



INFLUENCE OF GENISTEIN AND CAFFEINE ON ABSORBABLE SUTURE.

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ABSTRACT:

BACKGROUND: There are several reasons why sutures are utilized in surgery. Sutures are the materials that are most frequently utilized for this purpose. Absorbable sutures have the benefit of typically not needing to be removed. The pH and temperature of the tissues surrounding the suture affect how quickly this breakdown occurs. Although PGA is more powerful than silk, it loses a lot of strength over time in gums. Vicryl has low tissue reactivity, good tensile strength, and good knot security. It loses its tensile strength in 28 days. Food that we regularly eat contains secondary metabolites, which are organic compounds with low molecular weight that can be biosynthesized by any living thing utilising a variety of biosynthetic routes. Genistein and caffeine are two such metabolites that are widely consumed by Indians. Analyzing the impact of genistein and caffeine on absorbable sutures is our goal.

MATERIALS AND METHODS: Genistein and Caffeine were prepared as formulations and its antioxidant and anti inflammatory activity were evaluated individually and in combinations. Then the extract was coated in vicryl and PGA sutures of sample size 30 pieces (15 pieces in PGA and vicryl each). SEM analysis was done after coating. Tensile strength was analyzed for coated sutures before and after immersion in artificial saliva at day 1 and 14.

RESULTS: DPPH assay of antioxidant activity, genistein - caffeine formulation has very moderate (45%) antioxidant activity. In protein denaturation assay, anti inflammatory activity of genistein - caffeine has high (75%) anti inflammatory activity. Scanning electron microscope shows even coating of genistein-caffeine formulation on PGA sutures. vicryl coated with Genistein- caffeine shows maximum tensile strength at pH 4.4(

1439.45±0.87) . PGA sutures show decreased tensile strength after immersion in artificial saliva at pH 4.4 (630.68±0.54) than the control group.

CONCLUSION: Thus the study shows that absorbable suture which is coated with the genistein - caffeine was being tested under scanning electron microscope in which it shows even coating all over the surfaces. The tensile strength is higher in vicryl than in PGA before and after immersion in artificial saliva. The antioxidant activity, the formulation of genistein - caffeine formulation shows comparative effective activity in DPPH assay. In protein denaturation assay, the formulated extract genistein - caffeine has comparable moderate anti-inflammatory activity.

KEYWORDS: Genistein, Caffeine, Vicryl, Polyglycolic acid .

INTRODUCTION:

Skin closure is necessary for the majority of surgical and traumatic wounds in order to speed healing and prevent subsequent infections. This closure is typically performed by suturing, and the primary restoration of the skin and deeper layers is used to encourage wound healing. Suturing is superior to other procedures in that it has lower dehiscence rates and higher tensile strength(1). Currently, there are countless possibilities for sutures, so we may choose a suture type wisely, but it is important to comprehend the properties of various sutures. The best sutures should be simple to manage, offer adequate strength and secure knots, and be able to withstand changes in the wound such as swelling and recoil(2)). It should have very little potential to inflame or infect, be readily apparent, and be reasonably priced. Absorbable and non-absorbable sutures make up the first major group of sutures. In cases of profound temporary closure until the tissues heal or when it is difficult to remove, absorbable sutures are frequently used(3). Absorbable sutures are further divided into natural and synthetic varieties. Natural sutures disintegrate by proteolysis, whereas synthetic sutures break down by hydrolysis. Non-absorbable sutures are used for permanent ligation of internal tubular structures or arteries, hernia repair, and other long-term tissue closure procedures(4). Additional suture is divided

into monofilament and multifilament types. Monofilament sutures are made of a single filament and have a smaller surface area than multifilament sutures, which can be braided or twisted.

