bags and separation of the material was done as indicated by the current Brazilian legislation (Brasil, 2004, 2005); personal protective equipment (PPE) was used to avoid contact and contamination with the collected material. Thus, after the collection of garbage bags, the common waste was separated from pharmaceutical waste to discriminate and classify the medicines.

The pharmaceutical waste classified was placed in plastic bags and identified according to pharmaceutical classes: antibiotics, antifungals, antivirals, anthelmintics, anti-inflammatory medicine, analgesics, antispasmodics, digestive, antiulcer, antiemetic, anti-flatulent, laxative, vitamins, nutrient, mineral salts, antihypertensives, diuretics, cardiotoxic glycosides, antiarrhythmics, antidepressants, anxiolytics, antipsychotics, antiallergics, anti-vertigo, and vasodilators, expectorants, bronchial dilators, antivirals, antilipemic, hypoglycemics, hormones, herbal, homeopathic, and unidentified medicines.

Data related to pharmaceutical waste were tabulated and later analyzed, using the Microsoft Excel 2016 program.

## RESULTS AND DISCUSSION

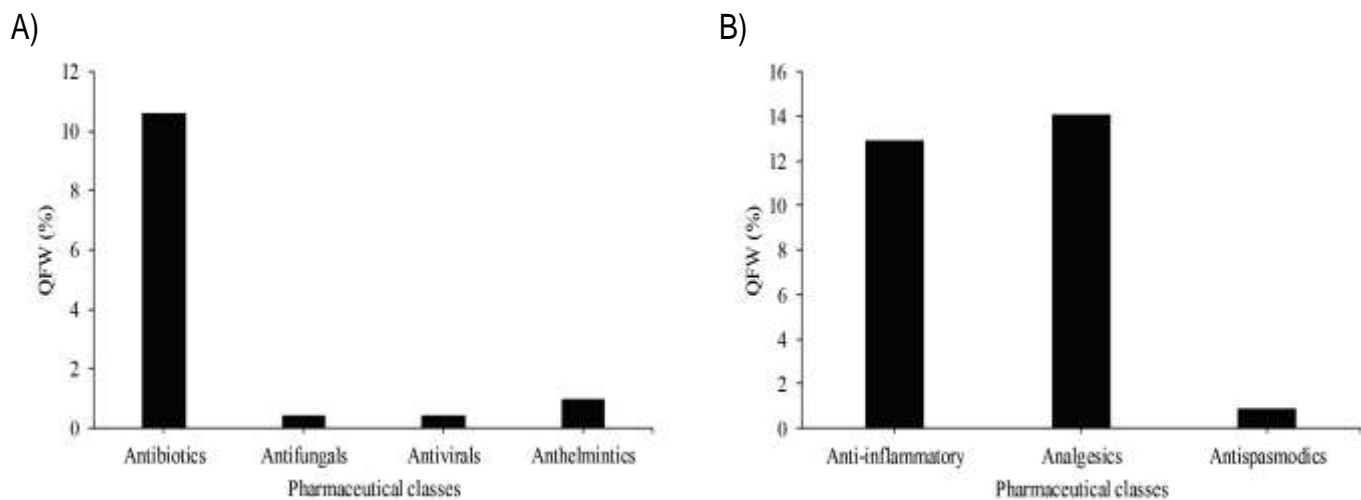
The disposal of medical waste as a function of the antibiotic, antifungal, antiviral, and anthelmintic pharmaceutical classes (Figure 1A) showed that the disposal of medicines from the antibiotic class is higher, representing approximately 10.6% of all medicines. Even though their marketing is restricted (requiring a prescription), their consumption is high, indicating that the prescription of this type of medicine by physicians may be considered relevant. The control of the market of these medicines was expected to reduce substantially its consumption, mainly due to the inhibition of self-medication; however, this has not been observed.

Ramos et al. (2017) evaluated the disposal of medicines and found that 78.9% of the respondents had this practice; the main reason reported for the disposal was the expiration date (62.9%) and the most discarded medicines were anti-infectious and antibiotics for systemic use (26.3%) and respiratory tract medicines (24.2%).