In dentistry, the tissue suture is used as the final stage of a surgical procedure to stabilize the wound lips, promote haemostasis, prevent food particles from accumulating on the incision site, and enable the first intended healing(5). A strong suture prevents the surgical wound from dehiscing or becoming unstable due to the displacement forces produced by muscle insertions, functional motions, and external agents. Synthetic polymers made of polyglycolic acid, like Dexon, lose all of their tensile strength within 25 days. Polyglactin 910 - polyfilament, like Vicryl, has low tissue reactivity, good tensile strength, and good knot security, however it loses tensile strength after 28 days. The protein from silkworms used in silk derivatives like Permahand, Ethicon, and Sof silk, Covidien is coated to reduce friction and water absorption(6). This material is simple to handle, has excellent knot security, and has a comparably decent tensile strength. Its considerable tissue reactivity, which results in tensile strength loss after months of use, makes it uncommon to use internally. The use of surgical silk is no longer necessary due to developments in sutures. However,

mucosal surfaces are still treated with it in dentistry(7).

Everyday foods that we eat contain secondary metabolites, which are organic compounds with low molecular weight that can be biosynthesized by any living thing utilising a variety of biosynthetic routes(8).Secondary metabolism is viewed in terms of evolution as a collection of specialised pathways that make use of a wide range of enzymes(9).Isoflavone genistein is a natural phytoestrogen that originates in Southeast Asia and is found in soybeans. In the colon, genistein is transformed into genistein glucuronide and sulphate, which together with genistein, are transported into veins by multidrug resistance-associated protein 3 transporters with a 100% absorption rate. Indians typically eat 30 to 40 mg of meals high in soybeans per day, which is below healthy limits. The chemical name for caffeine is 1,3,7-trimethylxanthine(10). The blood level of caffeine peaks between 15 minutes and 2 hours after consumption and is absorbed within around 45 minutes. Coffee, tea, and soda are a few examples of drinks that contain caffeine. Adults in India take 200 mg of caffeine on a daily average. When consuming 1.2 gms or more of caffeine in one dose, caffeine toxicity has been found more frequently(11).Our team has extensive knowledge and research experience that has translate into high quality publications(12–21)

It is important to comprehend the impacts of secondary metabolites on sutures and the material's ability to endure deterioration because they are found in the regularly ingested daily foods of the Indian populace. There has never been a study done before that looks at the impact of sutures and how

secondary metabolites affect the suture. As a result, the study's goal is to examine how genistein and caffeine affect absorbable sutures.

MATERIALS AND METHODS:

Artificial saliva preparation:

Artificial saliva of 1000 ml is prepared by mixing it with 8.035 g of Sodium chloride, 0.355 g of sodium bicarbonate,0.255g of potassium chloride,0.231g of potassium hydrogen phosphate,0.311g of magnesium chloride,40 ml of 1.0M of hydrochloric acid ,0.292g of calcium chloride,0.072g of sodium sulfate,6.118g of Trizma base and 1M hydrochloric acid and the prepared saliva was divided into 2 separate beakers of 500 ml and added to varying ph such as 7.4ph and 500ml with 4.4ph.

Preparation of extract :

Genistein is obtained in the form of soybean powder and caffeine was obtained from commercially available coffee powder bought from an organic shop. 30gms of each powdered soybean and coffee powder was weighed and added to 80 ml of distilled water .Then kept it in an orbital shaker for 24 hrs for obtaining concentrated genistein and caffeine.

Coating the sutures:

The extract prepared using genistein and caffeine were taken in different test tubes (Garg glass laboratory test tubes).The PGA (UNIGLIDE)and vicryl sutures (polyglactin 910) were taken and cut into 30 pieces each group named Group I (Control PGA),Group II (control vicryl),Group III (PGA coated with genistein -caffeine formulation) immersed at pH 4.4 artificial saliva, Group IV (PGA coated with genistein -caffeine formulation) (fig 1)immersed at pH 7.2

artificial saliva, Group V (vicryl coated genistein -caffeine formulation) immersed at pH 4.4 artificial saliva, Group VI (vicryl coated genistein -caffeine formulation) immersed at pH 7.2 artificial saliva. These pieces were kept in shaker for about 6 hours for the uniform coating in the suture materials. Then the sutures are kept in the atmospheric temperature to get dried completely and the suture material of size 1.5 mm were sent to analyze the anti-inflammatory and antioxidant activity. Suture measuring 10mm were subjected to tensile strength.

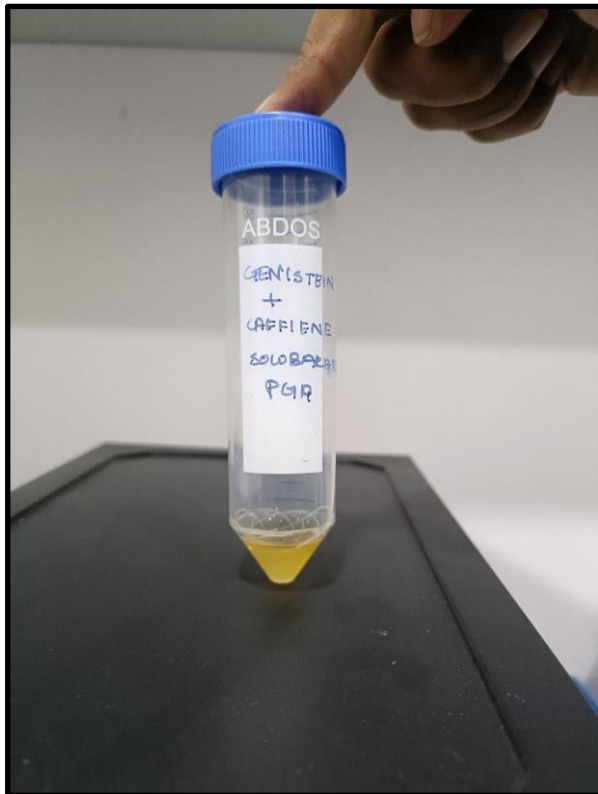


Fig 1: Sutures coated with Genistein and Caffeine.

RESULTS:

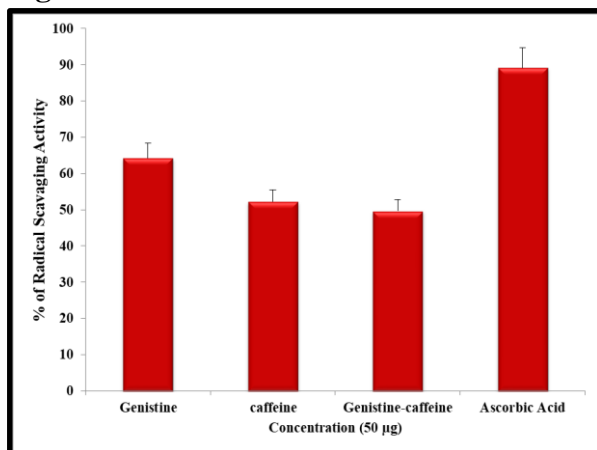
The antioxidant activity where genistein-caffeine formulations were analyzed in which the genistein have comparatively good antioxidant activity as the standard group (ascorbic acid). On protein denaturation assay the caffeine has good anti-inflammatory activity compared to the

genistein and the anti-inflammatory activity of caffeine is comparatively better than the standard group used (Diclofenac sodium). The Scanning electron microscope shows that the PGA sutures were evenly coated. The tensile strength of uncoated PGA (group I) was 1074.39Mpa whereas the tensile strength reduced after coating the suture with artificial saliva at pH 4.4 (group III) the tensile strength reduced to 630.68Mpa and at pH 7.2 (group IV) the tensile strength is about 784.23 Mpa which shows that tensile strength reduced after coating. But the coated vicryl (group V) has the higher tensile strength such when immersed in pH 4.4 the value is 1439.45Mpa and in pH 7.2 (group VI) the value is 1302.67 Mpa but the uncoated suture has a comparatively lesser tensile strength (976.83Mpa). ANOVA analysis of both the uncoated and coated PGA sutures at pH such as 4.4 and 7.2 has a p-value 0.03 which is statistically significant. Even the ANOVA analysis of uncoated and coated vicryl sutures at different pH has a p-value of 0.01 which is also found to be statistically significant. Coated vicryl at pH 4.4 (group V) has the highest Tensile strength (1439.81 MPa) on the 14 th day of immersion in artificial saliva because it has the lowest chance of getting degraded. In comparison, the coated vicryl at pH 7.2 has comparatively lesser tensile when immersed in artificial saliva and the degradation is comparatively faster. PGA coated at pH 4.4 and 7.2 (group III and group IV) suture samples show lesser tensile strength and therefore degradation rate is faster when immersed in artificial saliva at pH 4.4, 7.2. The ANOVA analysis of the PGA sutures showed a p-value of 0.03 which is statistically significant. ANOVA analysis of the vicryl sutures