The disposal of antifungals and antivirals in household waste was approximately 0.5%, whereas the disposal of anthelmintics was approximately 1% of the total pharmaceutical waste found. Most antiviral medicines present moderate environmental hazards, and all show degradation persistence; thus, the overall toxicity of these compounds can be classified as low to moderate (Wennmalm and Gunnarsson, 2005; Oliveira, 2015).

A proper collection and disposal of medicines helps to reduce the burden of medicine pollution when providing the medicine disposal in an environmentally safe location, which may also assist in preventing the accumulation of unnecessary medicines in homes to avoid medicine intoxication accidents, traffic of controlled medicines, and misuse of medicines (Medeiros et al., 2014).

Pinto et al. (2014) found that 91% of the total respondents dispose expired medicines from their homes in an environmentally incorrect manner; they dispose these medicines together with common or recyclable wastes and in running water, and only 4% dispose of medicines properly in health centers, pharmacies, or community centres.



**Figure 1.** Quantity of pharmaceuticals in household waste (QFW) as a function of (A) pharmaceutical classes—antibiotics, antifungals, antivirals, and anthelmintics (B) and anti-inflammatory medicine, analgesics, and antispasmodics.

The quantity of antispasmodics was 0.9%, which is close to that found for the anthelmintic medicine class. Despite some pharmaceutical classes presented low quantities in household waste, their cumulative power should be considered, since pharmaceutical chemical wastes have an inherent potential of environmental risk that extends to the micro pollutants, which may have serious consequences due to their cumulative effect (Heberer, 2002; Daughton, 2003).

The waste quantity of pharmaceuticals of the anti-inflammatory, analgesic, antibiotic classes was significant in the household waste evaluated, which were the predominant pharmaceutical classes of the medicines disposed of by the population (Figure 1B). Anti-inflammatory medicines and analgesics are readily available products and are generally purchased in large quantities by the population, which can be confirmed by the high quantity of these products in the household waste evaluated, which reached 13 and 14%, respectively.

The classes of the most discarded pharmaceuticals found by Rocha et al. (2009) were anti-inflammatory (13.2%) and analgesic (10.2%) ones. They reported that pharmaceuticals of both these classes are freely marketed in drugstores, and are only used when the user needs it, thus, they end up accumulating in homes, expiring, and should be discarded. They also reported that antimicrobials represented one-third of the most discarded group (9.2%). The disposal of pharmaceuticals of these classes found by Rocha et al. (2009) was similar to that found in the present study.

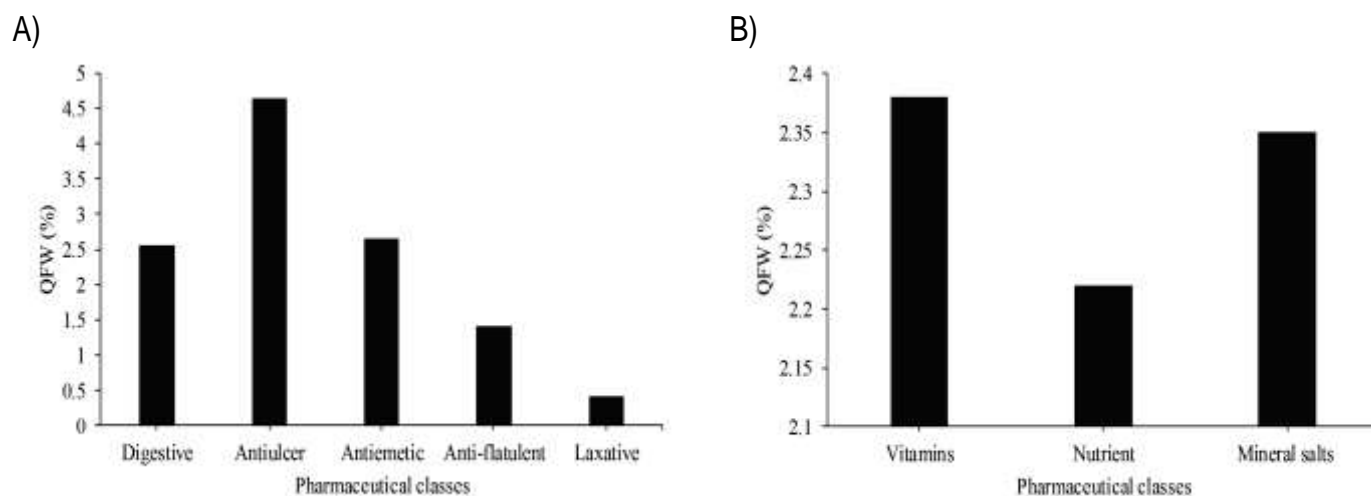
The disposal of medicines from the antiulcer class in household waste represented 4.65% (Figure 2A). Almeida et al. (2016) found that the most discarded