showed a p-value as 0.04 which is also statistically significant.

Fig 3 shows the antioxidant activity where genistein - caffeine formulations were analyzed in which the genistein have comparatively good antioxidant activity as the standard group (ascorbic acid). The maximum percentage of radical scavenging activity seen with genistein was found to be 65% whereas the standard group showed the maximum antioxidant activity of 90% compared to other secondary metabolites genistein has good antioxidant activity.

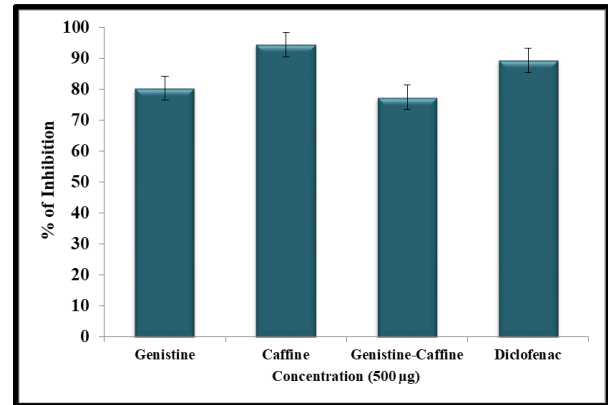
Fig 3:



Graph shows the antioxidant activity of prepared formulation (Genistein -caffeine)

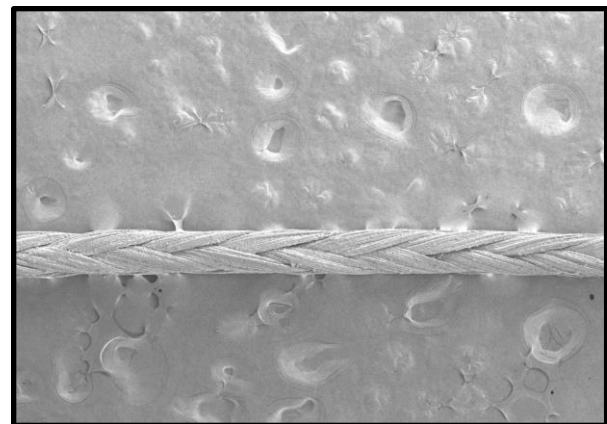
Fig 4 depicts the anti-inflammatory activity of Genistein and caffeine extract . On protein denaturation assay the caffeine has good anti inflammatory activity compared to the genistein and antiinflammatory activity of caffeine is comparatively better than the standard group used (Diclofenac sodium) . The maximum percentage of inhibition of caffeine coated on the sutures were observed as 95% whereas the standard group showed only 90%.

Fig 4:

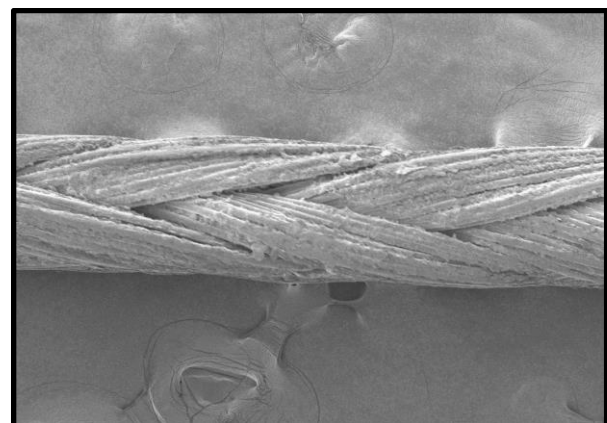


Graph shows the anti-inflammatory activity of prepared extract (Genistein -caffeine).

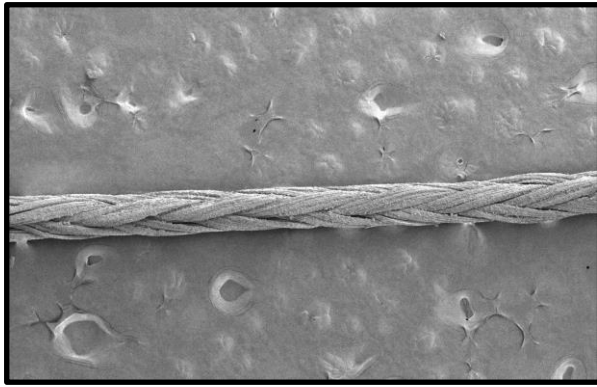
SEM ANALYSIS FOR THE SUTURES COATED WITH GENISTEIN AND CAFFEINE:



SEM analysis of Genistein and Caffeine coated PGA absorbable suture at the focal Length of x37.0



SEM analysis of Genistein and Caffeine coated PGA absorbable suture at the focal Length of x120.



SEM analysis of Genistein and Caffeine coated vicryl absorbable suture at the focal Length of x120.

The SEM analysis of PGA and vicryl sutures were done to analyze the surface roughness .Fig 4 shows the SEM analysis of PGA sutures were evenly coated but whereas the SEM analysis of vicryl sutures were coated as globules and it's not uniform.

SEM analysis of Genistein and Caffeine coated vicryl absorbable suture at the focal Length of x37.0.

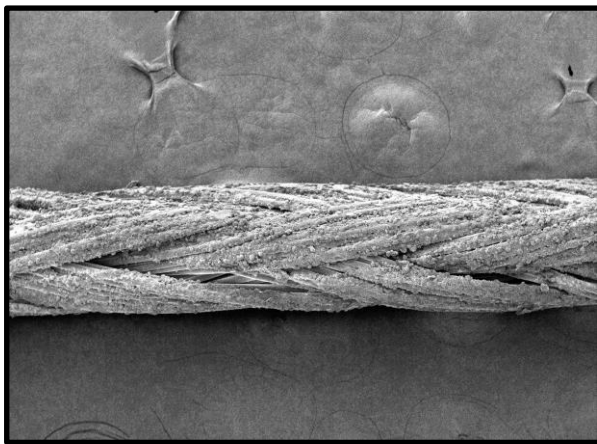


Table 1: Tensile strength of coated suture at different pH values compared to control uncoated values.

sample group	specimen label	MAXIMUM FORCE	Tensile stress at Tensile strength [Mpa]	Tensile strain (Displacement)at Break (standard)[%]
Group I	PGA (uncoated) (Control)	6.93±0.03	1074.39±0.11	9.45±0.15
Group II	VICRYL(uncoated) (Control)	6.29±0.22	976.83±0.67	13.67±0.45
Group III	PGA (Coated) (pH 4.4)	4.11±0.87	630.68±0.54	9.81±0.67
Group IV	PGA(coated) (pH 7.2)	5.05±0.17	784.23±0.03	4.66±0.87
Group V	VICRYL(coated) (pH 4.4)	9.29±0.64	1439.45±0.87	16.09±0.21

Group VI	VICRYL (coated) (pH 7.2)	8.38±0.34	1302.67±0.45	17.46±0.34
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Table 1 suggests the tensile strength of various coated and uncoated suture materials with different pH. All the data generated by the measurements were represented in the standard deviation and it is statistically analyzed with Spss software.