pharmaceuticals in pediatric units were from the antimicrobial (22.7%), electrolyte (14.8%), analgesic (14.6%), diuretic (9.5%), and antiulcer (6.7%) classes.

Pharmaceuticals from the digestive and antiemetic classes showed similar disposal in household wastes, which were 2.56 and 2.65%, respectively. This was probably because medicines from these pharmaceutical classes are consumed together by the population for digestive problems. Medicines from the laxative class were not significantly found in household wastes, showing a percentage of only 0.41%. Durães et al. (2015) evaluated pharmaceutical classes of medicines discarded in a university and found that the most pharmaceuticals were from the classes of the analgesics and antipyretics, representing 19% of the total quantity found, followed by the anti-inflammatory (11%), anti-flatulence (7%), laxative (5%), and antiemetic (4%) classes.

Disposal of vitamin, nutrient, and mineral salts supplements in household wastes showed little difference, with approximately 2.40, 2.22, and 2.35%, respectively (Figure 2B). Similar percentages of disposal were found for the digestive and antiemetic classes. Oliveira et al. (2015) applied a questionnaire in health facilities and found that 30.6% of the discarded pharmaceuticals were antihypertensives, followed by anticoagulants (10.15%), benzodiazepines (7.97%), antidiabetics (7.96%), and vitamins (2.06%).

Antihypertensives had a high disposal percentage in household waste, reaching approximately 11%; pharmaceuticals from this class were among the most commonly found in the household waste (Figure 3A). Diuretics also presented a significant percentage in the household wastes, approximately 4.12%; and the cardiotonic glycosides, and antiarrhythmic presented



**Figure 2.** Quantity of pharmaceuticals in household waste (QFW) as a function of (A) pharmaceutical classes—digestive, antiulcer, antiemetic, anti-flatulent, and laxative (B) and vitamins, nutrient, and mineral salts supplements.

lower quantities, only 0.29 and 0.51%, respectively. Baldoni et al. (2015) found that the profile of discarded expired pharmaceuticals by users of health units were predominantly from the antihypertensive (22.0%), oral hypoglycemic (10.7%), and antiplatelet agent (10.6%) classes.

The high quantity of discarded antihypertensives and diuretics may be related to non-adherence to the treatment by the patient, which may be due to side effects of these medicines, forgetfulness, fear of administering these medicines while using others, and unawareness of the need for treatment continuity (Barbosa and Lima, 2006; Oliveira et al., 2015), since medicines will reach the expiration date due to the interruption of the treatment and will be disposed as household waste. Most medicine users do not know what to do with expired medicines and are unaware of the damages caused by their inappropriate disposal (Carvalho et al., 2009).

The number of antidepressants within the pharmaceutical wastes evaluated was relevant (4.11%) (Figure 3B), and their disposal in household waste brings serious risks to the environment. Borrelly et al. (2012) conducted toxicity tests and found higher death of aquatic organisms (*Vibrio fischeri* and *Hyalella azteca*) when these species were in contact with antidepressant medicine. It is estimated that the pharmaceutical classes that cause most impacting are: antibiotics (76.6%), hormones (73.6%), and antidepressants (69.4%); these percentages represent the number of medicines of each class with inherent environmental risk (Rodrigues, 2009; Paut Kusturica et al., 2017).

The percentages of anxiolytic and antipsychotic pharmaceutical classes were low in the household

wastes evaluated, with averages not exceeding 0.30% (Figure 3B); however, Brazil is the third largest world consumer of benzodiazepine anxiolytic medicines, lagging behind only the United States and India (UN, 2018), the low percentage of anxiolytics found may be related to a greater adherence to treatment by patients.