So from the table it is seen that coated vicryl have better tensile Strength which means the degradation rate is comparatively less than the uncoated sutures but it's vice versa in PGA sutures.

Table 2: Tensile strength at 14th day of immersion

Group	specimen label	Tensile stress at break (standard)[MPa] (Degradation at 14th day)
Group I	PGA (uncoated)(Control)	1067.29±0.23
Group II	VICRYL (uncoated)(Control)	951.69±0.45
Group III	PGA (Coated) (pH 4.4)	636.56±0.46
Group IV	PGA (coated) (pH 7.2)	781.68±0.89
Group V	VICRYL (coated) (pH 4.4)	1439.81±0.76
Group VI	VICRYL (coated) (pH 7.2)	1291.93±0.24

Pairwise comparison of PGA and Vicryl groups through one way ANOVA analysis.

sample group	specimen label	p value
Group I,III,IV	PGA groups	0.037
Group II,V,VI	vicryl groups	0.045

The above mentioned table 2 shows the tensile strength at 14 th day of immersion .From the ANOVA analysis of the PGA sutures showed p value as 0.03 which is statistically significant. ANOVA analysis of the vicryl sutures showed p value as 0.04 which is also statistically significant.

DISCUSSION:

According to our study, caffeine has superior anti-inflammatory properties, while genistein, the control group, has somewhat higher antioxidant properties. Although the Indian populace uses these secondary metabolites often, our research will serve as a foundation for understanding the effects on suture materials. Previous research on genistein products demonstrates that it possesses anticancer and cytotoxic properties by promoting CIP2A downregulation, drastically reducing cell quantity, inducing cell arrest, and upregulating TNFR-1 protein and mRNA expression. Additionally, it turned on the p53 protein, caspase-3, and caspase-10(22).The preceding also demonstrates that genistein not only has greater antioxidant activity but also better anti-inflammatory activity. However, in our investigation, genistein and caffeine together had a less significant impact than the control group.(23) .

When analysing the suture's tensile qualities and degradation effects, it was discovered that the material's tensile strength decreased when fake saliva was applied at various pH levels. Study demonstrates that suture samples disintegrate more quickly under alkaline circumstances than under 7.4 pH conditions(24).The fact that the degree of degradation is higher in the case of the produced copolymer having both

components, strongly hydrophilic and weakly hydrophilic, than that of the homopolymer(25).

The sutures were the subject of numerous investigations with varying pH levels, but none of the studies examined the impact of secondary metabolites. According to studies, physical contact with biodegradable chemicals has little impact on the resistance of suture material integrity. However, other investigations examining the impacts of various media on resorbable suture materials have shown the opposite to be true(26).According to a recent study, flax fiber sutures exposed to moist conditions showed fiber debonding that resulted in fiber pullout, but dry flax fiber sutures showed brittle fracture. Previous research has shown that absorbable materials resist tension better than non-absorbable ones, with PGA showing the best performance both under normal conditions and after 5 to 7 days of immersion in Ringer's solution(27). Some studies have indicated that pH levels may have a greater influence on the performance of absorbable sutures than on non-absorbable ones(28).Both acidic and alkaline environments are able to accelerate the degradation of absorbable sutures.

CONCLUSION:

Thus the study shows that absorbable suture which is coated with the genistein - caffeine was being tested under scanning electron microscope in which it shows even coating all over the surfaces. The tensile strength is higher in vicryl than in PGA before and after immersion in artificial saliva.The antioxidant activity ,the formulation of genistein - caffeine formulation shows comparative effective activity in DPPH assay.In protein

denaturation assay , the formulated extract genistein - caffeine has comparable moderate anti inflammatory activity.

SCOPE OF FUTURE STUDY:

Further studies on the Genistein and caffeine on the various suture materials can be performed in vitro for better understanding.

ACKNOWLEDGEMENT:

The authors would like to acknowledge the help supported by the Department of periodontics and information technology of Saveetha dental College and hospitals and management for their constant assistance with the research.

CONFLICT OF INTEREST:

None to declare.

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