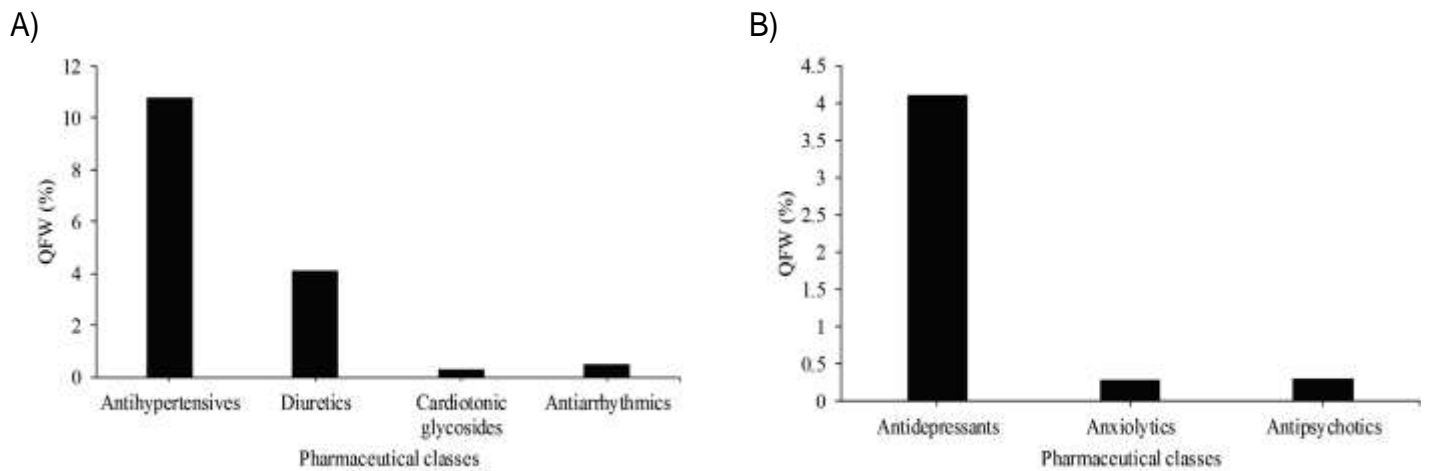
Antiallergics, antivertigo, and vasodilators presented percentages in household pharmaceutical waste of approximately 2% (Figure 4A); and expectorants, bronchial dilators, and antivirals presented 0.84, 0.66, and 0.42%, respectively (Figure 4B). Marques and Xavier (2018) attributed the high use of vasodilators, antipyretics, and analgesics by the population to unstable climate periods and rapid drops in temperatures, which favour the emergence of symptoms of cold; moreover, they also found a high use of antiviral, antibiotic, anti-inflammatory, and antiallergic medicines in these periods.

The quantity of antiallergics, anti-vertigo, vasodilators, expectorants, bronchial dilators, and antivirals in household wastes in central Brazil, where medicines from these pharmaceutical classes are used for treatment of respiratory diseases, is mainly related to high air temperature and low relative humidity, which was confirmed by Murara and Amorim (2010) who correlated the days that presented the highest occurrences of diseases with dry periods combined with high thermal amplitudes, or with days that had low relative humidity values.

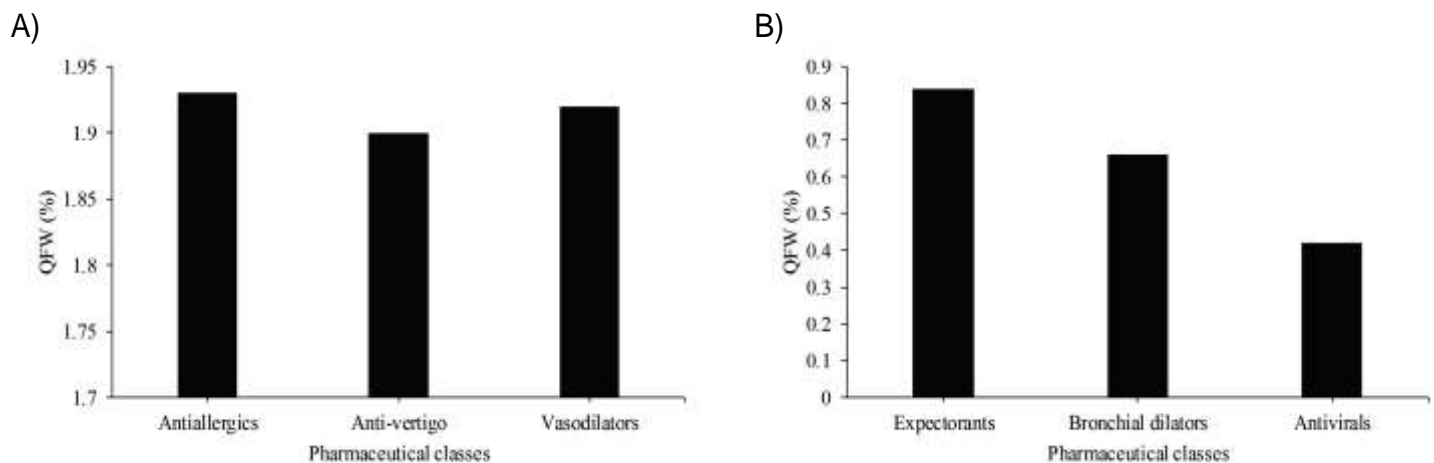
The percentage of antilipemic and hypoglycemic medicines found in household wastes was approximately 3.4 and 4.0%, respectively, and the presence of hormone medicines did not exceed 0.5% (Figure 5A).

Sodré et al. (2010) evaluated water supply samples in Campinas, SP, Brazil, and found that estrone and 17 $\beta$ -





**Figure 3.** Quantity of pharmaceuticals in household waste (QFW) as a function of (A) pharmaceutical classes—antihypertensives, diuretics, and cardiotonic glycosides, and antiarrhythmics (B) and antidepressants, anxiolytics, and antipsychotics.



**Figure 4.** Quantity of pharmaceuticals in household waste (QFW) as a function of (A) pharmaceutical classes—antiallergics, anti-vertigo, and vasodilators and (B) expectorants, bronchial dilators, and antivirals.

estradiol were detected below the limits of quantification. Also, stigmasterol had the highest concentration, followed by cholesterol and caffeine.

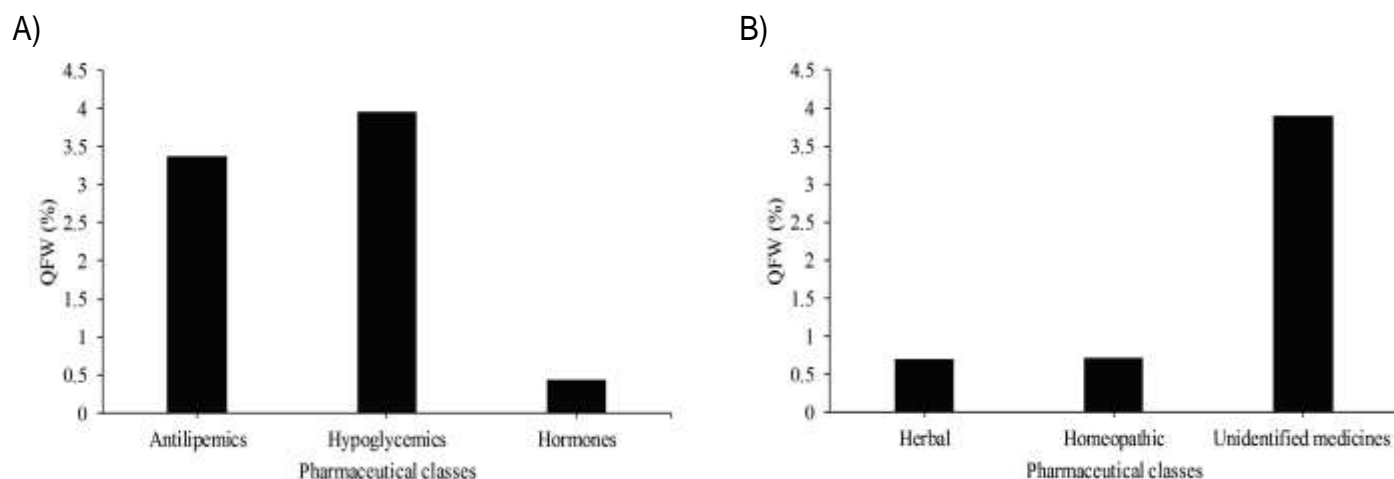
Regarding the disposal of hypoglycemic agents, Cavalcante et al. (2016) reported that there are no clear guidelines to control the disposal of collection containers; and, in some cases, the maximum capacity of the collection box is insufficient for the correct separation of the waste generated in a month, indicating that, in the absence of enough containers, the population discard part of their antidiabetic medicine waste in ordinary household waste.

According to Carvalho et al. (2009) in the United States, about 41 million citizens receive drinking water

contaminated with several pharmaceutical products, such as antibiotics and hormones.

Phototherapeutic and homeopathic medicines showed similar disposal in household wastes, with an average percentage of 0.7%. Guerrieri and Henkes (2017) conducted interviews about types of pharmaceuticals that the respondents used and had in their homes and found that 36.25% of them had only analgesics and antipyretics; 16.25% had antibiotic and anti-inflammatory medicines; 4.68% of respondents had others (e.g. contraceptive, homeopathic, and herbal medicines); and 1.56% had antidepressants.

The number of pharmaceuticals found was not large due to their high packaging degradation level; besides,



**Figure 5.** Quantity of pharmaceuticals in household waste (QFW) as a function of (A) pharmaceutical classes—antilipemic, hypoglycemics, and hormones and (B) herbal, homeopathic, and unidentified medicines.

the number of unidentified medicines was 3.90% (Figure 5B).

The most commonly found anti-inflammatory medicines in the household wastes evaluated were ibuprofen, nimesulide, and diclofenac. Considering these three anti-inflammatory medicines, ibuprofen represented 58.8% of the quantity found (Figure 6A). Xu et al. (2009) found that ibuprofen has a short residence time in different soils, indicating a high percolation potential, easily reaching groundwater.

Among the antibiotics found, amoxicillin and cephalixin represented 62.5% of the medicines from this pharmaceutical class (Figure 6B). Some of the most used classes such as antibiotics, which are used to prevent and treat bacterial infections, deserve to be highlighted since their intensive use has contaminated environmental matrices such as soil, water, sediment, plants, and animals with effects on the biota. After contaminating the environment, such drugs have the potential to cause adverse effects to the aquatic, terrestrial and also to humans (Botelho et al., 2015). According to a study in the United Kingdom, amoxicillin is one of the main substances considered by environmental monitoring studies because of the intense use, transport potential in the environment, and toxicity of these compounds (Capleton et al., 2006).

The most discarded medicines were dipyron monohydrate, ibuprofen, amoxicillin, nimesulide, and ranitidine hydrochloride. Among these pharmaceuticals, dipyron monohydrate and ibuprofen were more discarded than amoxicillin, nimesulide, and ranitidine hydrochloride, probably due to the high availability, continued use, and the low price of these medicines (Figure 6C). Bandeira et al. (2019) reported that the most common pharmaceuticals found in environments are:

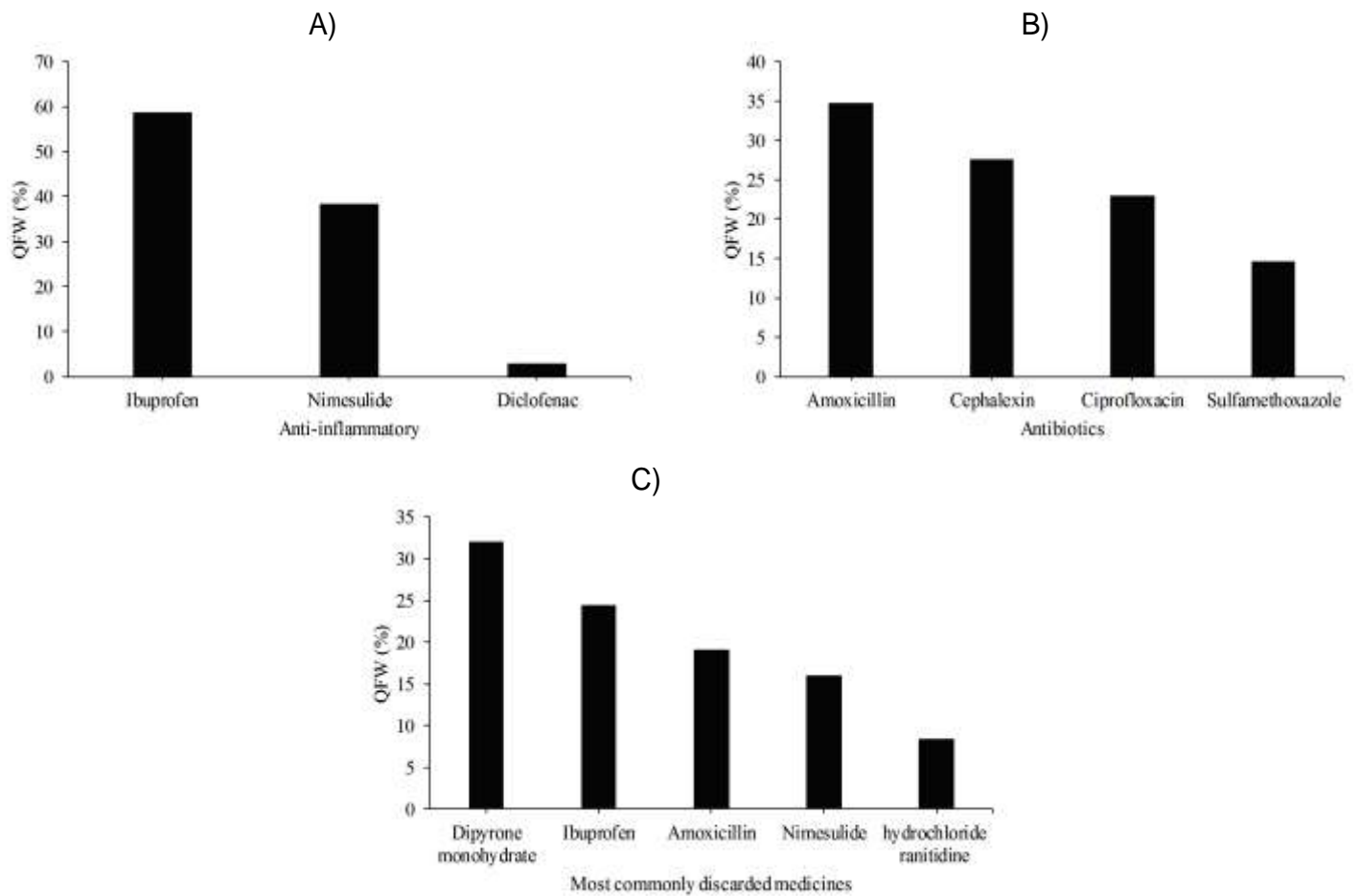
ibuprofen, paracetamol, dipyron, simvastatin, fluoxetine, and contraceptives; and the improper use of these pharmaceuticals can lead to health problems.

In a research conducted by Serafim et al. (2007), most respondents affirmed to use of dipyron-containing medicines; to have no-expired medicines in their homes (93.3%); and to dispose them together with the household waste (79%), thus emphasizing that this inappropriate disposal of medicines is a worrying factor as it may pose a health risk to children or people who may reuse them.

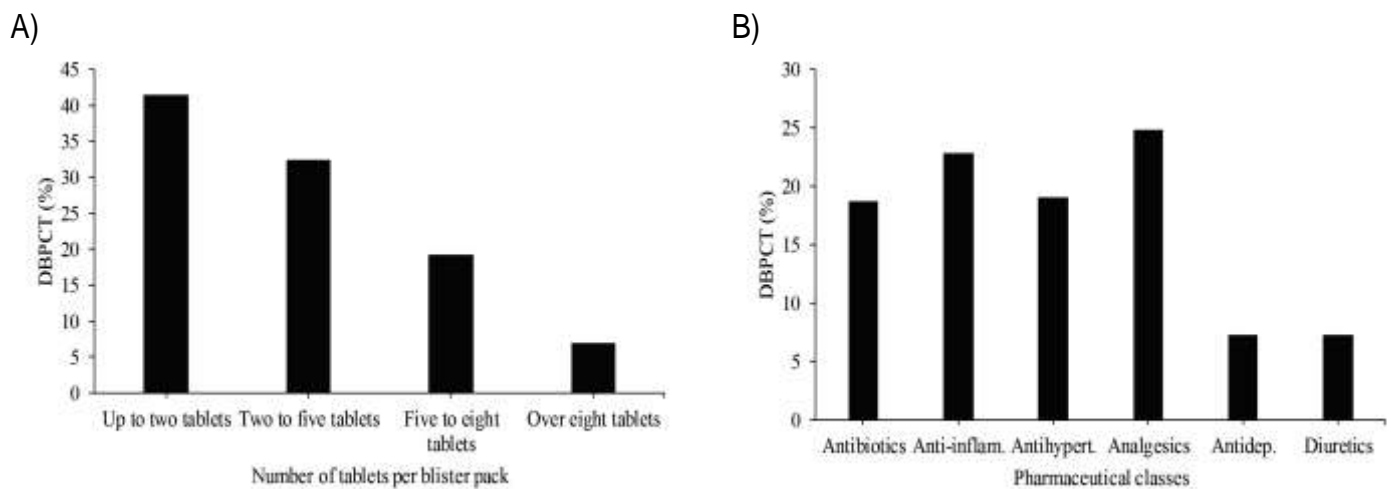
Figure 7A shows the percentages of discarded blister packs containing tablets as a function of the number of tablets per pack.

The percentages of discarded blister packs containing tablets were approximately 41.4% (up to two tablets), 32.5% (two to five tablets), 19.2% (five to eight tablets), and 7.0% (over eight tablets) (Figure 7A). Pinto et al. (2014) conducted similar study and found that most respondents (55%) dispose packs with small quantity, up to 4 tablets per year; however, the study pointed out that although this result seems insignificant, at the end of a year, about 1,300 tablets would be discarded in the environment, only in the context of the research.

Figure 7B shows the percentages of discarded blister packs containing tablets as a function of pharmaceutical classes. The most common pharmaceutical classes were antibiotics, anti-inflammatory, antihypertensives, analgesics, antidepressants, and diuretics. Analgesics, anti-inflammatory, and antibiotics represented 66.4% of these pharmaceuticals. The results found for analgesic and anti-inflammatory medicines were due to the high availability and low cost of many of the medicines from these two pharmaceutical classes. Analgesics, anti-inflammatory, steroid hormones, anti-parasites, and



**Figure 6.** Quantity of pharmaceuticals in household waste (QFW) as a function of (A) pharmaceutical classes—anti-inflammatory and (B) antibiotics and (C) the most commonly discarded medicines—dipyron monohydrate (dipyron M), ibuprofen, amoxicillin, nimesulide, and hydrochloride ranitidine.



**Figure 7.** Quantity of discarded blister packs containing tablets (DBPCT) as a function of (A) a number of tablets per blister pack (B) and quantity of discarded blister packs containing tablets as a function of pharmaceutical classes—antibiotics, anti-inflammatory, antihypertensives, analgesics, antidepressants, and diuretics.

antibiotics are among the medicines of environmental importance due to the quantity consumed, toxicity, and persistence in the environment (soil and water) (Carvalho et al., 2009; Salomão et al., 2019).

Household waste has a wide variety of debris. It is very common to find pharmaceuticals waste or similar dispensed by the population, which can be harmful to human health and the environment. This problem is mostly caused by people not knowing the risks of this waste and lack of a communication policy that can inform the population of the damage that this pharmaceuticals waste can cause to living and environmental beings in a region.

## Conclusion

The most disposed pharmaceuticals in household wastes are from the analgesic, anti-inflammatory, antihypertensive, and antibiotic pharmaceutical classes, with percentages above 10%. Antiulcer, diuretics, and antidepressants are the other pharmaceutical classes that present the highest quantity in household wastes.

The most commonly found medicine of the anti-inflammatory pharmaceutical class in household wastes is ibuprofen; and for the antibiotic class, it was the amoxicillin.

The medicines of most discarded blister packs containing tablets are from the analgesic, anti-inflammatory, and antibiotic pharmaceutical classes, and 73.9% of these blister packs have up to five tablets.

## CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

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